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Research Article

DIAGNOSTIC UTILITY OF CELL BLOCK IN SALIVARY GLAND NEOPLASM WITH FINE NEEDLE ASPIRATION CYTOLOGY AND ITS CLINICOPATHOLOGICAL CORRELATION

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A B S T R A C T

Introduction: Cell block [CB] technique employs the retrieval of small tissue fragments from a fine needle aspiration cytology specimen [FNAC] which are processed to form a paraffin block and viewed as histology sections. Aim of the study to compare the role of FNAC & CB in diagnosis of salivary gland neoplasm, in term of adequacy, cytomorphological details and diagnostic efficiency.
Material & Methods: Total 83 salivary gland cases were enrolled in the study and categorization done according to Milan System for reporting salivary gland cytopathology. Both FNAC smears and Cell block sections were prepared as standard protocol and examined.
Results & Discussion: Most common diagnosis was benign 52 cases (Category IVA; 62.7%) followed by malignancy 17 cases (Category VI; 20.5%) and non-diagnostic 4 cases (Category I; 4.82%). Only 1 (1.2%) case each was diagnosed as suspicious (Category V) and salivary gland neoplasm of uncertain malignant potential (Category IVB). 6 (7.24%) cases were diagnosed as non-neoplastic (Category II) and 2 (2.4%) as atypia of undetermined significance (Category III). Specificity and PPV of both the techniques (FNAC and Cell Block) were found to be 100%. Sensitivity, NPV and diagnostic accuracy of FNAC technique were found to be comparable to that of Cell block technique (63.6% vs72.7%, 86.7% vs 89.3%, 86.8%vs 91.7%). Cell block as a technique should be used in routine practice as it not only increases the diagnostic yield, but ancillary test and retrospective studies can also be done.

INTRODUCTION

Salivary gland cancers represent approximately 6% of head and neck cancers and about 0.3-0.5 of all malignancies.[1] Cell block [CB] is a method of preparing cytological material so that it can be processed as histology section and allows for multiple immunostains and other studies to be performed.[2] Fine needle aspiration cytology (FNAC) is a popular method and it is an accepted, sensitive, and specific technique in the diagnosis. [3, 4]

However, salivary gland lesions remain, one of the most challenging entity in cytopathology, because of the diversity of histologic subtypes and often overlapping morphologic features of the lesions. [5] To compare the diagnostic efficacy of fine needle aspiration cytology versus cell block technique in diagnosis of salivary gland neoplasm. To analyze results of both techniques in term of adequacy, cellularity, interpretation, concordance and pitfalls.

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MATERIAL AND METHODS

This study was conducted in the department of pathology in collaboration with department of ear, nose, and throat. This is prospective observational comparative study of two cytopreparatory techniques in the one year duration. Direct alcohol-fixed FNAC smears were stained using hematoxylin & eosin stain and air-dried smears with May-Grunwald Giemsa stain for Fine needle aspiration cytology smears. The cell block technique employs the retrieval of small tissue fragments from a FNAC specimen which are processed to form a paraffin block, sections cut and stained with haematoxylin and eosin stain. All FNAC smears and their corresponding cell blocks sections were examined by two cytopathologists independently and categorization done according to Milan system for Reporting Salivary Gland Cytopathology.

Statistical analysis was done using statistical package for the social sciences version 15.0 statistical analysis software SPSS inc. 2006, Chicago, USA. The values were represented in number (%) and mean ± standard deviation. The following statistical formulas were used mean, standard deviation, chi-square test, sensitivity, specificity, positive predictive value

(PPV), negative predictive value (NPV), and level of significance (P value). Study has been approved by the ethical committee of our university.

RESULTS

A total of 83 salivary gland cases were enrolled in the study who reported to our department during one year on the basis of Milan System diagnostic categories of salivary gland cytopathology. Most common age group between 3rd&5thdecade followed by 2ndto3rd decade of life. Majority of the cases were female (51.8%) and rest were male (45.8%).

Most common diagnosis on Milan System was benign 52 cases (Category IVA; 62.7%) followed by malignant 17 cases (Category VI; 20.5%) and non-diagnostic 4 cases (Category I; 4.82%). Only 1 (1.2%) case each was diagnosed as suspicious (Category V) and salivary gland neoplasm of uncertain malignant potential (Category IVB). 6 (7.24%) cases were diagnosed as non-neoplastic (Category II) and 2 (2.4%) as atypia of undetermined significance (Category III). [Table:1]

Table 1 Distribution of Study Population (n=83) according to Diagnosis based on Milan System.

SN	Diagnostic category	Diagnosis	No. of cases	Percentage
1-	I	Non-diagnostic	4	4.82
2-	II	Non-Neoplastic	6	7.24
3-	III	Atypia of Undetermined significance	2	2.4
4-	IV	Neoplastic	53	
	IVA	Benign	52	62.7
	IVB	Salivary gland neoplasm of uncertain malignant potentia	1	1.2
5-	V	Suspicious of malignancy	1	1.2
6-	VI	Malignancy	17	20.5

Non-diagnostic category(Category1) includes 4 cases of cystic lesions, 6 cases of non-neoplastic category(Category II) includes mainly 2 cases of sialadenosis and 4 cases of sialadenitis on both FNAC and cell block techniques and 2 cases of atypia of undetermined significance(Category3). Overall risk of malignancy in these categories was reported as none. Histopathology of category I,II,III was not available.

Table 2 Concordance of techniques between FNAC, Cell Block(CB) & Histopathology(HPE)

Techniques	Conc rdance		No concordance		No possible comment	
	No.	%	No.	%	No.	%
FNAC and CB	53	63.9	19	22.9	11	13.3
FNAC and HPE	31	37.3	10	12.0	42	50.6
CB & HPE	31	37.3	5	6.0	47	56.6
FNAC, CB & HPE	29	34.9	6	7.2	48	57.8

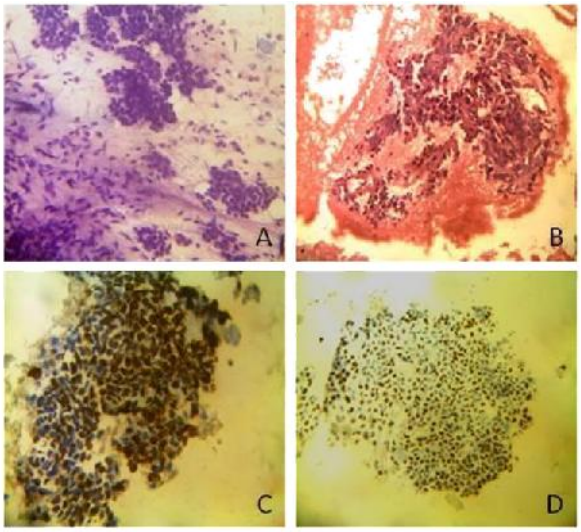
Out of total 83 cases, 69 cases on FNAC and 55 cases on cell block were diagnosed. The agreement in adequacy/inadequacy of FNAC and cell block was found for 69 (95.1%). Moderate level of agreement between Cell block and FNAC was observed. Agreement between the two techniques for adequacy was found to be statistically significant (p<0.001)**.**

Total benign 52 cases on FNAC were diagnosed as 35 cases of pleomorphic adenoma, 2 cases of warthin's tumor,1 case of each oncocytoma, basal cell adenoma, myoepithelioma and rest 12 cases were benign non-salivary gland tumor (Lipoma, Schwannoma, Lymphangioma and Hemangioma). Total malignant 17 cases on FNAC were diagnosed as 3 cases of mucoepidermoid carcinoma, 2 cases of adenoid cystic carcinoma, 1 case each of acinic cell carcinoma and salivary duct carcinoma, 2 cases of carcinoma ex pleomorphic adenoma and rest 8 cases were malignant non-salivary gland tumor (Squamous cell carcinoma and Non-Hodgkins lymphoma). Only 1 case of each diagnosed on FNAC as suspicious and salivary gland neoplasm of uncertain malignant potential was inadequate in cell block and histology was not available.

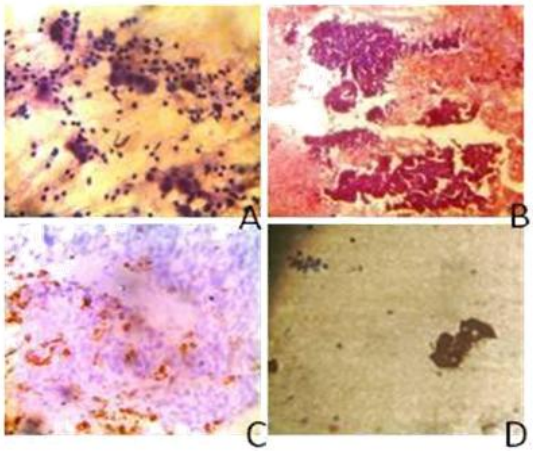
Table 3 Diagnostic accuracy of FNAC, Cell Block(CB), Immunocytochemistry (IHC) against Histopathology(HPE)

Technique	Sensitivity(%)	Specificity(%)	PPV(%)	NPV(%)	Diagnostic accuracy(%)
FNAC against HPE	63.6	96.3	87.5	86.7	86.8
Cell Block against HPE	72.7	100.0	100.0	89.3	91.7
FNAC against CB	85.7	100.0	100.0	95.2	96.3
ICC	80.0%	100.0%	100.0%	90.5%	93.1%.

Out of 55 cases of cell blocks,15(27.3%) were diagnosed as malignant and rest 40 cases (72.7%) were diagnosed as benign. Immunocytochemistry (ICC) was done on cell block in 41 cases, 27 (65.9%) were classified as benign and rest 14 cases (34.1%) were diagnosed as malignant.



1. [A and B] FNAC and Cell Block smears [A & B] of pleomorphic adenoma (May-Grunwald Giemsa, ×10 and H&E Stain, ×20), [C and D] CK7 &P63 Immunocytochemical stain stain on cell block (IHC Stain, ×20)



2. [A and B] FNAC and Cell Block smears[A &B} Warthin's Tumour (May-Grunwald Giemsa, ×10 and H&E Stain, ×20), [C and D] LCA and CK7 Immunocytochemical Stain on cell block positive in lymphoid cells (IHC Stain, ×20)

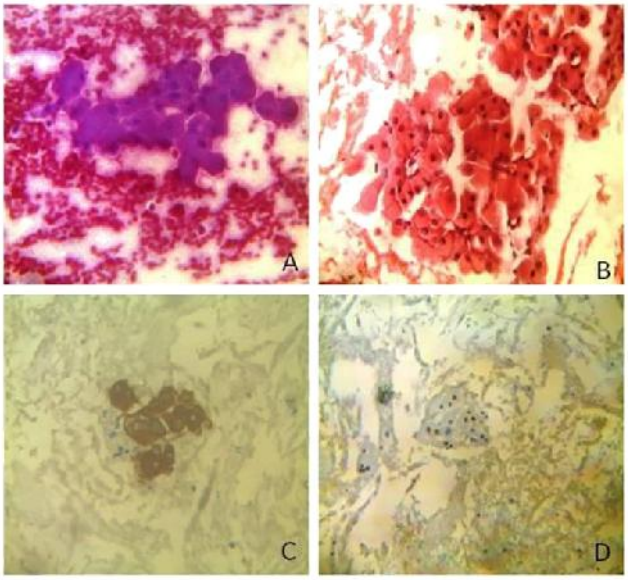
Remaining 14 blocks were exhausted for ICC panel. Histopathology of 41 cases was available and classified 30 (73.2%) cases as benign and rest 11 cases (26.8%) were

diagnosed as malignant. Correlation of FNAC, Cell block technique with histological diagnosis and ICC in 41 cases only, due to non-availability of histology in some cases and non-diagnostic or inadequacy of samples in cell block in other cases. Overall risk of malignancy reported was 26.8% in category 4a. [Table4]

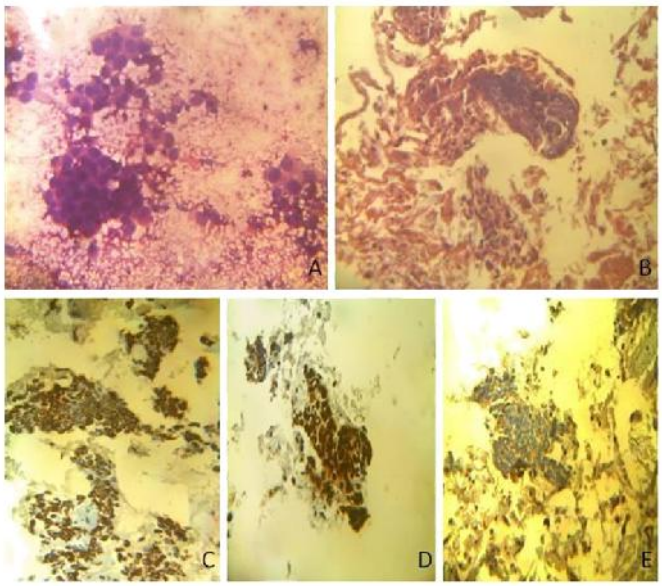
21cases (38.2%) were found to be almost similar by both the techniques. Therefore, cell block technique was found to be better with respect to cellularity in majority of the cases.

Table 4 Histopathological follow up of Diagnostic Categories

Category	Cat1(%)	2(%)	3(%)	4a(%)	4b(%)	5(%)	6(%)	Total
No. of cases	4(4.8%)	6(7.2%)	2(2.4%)	52(62.7%)	1(1.2%)	1(1.2%)	17(20.5%)	83
No. of cases with Histopathology follow up	0(0.0%)	0(0.0%)	0(0.0%)	41(74.5%)	0(%)	0(%)	14(25.4%)	55(66.2%)
Benign neoplastic	0(0.0%)	0(0.0%)	0(0.0%)	30(73.1%)	0(0.0%)	0(0.0%)	1(7.1%)	31(56.3%)
Malignant	0(0.0%)	0(0.0%)	0(0.0%)	11(26.8%)	0(0.0%)	0(0.0%)	13(92.8%)	24(43.6%)
Risk of malignancy	0(0.0%)	0(0.0%)	0(0.0%)	26.8(%)	0(0.0%)	0(0.0%)	92.8%	43.6%

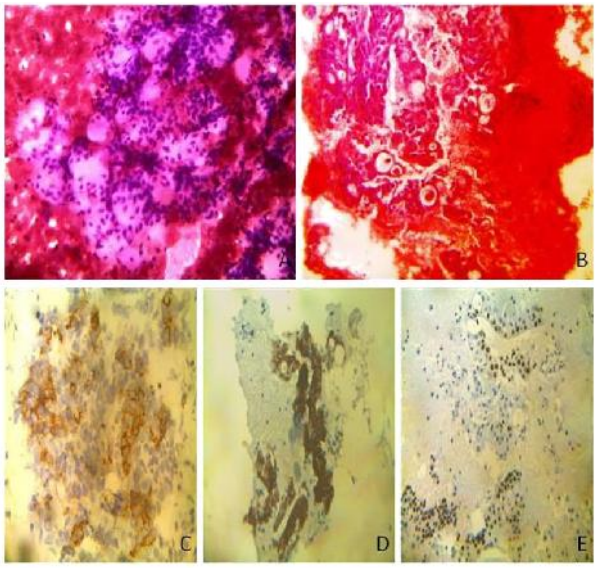


3. FNAC and Cell Block smears [A &B] of Oncocytoma (May-Grunwald Giemsa, x40 and H&E Stain, ×40), [C and D] CK7 &P63 Immunocytochemical stain positive on cell block (IHC Stain, ×20)



4. [A and B] FNAC smears and Cell Block smear of Mucoepidermoid Carcinoma (May-Grunwald Giemsa, ×20 and H&E Stain, ×20). [C and D] CK7, P63 &P40Positive on cell block (IHC Stain, ×20)

Cellularity was found to be better in cell block in majority of cases (n = 28; 50.9%) while cellularity in (n=6; 10.9%) cases were found to be better in FNAC technique. The results of rest



5. [A and B] FNAC smears and Cell Block smear of Adenoid Cystic Carcinoma (May-Grunwald Giemsa, ×20 and H&E Stain, ×20). [C and D] CK7, CD117 &p63 positive on cell block (IHC Stain, ×20)

Architectural pattern details of 35 (63.6%) cases were found to be similar by both the techniques while better results by FNAC technique were obtained in 12 (21.8%) cases and better results by cell block technique were obtained in 8 (14.6%) cases. Architecture was better seen in FNAC as compared to cell block technique. On FNAC, most common architectural pattern was glandular (84.0%) followed by sheets & clusters (13.0%) while least common pattern was cribriform pattern (3.0%). On Cell block, sheets & clusters (65.4%) was most common pattern followed by glandular, acinar pattern (14.5%) and rest cribriform pattern (1.8%) pattern.

Chondromyxoid stroma was better observed by FNAC technique in 38 (69.1%) cases, where as rest 17 (30.9%) cases results of FNAC and Cell block technique were found to be similar. Background details were better observed in all the cases by cell block technique. The results of both the techniques were found to be similar in 15 (27.2%) cases.

Maximum concordance was found between FNAC & Cell block (63.9%) followed by FNAC & HPE and Cell Block & HPE (37.3%). Minimum concordance was found between FNAC, Cell block & HPE (34.9%). Concordance with histopathological diagnosis by both the techniques was not found to be statisticly significant and was due to the inadequacy of samples in cell block. Comparison of histological diagnosis, FNAC and Cell block was available in

29 cases only due to non-availability of histology in some cases and inadequacy of samples in cell block technique in other cases. [Table:2]

The 35 cases of pleomorphic adenoma diagnosed on FNAC were found to be reported as 31 cases of pleomorphic adenoma, 1 case as mucoepidermoid carcinoma, and 3 cases as adenoid cystic carcinoma on cell block technique. Histopathology of 25 cases (83.3%) of pleomorphic adenoma were available and diagnosis were remains the same. One case of each was diagnosed as mucoepidermoid carcinoma and adenoid cystic carcinoma on cell block which was reported as same on histopathology. However, remaining 8 cases histopathology was not available. However, ease of diagnosis was better with cell block technique (77.5%) as compare to FNAC technique (67.3%). In pleomorphic adenoma for further confirmation of diagnosis we applied CK7 and P63 on cell block. CK7 show diffuse cytoplasmic positivity and myoepithelial cells show diffuse nuclear positivity for P63. [Fig: 1]

Histologically proven 2 cases of Warthin's tumor, diagnostic efficacy was similar in both the techniques. We applied immunocytochemistry on cell block, LCA came out to be positive in background lymphoid cells and CK7 showed cytoplasmic positivity in epithelial cells.

In one case of histopathology proven oncocytoma both the techniques diagnose accurately. We also applied immunocytochemistry on the cell block. Oncocytes shows strong diffuse cytoplasmic positivity for CK7 and negative for CK20, while positive nuclear staining for P63. Fig:2 In one case of each basal cell adenoma and myoepithelioma diagnosed on FNAC, inadequate in cell block and histology was not available. Overall risk of malignancy in category IVB & V was reported as none. Histopathology of these categories was not available.[Table4]

Out of total 17 malign nt cases on FNAC, 3 cases of mucoepidermoid carcinoma were diagnosed. These 3 cases were again diagnosed as mucoepidermoid carcinoma on cell block, while 2 out of these 3 cases were diagnosed as mucoepidermoid carcinoma on histopathology. One case could not be found and was not available for histopathological diagnosis. Diagnostic efficacy was similar in both the techniques; however, ease of diagnosis was more with cell block (21.4%) as compared with FNAC (17.6%). In mucoepidermoid carcinoma, tumor cells show strong diffuse nuclear positivity for P63 and membraneous positivity for S-100 on immunocytochemistry. 2 cases were diagnosed as adenoid cystic carcinoma on FNAC, while these cases were diagnosed again as adenoid cystic carcinoma on cell block. One out of these two cases was diagnosed as mucoepidermoid carcinoma, while another case was diagnosed as adenoid cystic carcinoma on histopathology. Immunocytochemistry showed CD117 positivity in luminal cells and p63 positivity in myoepithelial cell which confirms the diagnosis. [Fig: 3]

One case was diagnosed as acinic cell carcinoma on FNAC, but its cell block and histopathology were not available. One case was diagnosed as salivary duct carcinoma on FNAC. This case was also diagnosed as salivary duct carcinoma on cell block and its histopathology was not available as the patient did not get operated. Both the techniques diagnosed it accurately; however, ease of diagnosis was more with cell block (7.14%) as compared with FNAC (5.9%). Cell block was exhausted for immunocytochemistry. Two cases were diagnosed as carcinoma ex pleomorphic adenoma on FNAC. One out of these two cases was reported as mucoepidermoid carcinoma[Fig:4], while another case was reported as suspicious for malignancy on cell block. Histopathologically one cases reported as mucoepidermoid carcinoma and other reported as pleomorphic adenoma.

Ease of interpretation was better with cell block as compared to FNAC. We had applied immunocytochemistry on cell block of carcinoma ex pleomorphic adenoma; CK7 and β-catenin were positive which confirms the diagnosis. The rest of 8 cases were diagnosed as non-salivary gland tumors like metastatic squamous cell carcinoma and non-Hodgkins lymphoma on FNAC and cell block which was histologically proven. The histopathology was taken as the gold standard technique. Specificity and PPV of both the techniques (FNAC and Cell Block) were found to be 100%. Sensitivity, NPV and diagnostic accuracy of FNAC technique were found to be comparable to that of cell block technique (63.6% vs72.7%, 86.7% vs 89.3%, 86.8%vs 91.7%). [Table:3] Adequacy, cellularity and ease of interpretation were found to be better in cell block while architectural pattern and chondromyxoid stroma were found to be better on FNAC. Overall risk of malignancy reported was 92.8% in category 6. [Table: 4]

DISCUSSION

One of the constraints of the conventional fine needle aspiration (FNA) smear is the limited material available for adjuvant diagnostic investigations. The use of the cell block technique enables the retrieval of small tissue fragments in a fluid specimen which are processed to form a paraffin block. It has been widely accepted that this method of analysis increases the cellular yield and improves diagnostic accuracy. [6,7] The ability to obtain numerous sections allows for multiple immunostains and other studies to be performed akin to paraffin sections produced in histopathology.[8]

The use of cell blocks has been widely advocated in the diagnostic work-up of patients with masses amenable to FNA since they provide diagnostic architectural information which complement FNA smears. [9]

Mean age of cases was 40.04±16.68 years. Most common age group was 31-40 years (25.3%) followed by 21-30 years (19.3%). Similar results were concluded by Sharma *et al*. (2015). [10] Majority of females (51.8%) was affected than rest was male (45.8%). In present study parotid gland was the most frequently involved salivary gland accounting for 82.5% of lesions followed by submandibular gland comprising of 7.5%. This finding was also seen with other studies like Vikram *et al* [11] and Jain *et al*. [12] Salivary gland lesions were enrolled in this study reported according to Milan System for reporting salivary gland cytopathology diagnostic categories. [13]

On FNAC, out of 69 cases 75.4% specimens were diagnosed as benign and rest 24.6% as malignant cases. On Cell block, out of 55 cases, 14 (25.5%) were diagnosed as malignant and rest 41 (73.5%) cases were diagnosed as benign which was further confirmed by histopathology and immunocytochemistry. Lundberg *et al*. (2016) performed the retrospective study via analyzing the histo-fragments by immunocytochemical markers in fine needle aspiration cytology samples from salivary gland lesions. Cell blocks which are paraffin-embedded aggregates of cytopathological

single-cell samples have revolutionized cytopathology. Immunocytochemistry identifies cellular differentiation, which is relevant for the diagnosis of some of the more unusual salivary gland tumors. [14]

Only 41 cases of category 4a, histologically correlated and classified 30 (73.2%) cases as benign and rest 11(26.8%) cases were classified as malignant. Pleomorphic adenoma is the most common benign tumor where as the mucoepidermoid carcinoma is the most common malignant tumor among all salivary gland tumors diagnosed by FNAC, Cell block and HPE which was similar to the study done by young *et al*, Nanda *et al*, Anand *et al*. [15-17] Agreement between the two techniques for adequacy was found to be statistically significant (=0.570; p<0.001) Cellularity was found to be better in cell block in majority of cases (n = 28; 50.9%) while cellularity in 6 (10.9%) cases were found to be better in FNAC technique. The results of rest 21cases (38.2%) were found to be almost similar by both the techniques. This was concordant with the study done by Varsegi *et al*. [18]

On FNAC, most common architectural pattern was glandular (84.0%) followed by sheets & clusters (13.0%) while least common pattern was cribriform pattern (3.0%). On Cell block, sheets & clusters (65.4%) was most common pattern followed by glandular, acinar pattern (14.5%) and rest cribriform pattern (1.8%) pattern. These findings were concordant with study done by Akalin *et al*. [19]

Maximum concordance was found between FNAC & Cell block (63.9%) followed by FNAC & HPE and Cell Block & HPE (37.3%). Minimum concordance was found between FNAC, Cell block & HPE (34.9%). Concordance with histopathological diagnosis by both the techniques was not found to be statistically significant and was due to the inadequacy of samples in cell block.

In our study, we had applied immunocytochemistry on cell block on various cases. In pleomorphic adenoma, CK7 show diffuse cytoplasmic positivity and myoepithelial cells show diffuse nuclear positivity for P63. Similar findings were seen in the study of C. Mythily and B Saranya (2017). [20]

In mucoepidermoid carcinoma, tumor cells show strong diffuse nuclear positivity for P63 and membraneous positivity for S-100. In acinic cell carcinoma and we found that CD117 show membraneous positivity and CK7 show cytoplasmic positivity while ER/PR shows negative immunostaining. Two cases were diagnosed as carcinoma ex pleomorphic adenoma on FNAC (11.8%).One out of these two cases was reported as mucoepidermoid carcinoma, while another case was reported as suspicious for malignancy on cell block. Histopathologically one cases were reported as mucoepidermo id carcinoma and other one reported as pleomorphic adenoma.. The rest of the 8 cases were diagnosed as non-salivary gland tumors like metastatic squamous cell carcinoma, non-Hodgkin's lymphoma on FNAC. and cell block which was histologically proven. Maximum concordance was found between FNAC & Cell block (63.9%) followed by FNAC & HPE and Cell Block & HPE (37.3%). Minimum concordance was found between FNAC, cell block & HPE (34.9%).

Diagnostic accuracy of FNAC as compared to Cell block was 96.3% along with sensitivity, specificity, PPV, NPV were 85.7%, 100.0%, 100.0% and 95.2% respectively. Diagnostic accuracy of FNAC as compared to histopathology was 86.8%. Sensitivity, specificity, PPV, NPV were 63.6%, 96.3%, 87.5% and 86.7% respectively. These findings were concordant with study done by Omhare *et al* in 2014 and Rossi ED *et al* in 2016. [21, 22]

Diagnostic accuracy of cell block as compared to histopathology was 91.7%. Sensitivity, specificity, PPV, NPV were 72.7%, 100.0%, 100.0% and 89.3% respectively. Diagnostic accuracy of Immunocytochemistry as compared to histopathology was 93.1% along with sensitivity, specificity, PPV, NPV were 80.0%, 100.0%, 100.0% nd 90.5% respectively.

Adequacy, cellularity and ease of diagnosis were found to be better in cell block while architectural pattern and chondromyxoid stroma were found to be better on FNAC. However, ease of interpretation in salivary gland tumors, especially pleomorphic adenoma is better with cell block as compared to FNAC due to better visualization of morphological details, and better cellularity.Limitations of the present study was a time bound study and one important limitation of our study was the small number of sample size, very low number of malignant cases. Histological correlation was not available in all the categories. In term of adequacy, cellularity and ease of interpretation, cell block is better than FNAC. Cell block and FNAC both have excellent specificity. To conclude that cell block technique should be used in routine practice as it not only increases the diagnostic yield, but ancillary test and retrospective studies can also be done.

CONCLUSION

To conclude that cell block technique should be used in routine practice as it not only increases the diagnostic yield, but ancillary test and retrospective studies can also be done.
Thus, the combined use of both the techniques increases the diagnostic accuracy and helps the surgeon for appropriate management of the patients.

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