

# Comparative Analysis of Cell Block Preparation Versus Smear Examination in the Fine Needle Aspirates of Head & Neck Lesions with Application of IHC Markers on Cell Block Preparation

Kanika Wadhwa<sup>1</sup>, Permeet Kaur Bagga<sup>2</sup>, Bikramjit Singh<sup>3</sup>, Surinder Paul<sup>4</sup>

## ABSTRACT

**Introduction:** Diagnostic cytology is the science of interpretation of cells that are exfoliated from the epithelial surfaces or removed from various tissues. The aim of this study was to assess the utility of cell block in increasing the cytodiagnosis of fine needle aspirates of head and neck lesions and to apply immunohistochemical markers on cell blocks.

**Material and methods:** Total sample of 50 patients of head and neck lesions were received in the Department of Pathology, after approval from the Institutional Ethics Committee. Informed consent of the patient was taken. Relevant history of the patient was taken as per the written proforma. Patients of all age group presenting with head and neck lesions underwent FNAC and histopathological examination was included in the study.

**Results:** Thus FNAC served better than cell block in determining the cellularity ( $\kappa$  – statistic = -0.04,  $P$  0.0002) while on morphological preservation grounds, superior nuclear and cytoplasmic characteristics were observed in cell block in comparison to FNAC ( $\kappa$  – statistic = -0.08). Overall Sensitivity and positive predictive value of cell block method with immunohistochemistry (96% and 100% respectively) proved to be better as compared to FNAC alone (88.8% and 95.65%).

**Conclusion:** The diagnostic value of a Cell Block technique with immunohistochemistry is found to be superior to FNAC smears for the diagnosis of benign and malignant lesions of head and neck region. Taking into consideration the advantages of Cell Block method an excellent complementary tool for improving cytodiagnosis, we can recommend that cell blocks preparations should be routine practice so as to augment the information that is obtained solely from FNAC smear cytology.

**Keywords:** Cell Block; FNA, Head And Neck Lesions

## INTRODUCTION

Diagnostic cytology is the science of interpretation of cells that are exfoliated from the epithelial surfaces or removed from various tissues. The advantages are it is non-invasive, simple and helps in faster reporting and is relatively inexpensive. The accurate identification of cells as either malignant or reactive mesothelial cells is a diagnostic problem in conventional cytological smears. The main challenge to a cytopathologist in the present era of personalized treatment is to be able to devise techniques that can provide more information with less tissue available.<sup>1</sup>

FNAC is a simple, relatively safe, minimally painful, rapid and nonoperative procedure which has proven to show high

sensitivity and specificity in various lesions. Still some drawbacks are encountered due to cellular overlapping, delaying artifact, suboptimal processing, preparatory cytotechnique and most important its usually seen in various studies that the cytological examination of fluids by means of smears, however carefully prepared, leaves behind a large residue that is not further investigated but that might contain valuable diagnostic material. This residual material can be very useful in increasing diagnostic yield by the cell block method.<sup>2</sup>

The cell block (CB) technique is one of the oldest and complementary methods for the evaluation of body cavity fluids, Cell block technique is a mini formalin-fixed paraffin-embedded (FFPE) biopsy obtained from fine-needle aspirate or fluid sediment.<sup>3</sup> Preservation of cytological material in the cell block for IHC and molecular studies adds to its diagnostic accuracy and enables long-term archiving for future analyses.<sup>4,5</sup> The aim of this study was to assess the utility of cell block in increasing the cytodiagnosis of fine needle aspirates of head and neck lesions and to apply immunohistochemical markers on cell blocks.

## MATERIAL AND METHODS

The present study was conducted in 50 patients of head and neck lesions including oral cavity received in the Department of Pathology, after approval from the Institutional Ethics Committee. Informed consent of the patient was taken. The samples included were: Smears prepared from FNAC, Sections prepared from biopsied material and Cell blocks prepared from FNAC material. After this, cell blocks of selected cases were subjected to immunohistochemical

<sup>1</sup>Junior Resident, Department of Pathology, Government Medical College, Amritsar, <sup>2</sup>Associate Professor, Department of Pathology, Government Medical College, Amritsar, <sup>3</sup>Assistant Professor, Department of Oncosurgery, Government Medical College, Amritsar, <sup>4</sup>Professor, Department of Pathology, Government Medical College, Amritsar, India

**Corresponding author:** Dr. Permeet Kaur Bagga, Associate Professor, Department of Pathology, Government Medical College, Amritsar, India

**How to cite this article:** Kanika Wadhwa, Permeet Kaur Bagga, Bikramjit Singh, Surinder Paul. Comparative analysis of cell block preparation versus smear examination in the fine needle aspirates of head and neck lesions with application of IHC markers on cell block preparation. International Journal of Contemporary Medical Research 2019;6(6):F45-F49.

**DOI:** <http://dx.doi.org/10.21276/ijcmr.2019.6.6.42>

staining using PAN-CK, LCA, Vimentin and S-100.

The scoring criteria was used according to that described by Bhatia P et al. for grading on basis of cellularity of the slide, score 0,+1,+2,+3 were used which were interpreted as no cells, low (<10%), moderate (10-50%), and high (>50%) respectively.<sup>6</sup>

## RESULTS

As per distribution of sample, out of total 50 samples majority i.e 42% were received from thyroid lesions, while other sites in descending order were: both salivary gland and lymphnodes 22%, lateral border of tongue 12% and least was lip 2%. Age and Gender distribution in the present study shows that maximum number of study subjects fall under 41-50 years and 51-60 years with equal frequency (28%), with male predominance (54%) and Male: Female Ratio as: 1.2:1.

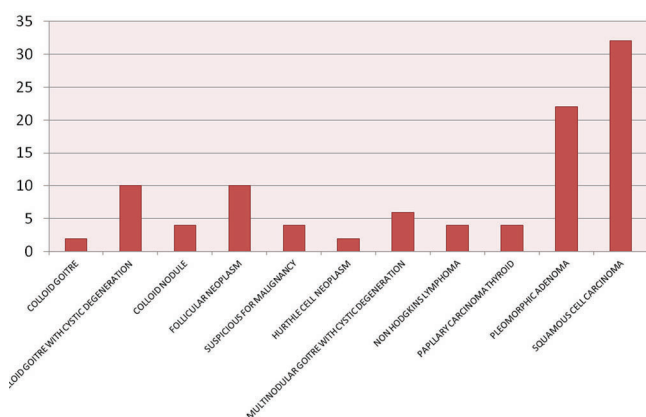
When both the methods were compared on grounds of morphological preservation it was seen that with FNAC morphological preservation was well preserved in 86% of the samples in comparison to 96% cases of Cell block technique. kappa statistic value showed that superior nuclear and cytoplasmic characteristics were observed in cell block in comparison to FNAC ( $\kappa$  - statistic= -0.08)(Table-1). Fnc diagnosis of various tissue sample shown graphically

	%Age	95% CI
Sensitivity	96%	79.65% to 99.90%
Specificity	100%	81.47% to 100.00%
Positive predictive value	100%	-
Negative predictive value	94.74%	72.51% to 99.19%
Accuracy	97.67%	87.71% to 99.94%

**Table-1:** Sensitivity and specificity of cell block with immunohistochemistry to diagnose between benign and malignant lesions

Statistical analysis	FNAC	Cell block with IHC
Sensitivity	88.8%	96%
Positive predictive value	95.65%	100%
Accuracy	92%	97.67%

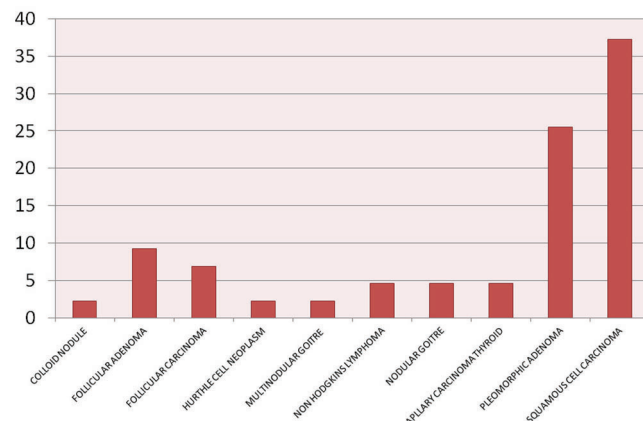
**Table-2:** Comparison of sensitivity and positive predictive value of both methods



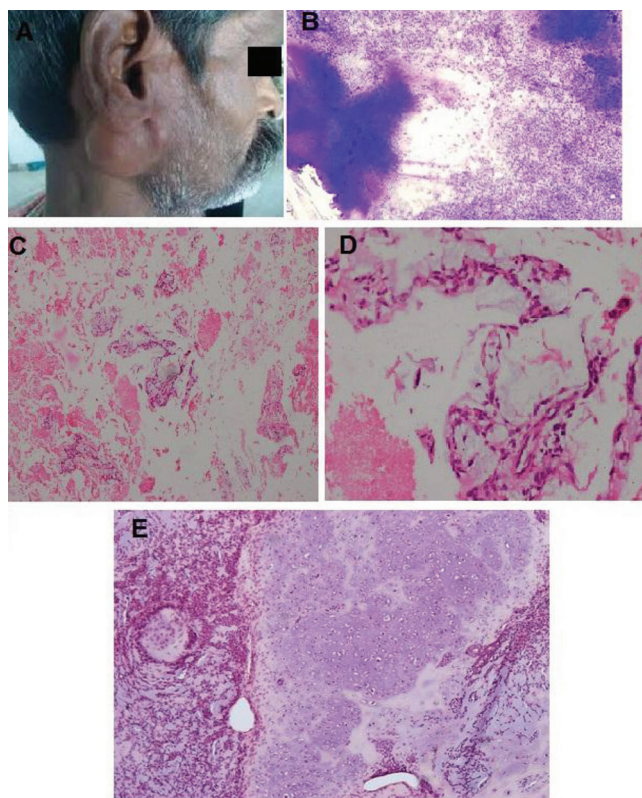
**Figure-1:** Diagnosis of tissues according to fnac in the study sample

in figure-1.

IHC was applied on malignant / suspicious of malignancy cases in the present study. Results of IHC staining on cell block showed that all 25 cases of cell blocks showed positive staining with the IHC markers used in the study namely PAN CK, LCA and negative staining with Vimentin and S-100.



**Figure-2:** Diagnosis of tissues according to cell block in the study sample



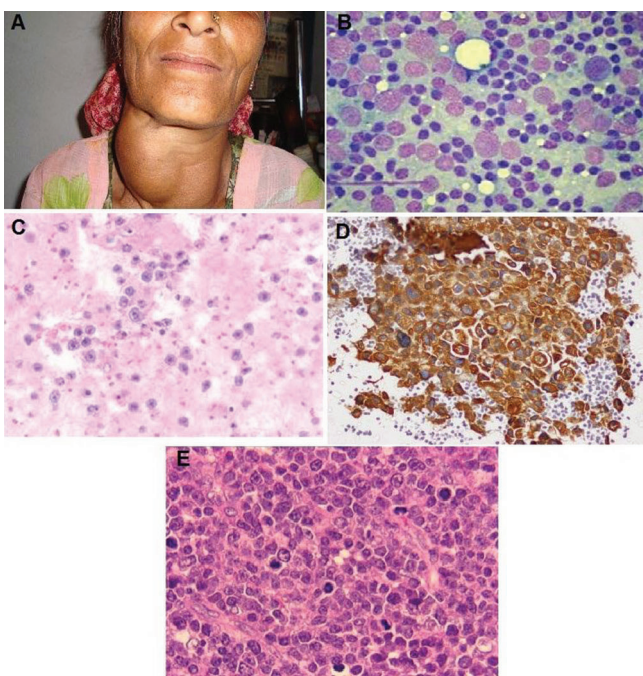
**Figure-3:** A. Middle aged male presented with swelling at angle of mandible, below ear lobe; B. Microphotograph showing abundant chondromyxoid matrix admixed with ductal and mesenchymal cells in the fnac smears of pleomorphic adenoma (H&E, x100); C. Microphotograph showing clusters of oval to spindled cells with the background of chondromyxoid matrix in the cell block preparation of pleomorphic adenoma (H&E, x100); D. Microphotograph showing clusters of oval to spindled cells with the background of chondromyxoid matrix in the cell block preparation of pleomorphic adenoma (H&E, x400); E. Microphotograph of histopathology of pleomorphic adenoma (H&E, x100)



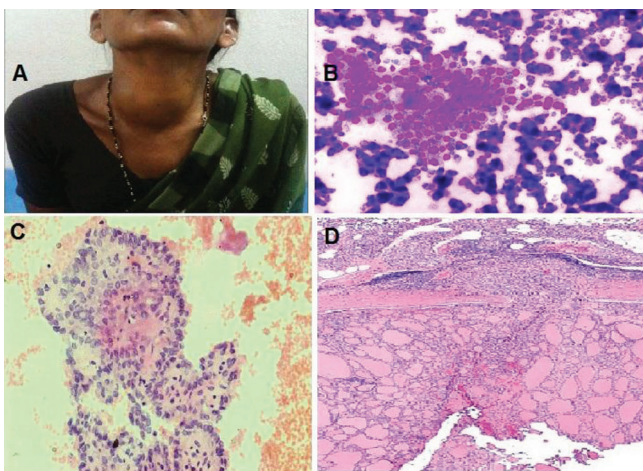
23 cases out of 25 cell blocks were positive for PAN CK immunostaining, which were categorized as: 16 cases of Squamous cell carcinoma, 2 cases of Papillary carcinoma of thyroid, 4 of Follicular carcinoma, and 1 of Hurthel cell carcinoma. While all these cases were negative with other immunohistochemical markers used. With LCA only 2 out of 25 cases of non hodgkins lymphoma cell blocks were positive

while they were negative for other immunohistochemical markers used. Both vimentin and S-100 showed no case of positive staining on cell block.

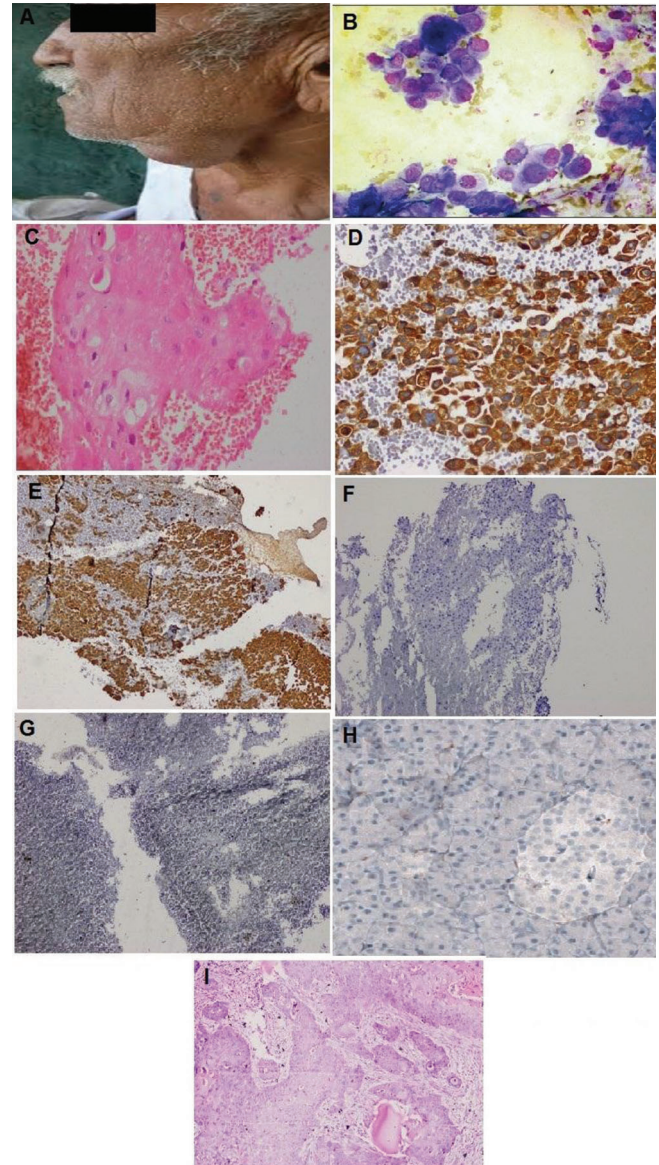
Overall, Cell block method along with IHC on comparison to histopathological diagnosis, showed correlation concordance in 97.6% cases with 96% sensitivity in diagnosing malignant



**Figure-4:** A. Middle aged female presenting with a neck mass slightly right lateral of the midline; B. Microphotograph of fine needle aspirate (FNA) smear of non-hodgkins lymphoma showing population of large lymphoid cells (Giemsa, x400); C. Microphotograph of cell block of non-hodgkins lymphoma (H&E, x100); D. Microphotograph showing LCA positivity in the cell block of non-hodgkins lymphoma (H&E, x400); E. Microphotograph of tissue section of non-hodgkins lymphoma showing proliferation of atypical lymphoid population (H&E, x400)

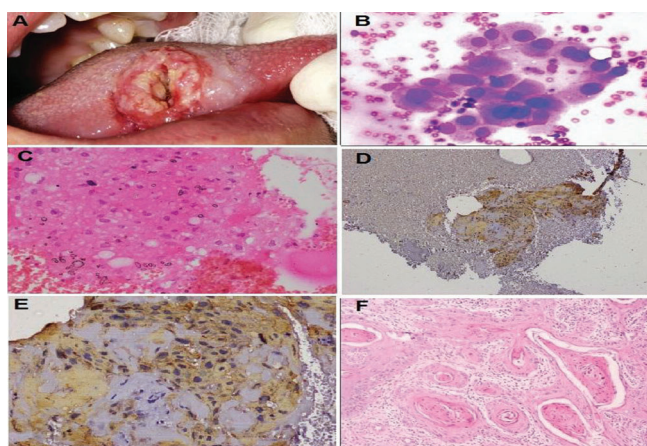


**Figure-5:** A. Middle aged female presented with thyroid swelling; B. Microphotograph of FNA smear showing cluster of thyroid follicle cells (H&E, x400); C. Microphotograph of cell block showing thyroid follicle cells (H&E, x100); D. Microphotograph of tissue section of follicular carcinoma showing focus of capsular invasion (H&E, x100)



**Figure-6:** A. Elderly male presenting with multiple cervical swellings; B. Microphotograph of FNA smears of SCC showing clusters of atypical epithelial cells (Giemsa, x400); C. Microphotograph of cell block preparation SCC showing atypical cells with abundant cytoplasm (H&E, x400); D. Microphotograph of IHC preparation of cell block showing cytoplasmic positivity of pan-CK (H&E, x400); E. Microphotograph of IHC preparation of cell block showing cytoplasmic positivity of pan-CK (H&E, x100); F. Microphotograph of IHC of cell block showing negative staining for LCA marker in a case of SCC (H&E, x100); G. Microphotograph of IHC of cell block showing negative staining for vimentin marker in a case of SCC (H&E, x100); H. Microphotograph of IHC of cell block showing negative staining for S-100 marker in a case of SCC (H&E, x100); I. Microphotograph of histopathology of squamous cell carcinoma showing nest of atypical squamous cells with evidence of keratin pearls (H&E, x100)





**Figure-7:** A. Ulcer right lateral border of tongue with heaped up edges; B. Microphotograph of fnac smear of squamous cell carcinoma showing atypical squamous with abundant cytoplasm, nuclear pleomorphism.(H&E,x400); C. Microphotograph of cell block of squamous cell carcinoma .(H&E,x100); D. Microphotograph of cell block of squamous cell carcinoma showing cytoplasmic positivity for pan-ck immunohistochemical staining.(H&E,x100); E. Microphotograph of cell block of squamous cell carcinoma showing cytoplasmic positivity for pan-ck immunohistochemical staining.(H&E,x400); F. Microphotograph of histopathology of squamous cell carcinoma showing nest of atypical squamous cells with evidence keartin pearls.(H&E,x100)

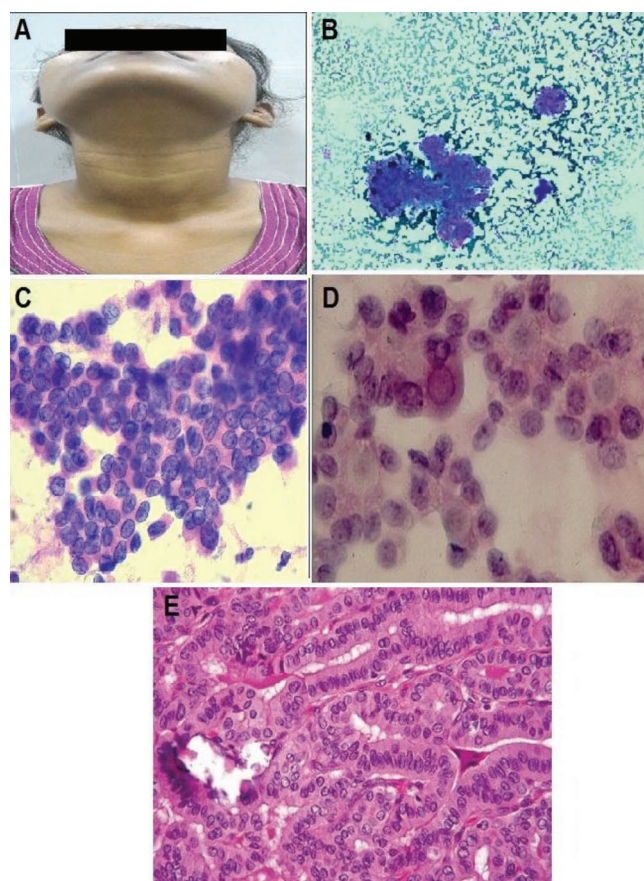
lesions within the study sample. Cell block diagnosis of the tissue shown graphically in figure 2.

Further, On correlating FNAC diagnosis with Cell block confirmed by immunohistochemistry, it was observed that 6 cases of colloid goiter and 1 of adenomatous goiter were unable to diagnose on cell block due to low cellularity. Colloid material could only be diagnosed in FNAC. It was observed that all 5 cases of Multinodular goiter and 2 cases of follicular adenoma were consistent in both investigations. 3out of 4 cases of follicular carcinomas were diagnosed as follicular neoplasm without being able to differentiate between benign and malignant on FNAC, where as these 3 cases were diagnosed as follicular neoplasm with atypia on cell block. Remaining 1 case was correctly diagnosed on FNAC as suspicious of malignancy whereas on cell block this case was diagnosed as follicular adenoma. Single case of hurthle cell carcinoma was consistent in both investigations. All 2 cases of papillary carcinoma thyroid, 2 cases of non hodgkins lymphoma, 11 cases of pleomorphic adenoma and 16 cases of squamous cell carcinomawere consistent in both investigation methods (Figure 3-8). Total 4 cases were found to be inconsistent between the two investigations. Comparative analysis of both the methods are given in table-2.

## DISCUSSION

The present study included a total of 50 specimens which were subjected to the Cytological smear and the Cell block techniques. After this, both cell blocks and tissue sections were subjected to immunohistochemical staining using PAN-CK, LCA, Vimentin and S-100.

Age and Gender distribution in the present study shows that



**Figure-8:** A. Young female presenting with prominent thyroid nodule; B. Microphotograph of fnac smears of papillary carcinoma thyroid showing prominent papillae formation.(H&E,x100); C. Microphotograph of fnac smear of papillary carcinoma thyroid showing thyroid follicle cells with prominent nuclear membrane, prominent nucleoli and nuclear grooving in few cells, "orphan annie eye nuclei".(H&E,x400); D. Microphotograph of cell block of papillary carcinoma thyroid.(H&E,x400); E. Microphotograph of histopathology of papillary carcinoma thyroid showing prominent papillae formation with fibrovascular core

maximum number of study subjects fall under 41-50 years and 51-60 years with equal frequency (28%), with a Male predominance and male: female Ratio of 1.2:1. Similar to our results, Shekhar H et al. reported that in their study age ranged from 1 to 75 years in which 57% were male and 43% were female. Maximum incidence observed in the age group of 31 to 40 years of age.<sup>7</sup> Rathod G et al also reported similar results from their study with a male:female ratio of 1.43:1.<sup>8</sup> Mair et al. described a point scoring system which graded slides on the basis of amount of cellular material as minimal, sufficient for diagnosis and abundant, while retention of appropriate morphology and architecture was scored as minimal, moderate and excellent.<sup>9,10</sup> In accordance with our results, Shehnaz Khan et al also reported similar results and suggested that the cell block samples are best used as an adjunct for IHC and not for primary cytological diagnoses.<sup>11</sup> Thapar M. et al also reported that the cell block technique gave better diagnostic quality at a higher rate, giving results of textbook quality. The cell block technique not only increased the positive results, but also helped to demonstrate

better architectural patterns which could be of great help in approaching the correct diagnosis of the primary site.<sup>10</sup> Similar observations were noticed by Maurice L et al and Berg T et al who concluded that studies that organoid arrangements and the pathognomic of malignancy are better seen in cell blocks than in smears.<sup>12,13</sup> Low cellularity in cell blocks could be explained on the basis that the degeneration of cells in the cell block samples may be attributed to a delay in immersing the cell block specimen into fixative immediately after collection and variation in FNA technique amongst personnel.<sup>11</sup>

In accordance to our study Xiangning Wang et al reported that for FNAC smears, the sensitivity was 76%, but the specificity and accuracy were only 68% and 71%, respectively.<sup>14</sup> In contrast, the sensitivity, specificity and accuracy of the CB with immunohistochemistry technique were 88%, 87%, and 88%, respectively, and much higher than that of cytologic smears, indicating that this technique is better in distinguishing benign lesions from malignant lesions. Various other authors like Bhanvadia VM et al<sup>15</sup>, Basnet S et al<sup>16</sup>, Taft et al<sup>17</sup> and Patil RN et al<sup>18</sup> reported similar results which goes in accordance to ours.

Barsagade et al used cell block as an adjuvant to FNAC and there was increase in adequacy rate of 87.40% as compared to conventional.<sup>19</sup> According to Nathan et al. Cell blocks are adjuvant to FNAC smears for establishing a more definite cytopathologic diagnosis. They observed improvement in 15.2% cases when cell blocks were studied with smears.<sup>20</sup>

## CONCLUSION

The diagnostic value of a Cell Block technique with immunohistochemistry is found to be superior to FNAC smears for the diagnosis of benign and malignant lesions of head and neck region. Taking into consideration the advantages of Cell Block method an excellent complementary tool for improving cytodagnosis, we can recommend that cell blocks preparations should be routine practice so as to augment the information that is obtained solely from FNAC smear cytology.

## REFERENCES

1. Ceelan G, The Cytologic diagnosis of ascetic fluid. *Acta Cytol.* 1964; 8:175-85
2. Gupta N, Sekar A, Rajwanshi A. Role of FNAC, fluid specimens, and cell blocks for cytological diagnosis of lung cancer in the present era. *J Cytol* 2015;32:217-22
3. Sanz-Santos J, Serra P, Andreo P, Llatjós M, Castellà E, Monsó E. Contribution of cell blocks obtained through endobronchial ultrasound-guided transbronchial needle aspiration to the diagnosis of lung cancer. *BMC Cancer* 2012;12:34.
4. Wagner DG, Russell DK, Benson JM, Schneider AE, Hoda RS. Cellient automated cell block versus traditional cell block preparation: A comparison of morphologic features and immunohistochemical staining. *Diagn Cytopathol.* 2011;39:730-6
5. Richardson HL, Koss LG, Simon TR. An evaluation of concomitant use cytological and histological technique in recognition of cancer in exfoliate material from various sources. *Cancer* 1995;8:948-50.
6. Bhatia P, Dey P, Uppal R, Shifa R, Srinivasan R, Nijhawan R. Cell blocks from scraping of cytology smear: comparison with conventional cell block. *Acta Cytol.* 2008;52:329-33.
7. Shekhar Himanshu, Kaur Ashmeet, Agarwal Pallav, Pancharia Aruna, Jadeja Poojaba. Fine needle aspiration cytology in head and neck swellings: a diagnostic and therapeutic procedure. *Int J Res Med Sci.* 2014;2:1667-1671
8. Rathod GB, Parmar P. Fine needle aspiration cytology of swellings of head and neck region. *Indian J Med Sci* 2012;66:49-54
9. Mair, Dunbar F, Becker PJ, Du Plessis W. Fine Needle cytology: Is aspiration suction necessary. A study of 100 masses in various sites? *Acta Cytol.* 1989;33:809-13
10. Thapar M, Rajiv K M, Sharma Amit, Goyal V. Critical Analysis of cell block versus smear examination in effusions. *J Cytol.* 2009; 26: 60-4
11. Khan Shehnaz, Omar Tanvier, Michelow Pamela. Effectiveness of the cell block technique in diagnostic cytopathology. *J Cytol.* 2012; 29: 177-182
12. Maurice L, Perou AC. Malignant cells in serous effusions. *AMA Arch Pathol.* 1955;27:53-60.
13. Berg T, Hom BF. Studies on effusions II: A simple technique for the discovery of cancer cell in neoplastic exudates. *Cancer.* 1965;3:36-42.
14. Xiangning Wang, Jiang Qian, Yifei Yuan, Bo Ping, Liqing Feng, Yingwen Bi, et al. Accuracy of fine needle aspiration biopsy processed by cytologic smear and cell block techniques for the diagnosis of lacrimal gland tumors: a study of 48 cases. *Int J Clin Exp Pathol* 2014;7:3684-3693
15. Bhanvadia VM, Santwani PM, Vachhani JH. Analysis of diagnostic value of cytological smear method versus cell block method in body fluid cytology: study of 150 cases. *Ethiop J Health Sci.* 2014;24:125-31.
16. Basnet S, Talwar OP. Role of cell block preparation in neoplastic lesions. *Journal of Pathology of Nepal.* 2012;2:272-276. 3. Basnet S, Talwar O. Role of cell block preparation in neoplastic lesions. *Journal of Pathology Nepal* 2012;2:272-6.
17. Taft PD, Kinish MJ, Naylor B. Cells of Squamous cell carcinoma in pleural, peritoneal and pericardial fluids: Origin and morphology. *Acta Cytol.* 1960;33:245-53.
18. Patil Revati N, Mahore Sadhana D, Kohle Hrushikesh S, Bothale Kalpana A, Gowardhan Vidula P. Cell Block Technique: An Effective Tool in Diagnostic Cytopathology. *IOSR Journal of Dental and Medical Sciences* 2016;15:99-106.
19. Barsagade, Ashwinkumar. Diagnostic Utility of Cell Block Preparation of Fine Needle Aspiration Material. 2017.5.12.48.
20. Nathan NA, Narayan E, Smith MM, Horn MJ. Cell Block Cytology. *Am J Clin Pathol.* 2000;1;114:599-606.

**Source of Support:** Nil; **Conflict of Interest:** None

**Submitted:** 01-05-2019; **Accepted:** 01-06-2019; **Published:** 26-06-2019