



ISSN: 0976-3031

Available Online at <http://www.recentscientific.com>

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 9, Issue, 2(K), pp. 24543-24552, February, 2018

**International Journal of
Recent Scientific
Research**

DOI: 10.24327/IJRSR

Research Article

SIGNIFICANCE OF STUDY OF FINE NEEDLE ASPIRATION CYTOLOGY AND CELL BLOCK PREPARATION IN NECK SWELLINGS AND BREAST LESIONS

Pravin M.Meshram^{1*}, Kishor A.Lonkar², Anjali S. Kulkarni³ and Rajan S. Bindu⁴

¹Department of Pathology, Government Medical College, Nagpur (M.S.), India

²Blood Bank Incharge, General Hospital, Washim (M.S.), India

^{3,4}Department of Pathology, Government Medical College, Aurangabad (M.S.), India

DOI: <http://dx.doi.org/10.24327/ijrsr.2018.0902.1680>

ARTICLE INFO

Article History:

Received 15th November, 2017

Received in revised form 25th

December, 2017

Accepted 23rd January, 2018

Published online 28th February, 2018

Key Words:

Fine needle aspiration cytology (FNAC),
Cell block, Neck swellings, Breast lesions.

ABSTRACT

Background: This study was to understand significance of fine needle aspiration cytology-conventional smear and cell block preparation, to compare morphological features and to evaluate the diagnostic utility of cell block along with fine needle aspiration cytology and its effectiveness in routine use in cyto-diagnosis of neck swellings and breast lesions.

Methods: In present study, FNAs performed in Minor OT (OPD patient), sixty six fine needle aspirates from neck and breast lesions were submitted to the cytology section and FNAC remnants were taken for cell block preparation.

Results: Positive correlation between cell block and FNAC smear for malignant and benign lesions was seen in 86.67% and 81.82% cases respectively. Absolute concordance was found in 84.12% between both techniques and the strength of agreement was good.

Conclusions: Tissue architecture is well appreciated in cell blocks. Cell blocks can be used for special stains and for immunohistochemistry which can be carried out on sections and can diagnose certain tumors without requiring surgical interventions. Combined use of FNAC smear and cell-block can be useful for establishing a more definitive cytopathologic diagnosis. The disadvantage with cellblock technique is delay in the in the diagnosis when compared to FNAC smear and sometimes risk of losing material during processing.

Copyright © Pravin M.Meshram *et al*, 2018, this is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Diagnostic cytology is the science of interpretation of cells that are either exfoliated from epithelial surfaces or removed from various tissues. George N Papanicolou introduced Cytology as a tool to detect cancer and pre-cancer in 1928. Fine-Needle Aspiration Cytology/ Biopsy (FNAC/FNAB) is now a widely accepted diagnostic procedure, which has largely replaced open biopsy.¹ This method is common investigation in the diagnosis of superficial lumps that are easily palpable. Breast, Thyroid and superficial Lymph node are the most common organs subjected to the procedure.^{1,2,3}

The advantages of diagnostic cytology are that it is a non-invasive, simple procedure, helps in faster reporting, is relatively inexpensive, has high population acceptance and facilitates cancer screening in the field.^{1,2,4,5,6,7} Sometimes FNA alone does not yield sufficient information for precise diagnosis and the risk of false negative or intermediate diagnosis always exists. In order to overcome these problems,

cell block technique has been resorted to make the best use of the available material.⁸

Cell block technique is simple, reproducible and safe.⁹ Various methods for preparing paraffin embedded cell blocks from fine needle aspiration cytology mainly include direct transfer of all centrifuged cellular material wrapped in lens paper or embedding in plasma or agar and then processing as a routine histological specimen.⁸ Cell block preparation in a way mimics the histopathological sections, thus help in sub classifying various neoplastic lesions^{8,10} and might be crucial for making a definitive diagnosis.¹¹

This study is to understand significance of fine needle aspiration cytology-conventional smear and cell block preparation, to compare morphological features of conventional smear with slides of cell block technique and to evaluate the diagnostic utility of cell block along with fine needle aspiration cytology and its effectiveness in routine use in cyto-diagnosis of neck swellings and breast lesions.

*Corresponding author: Pravin M.Meshram

Department of Pathology, Government Medical College, Nagpur (M.S.), India

METHOD

Present study was conducted during period December 2012 to November 2014 in Department of Pathology. FNAs were performed in Minor OT (OPD patient), sixty six fine needle aspirates from neck and breast lesions were submitted to the cytology section and FNAC remnants were taken for cell block preparation.

Patients referred for fine needle aspiration cytology of neck swellings and breast lesions were included. And Swellings other than neck region and breast lesion, Non-palpable swellings were excluded from study.

FNAs were carried out in presence of surgeon. All universal precautions followed during the procedure.

Preparation, Fixation and Pap Staining

Immediately after withdrawing, needle detached, air drawn into the syringe, needle reattached and material in the needle expressed onto a slide. Needle tip was brought into light contact with the slide and the aspirate was carefully expressed without spraying into the air. Such aspirates were smeared immediately using another slide and dropped into the fixative. Material diluted with blood spreaded like a peripheral smear, where particles tend to come to the edge of the smear. Larger particles crushed gently by firm flat pressure.

Slide stained with pap stain after fixation.

PAP Staining¹

1. 90% Ethanol (fixation) - 15 minutes (mt)
2. 80% Ethanol - 2 mt.
3. 60% Ethanol - 2 mt.
4. Distilled water - 5 dips
5. Distilled water - 5 dips
6. Haematoxylin stain - 2 mt.
7. 0.05% HCl solution - 2 mt.
8. Running tap water (Bluing) - 10 mt.
9. 60 % Ethanol - 2 mt.
10. 80% Ethanol - 2 mt.
11. 80% Ethanol - 2 mt.
12. 95% Ethanol - 2 mt.
13. OG-6 stain - 2 mt.
14. 95% Ethanol - 2 mt.
15. 95% Ethanol - 2 mt.
16. 95% Ethanol - 2 mt.
17. EA-36 Stain - 2 mt.
18. 95% Ethanol - 2 mt.
19. 95% Ethanol - 2 mt.
20. 95%Etanol - 2 mt.
21. 95% Ethanol - 2 mt.
22. Absolute Ethanol - 2mt.
23. Absolute Ethanol - 2 mt.
24. Absolute Ethanol - 2 mt.
25. Absolute Ethanol+ Xylene (1:1) - 2mt.
26. Xylene - 5 mt.
27. Xylene - 5 mt.
28. Xylene - till clear
29. Mounting in D.P.X

Cell Block Preparation

After preparation of smear, the needles and syringes used to obtain fine-needle aspirates were rinsed in 10 ml of 50% ethanol in a specimen container. Any residual clot or tissue in the hub of needles was removed with the aid of another needle and rinsed in 50% ethanol. The entire material was centrifuged in a 10 ml disposable centrifuge tube at 4,000 rpm for 6 minutes to create cell pellet. The cells transferred to flat bottom and again centrifuged at 4,000 rpm for 6 minutes to form a cell button. The supernatant fluid was decanted and the deposit fixed in freshly prepared Nathan alcohol formalin substitute (NAFS) consisting of 9 parts of 100% ethanol and 1 part of 40% formaldehyde. The fixed cell button, at the end of 45 minutes' fixation, was recentrifuged at 4,000 rpm for 6 minutes. The tubes are then removed vertically from the centrifuge without disturbing the sedimented pellet with cells. Supernatant is poured off taking care not to disturb the flat layer of sediment cells at the bottom.¹² The cell button is resuspended in 0.9% molten agar at 50°C and quickly placed in centrifuge again. It is spun at 4000 rpm for 2 minutes and then refrigerated at 4°C for 30 minutes to harden agar.¹³

Removal of the cell block as a button of agar with specimen for final processing

1. The solidified agar disk, with the layer of concentrated specimen at the bottom is dislodged from the flat bottom tube by squirting 10% formalin through a 23 gauge needle with the syringe.
2. The needle is inserted along the side of the tube at the periphery of solidified agar disc with specimen.
3. The needle is rotated along the side of the tube while formalin is slowly pushed through the syringe. This results in the separation of the agar button along with concentrated specimen in it from the flat bottom of the tube.
4. The cell block (agar button with specimen cells) is then placed in a labeled cassette and stored in 80% ethanol until ready for processing.¹³

Further processing is done in automatic tissue processor using 13 hours processing schedule as follows:

80% Ethanol with one change	- 2.5 hours
95% Ethanol	- 1hour
100% Ethanol 4 times	- 1hour each
1:1 Ethanol/ Xylene	- 1hour
Xylene 3 times	- 1hour each

Paraffin wax, 60°C, vacuum impregnation at 20 lbs for 1.5 hours.

The disk is embedded in paraffin with the cell button side down as cutting surface. Three to four micrometer thickness sections were cut when cell button is exposed and clearly visible.

H and E staining¹⁴

The cell block paraffin sections were taken through following steps:

Xylol (to dissolve wax)	- 5 minutes
Xylol	- 5 minutes
Absolute Ethyl alcohol	- 15 dips
Absolute Ethyl alcohol	- 25 dips
95% Ethyl alcohol	- 15 dips

80% Ethyl alcohol	- 15 dips
70% Ethyl alcohol	- 15 dips
Wash with distilled water	- 15 dips
Mayer's hematoxilin	- 2 minutes
Wash in running tap water	- 1 minute until excess stain removed
Wash in running tap water	- 30 seconds
Wash in tap water	- 15 dips
Eosin	- 20 seconds
Wash in running tap water	- 1 minute
95% Ethyl alcohol	- 15 dips
95% Ethyl alcohol	- 15 dips
Absolute Ethyl alcohol	- 15 dips
Absolute Ethyl alcohol	- 1 minute
Xylol	- 15 dips
Xylol	- 15 dips
Xylol	- 5 to 10 minutes
Slides were mounted in DPX	

Both smears and sections were examined separately.

Microscopic Evaluation of Smears¹⁵

The following criteria such as cellularity, morphological and architectural preservation, and presence of background were used for cyto-morphological pattern. Comparative evaluation of morphological features of conventional FNAC smear with cell block smear done and tabulated.

A comparison between the grading of cellularity, morphological and architectural preservation, and presence of background staining were performed on paired FNA smears and cell block samples according to the grading system as shown in Table. Each arm of the paired analysis was performed without knowledge of the grading outcome of the other. The FNA smears were assessed separately and at different times to that of their paired cell block samples. The cellularity, morphology and architecture of each cell block sample were evaluated using the hematoxylin and eosin (H and E) stain and that of the FNA smear was evaluated using the Pap stain.

Cellularity was qualitatively graded with score from 0 to 3+ depending on presence of tumor cells. Cellularity graded as acellular with no cells, low cellular with tumor cells less than 10% of cells present, moderately cellular with tumor cells 10-50% of cells present and high cellular with tumor cells more than 50% of cells present.

Morphological preservation was scored 0 or 1+ depending on presence or absence of clear nuclear chromatin, nuclear margin, cytoplasm contents and cytoplasmic membrane. Architectural preservation was scored 0 or 1+ depending on presence or absence of tissue architecture as evidenced by cellular relationship with each other e.g. presence of papillae in papillary carcinoma of thyroid.

Background staining was scored 0 or 3+ depending on presence or absence of background staining in relation to the smear / section, 0 with no staining background, 1 with less than 10% staining of smear / section, 2 with 10-50% staining of smear / section, 3 with more than 50% staining of smear / section.

Table 1 The grading system

Score	Description
Cellularity	
0	No cells
1+	Low
2+	Moderate
3+	High
Morphological preservation	
0	Poorly preserved
1+	Well preserved
Architectural preservation	
0	Absent
1+	Present
Background staining	
0	No background
1	Mild background staining
2	Moderate background staining
3	Severe background staining

The diagnosis was made as benign, malignant or suspicious and all possible cases followed.

RESULTS

The present study was carried out for a period of 24 months in Department of pathology of a tertiary care centre. 66 cases were enrolled in the study. In present study, fine needle aspirations from 39 neck swellings and 27 breast lesions were done. A total of 66 randomly obtained specimens were processed as cellblocks from remaining material in needles and syringes after preparation of smears.

Table 2 Diagnostic material in FNAC Smear and Cell block

Method	Diagnostic material present (out of 66)	Percentage (%)
FNAC Smear	61	92.42
Cell block	56	84.85

Out of 66 randomly taken FNACs diagnostic material obtained in 61 (92.42%) smears and in 56 (84.85%) cell blocks.

Table 3 Adequacy of material on FNAC & Cell block

FNAC Smear	Cell Block		Total
	Adequate	In-adequate	
Adequate	54	7	61
In-adequate	2	3	5
Total	56	10	66

Out of total 66 cases, 54 shows adequate material for diagnosis on both FNAC and cell block, 2 smears were hemorrhagic on FNAC but diagnostic on cell block, 7 cases has adequate material for diagnosis on FNAC but inadequate material for diagnosis on cell block, while 3 cases has inadequate material for diagnosis on both FNAC and cell block.

Table 4 Distribution of fine needle aspirations from various sites

Site	Number	Percentage (%)
Lymph node	16	24.24
Thyroid	23	34.85
Breast	27	40.91
Total	66	100.00

A total of 66 randomly obtained specimens were processed as cellblocks. Site wise distribution of total 66 randomly obtained specimens were as above.

Table 5 Age wise distribution of 66 cases

Age (in years)	FNAC	Percentage (%)
0-10	2	3.03
11-20	6	9.09
21-30	15	22.73
31-40	17	25.76
41-50	13	19.69
51-60	6	9.09
61-70	6	9.09
>71	1	1.52
TOTAL	66	100%

The above table states that 25.76% cases fell in the 31-40 year age group, 21-30 years with 22.73%, followed by 41-50 years age group with 19.69% , 61-70years with 12.12%, 11-20 years, 51-60 years and 61-70 years with 9.09%, 0-10 years with 3.03% and >71 years age group with 1.52%

Table 6 Sex wise distribution of fine needle aspirates

Sex	Number	Percentage (%)
Male	19	28.79
Female	47	71.21
Total	66	100

Out of total 66 cases, 19 cases were males and 47 cases were females. The male: female ratio was 1:2.47.

Table 7 Distribution of sites of fine needle aspirates in males

Type of fine needle aspirates	Males	
	Number	Percentage (%)
Lymph node	11	57.89
Thyroid	8	42.11
Breast	0	0
Total	19	100

In males lymph nodes accounts for 57.89% cases followed by thyroid 42.11% cases.

Table 8 Distribution of sites of fine needle aspirates in females

Type of fine needle aspirates	Female	
	Number	Percentage (%)
Lymph node	5	10.64
Thyroid	15	31.91
Breast	27	57.45
Total	47	100

In females Breast aspirates accounted for 57.45% cases, followed by thyroid aspirates 31.91% cases, followed by lymph node aspirates 10.64% cases as shown in table.

Table 9 Results with fine needle aspiration smears and blocks

FNAC	CELL BLOCK				Total (%)
	Malignant (%)	Benign (%)	Suspicious (%)	No Diagnosis (%)	
Malignant (%)	26 (39.4)	0 (0)	0 (0)	2 (3.0)	28 (42.4)
Benign (%)	0 (0)	27 (40.9)	1 (1.5)	5 (7.6)	33 (50.0)
Suspicious (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
No Diagnosis (%)	1 (1.5)	1 (1.5)	0 (0)	3 (4.5)	5 (7.6)
Total (%)	27 (40.9)	28 (42.4)	1 (1.5)	10 (15.2)	66 (100)

Out of total 66 cases, on FNAC smear sections a diagnosis of malignancy was given in 28(42.4%) cases and diagnosis of benign was given in 33 (50%) cases, and 5 cases (7.6%) were not diagnosed. On cell block sections a diagnosis of

malignancy was given in 27 (40.9%) cases, diagnosis of benign was given in 28(42.4%) cases, 1 case (1.5%) was suspicious and 10 (15.2%) cases were not diagnosed.

Table 10 Distribution of cases on combination of FNAC and cell block

FNAC's	Number of cases	Percentage
Benign	33	50.00
Malignant	30	45.45
No Diagnosis	3	04.55
Total	66	100

Thus 56 (84.84%) cases were adequate for diagnosis on cell block, 61 (92.42%) cases were adequate for diagnosis on FNAC smear. 63 (95.45%) cases of which 30 (45.45%) malignant and 33 (50%) benign were adequate for diagnosis when both FNAC smear and cell block were combined.

Table 11 Site wise distribution of Benign & Malignant cases

Site	Benign (%)	Malignant (%)
Lymph node	6 (18.2)	9 (30.0)
Thyroid	16 (48.5)	6 (20.0)
Breast	11(33.3)	15 (50.0)
Total	33	30

Out of 33 cases of benign, 16 cases were from thyroid followed by 11 cases were from breast and 6 cases were from lymph node. Out of 30 cases of malignancy, 15 cases were from breast followed by 9 cases were from lymph node and 6 cases were from thyroid.

Table 12 Correlation between cell block and FNAC smear diagnosis

Site	Benign	Malignant	Total (%)
Positive correlation	27	26	53 (84.13)
Non-correlation	6	4	10 (15.87)
Total	33	30	63 (100)

$\kappa=0.683$, 95% Confidence interval (0.503-0.863), $P < 0.001$
Cell block and fine needle aspiration were reported separately and correlation between their diagnoses tabulated as above. Out of 63 diagnosed cases positive correlation was found in 53 cases and non-correlation in 10 cases. Out of these 53 correlated cases, 27 cases were benign and 26 cases were malignant. Out of 10 non-correlated cases, 6 cases were benign and 4 cases were malignant.

Absolute concordance was found in 84.13% between both techniques ($\kappa=0.683$). The strength of agreement was good.

Table 13 Correlation between cell block and FNAC smear diagnosis for benign lesions

Site	Benign	Percentage (%)
Positive correlation	27	81.82
Non-correlation	6	18.18
Total	33	100

Out of 33 cases positive correlation for benign lesion was found in 27 (81.82%) cases. Non-correlation for benign lesions was found in 6 (18.18%) cases. Out of these 6 cases, 1 case was hemorrhagic on FNAC smear and benign on cell block, while other 5 cases were benign on FNAC smear and acellular on cell blocks.

Table 14 Correlation between cell block and FNAC smear diagnosis for malignant lesions

Site	Malignant	Percentage (%)
Positive correlation	26	86.67
Non-correlation	4	13.33
Total	30	100

Out of 30 cases positive correlation for malignant lesions were found in 26 (86.67%) cases. Non-correlation for malignant lesions were found in 4 (13.33%) cases. Out of these 4 cases, 1 case was hemorrhagic on FNAC smear and malignant on cell block, 1 case was benign on FNAC smear and suspicious on cell block which was later confirmed malignant on histopathology, while other 2 cases were malignant on FNAC smear and acellular on cell blocks.

Table 15 FNAC smear diagnosis

Site of FNAC	No. of cases	FNAC smear Diagnosis						
		Confirmed		Disproven		Not proven		Lost to follow up
		Histologic	Clinical	Histologic	Clinical	Insufficient evidence on FNAC		
Lymph node	16	7	5	0	0	1	3	
Thyroid	23	13	0	2	0	2	6	
Breast	27	19	1	1	0	2	4	
Total	66	39	6	3	0	5	13	

Out of 66 cases, 55 cases were diagnosed, 1 case was suspicious on cell block and 61 cases were diagnosed on FNAC smear. 63 cases of which 30 malignant and 33 benign were diagnosed when both FNAC smear and cell block were combined, while 3 cases were not diagnosed on both FNAC and cell block due to inadequate material.

For FNAC smear diagnosed 15 cases of lymph node lesions, histopathological & clinical diagnosis was available in 12 cases. 6 malignant and 6 benign cases confirmed by histopathological & clinical diagnosis.

For FNAC smear diagnosed 21 cases of thyroid lesions, histopathological diagnosis was available in 15 cases. 4 malignant and 9 benign cases confirmed by histopathological diagnosis, while 2 cases were false negative on FNAC smear.

For FNAC smear diagnosed 25 cases of Breast lesions, histopathological & clinical diagnosis was available in 21 cases. 11 malignant and 9 benign cases confirmed by histopathological & clinical diagnosis, while 1 case was false negative on FNAC smear.

Thus total 61 cases were diagnosed on FNAC smear. For 44 of the cases, biopsy or surgically removed tissue was available for confirmation of diagnosis. In 38 cases, the FNAC smear diagnosis was confirmed by histopathology. There were 3 false-negative aspirates. Clinical follow-up was used to evaluate the cytological diagnosis in 6 cases. In 6 cases, the FNAC smear diagnoses were clinically confirmed. In 13 cases, patients were lost to follow up.

Table 16 Comparison of FNAC with Histopathological & Clinical Diagnosis

FNAC	Histopathological & Clinical Diagnosis	
	Malignant	Benign
Malignant	21	0
Benign	3	24

Thus for FNAC smear diagnosed total 61 cases of lymph node, thyroid and breast lesions, histopathological & clinical diagnosis was available in 48 cases. 21 malignant and 24 benign cases confirmed by histopathological & clinical diagnosis, while 3 cases were false negative on FNAC smear.

Table 17 Statistical parameters of FNAC smear

Statistic Parameter	FNAC smear	95% Confidence Interval
Accuracy (%)	93.8	86.90-100.0
Sensitivity (%)	87.5	74.27-100.0
Specificity (%)	100	86.20-100.0

Thus in lymph node, thyroid and breast lesions accuracy, sensitivity, specificity of FNAC smear was as follows. The overall predictive values of cytological positivity and negativity were 100% and 88.9% respectively.

Table 18 Cell Block smear diagnosis

Site of FNAC	No. of cases	Cell Block smear Diagnosis						
		Confirmed		Disproven		Not proven		Lost to follow up
		Histologic	Clinical	Histologic	Clinical	Insufficient evidence on Cell Block		
Lymph node	16	6	5	0	0	2	3	
Thyroid	23	13	0	1	0	3	6	
Breast	27	16	1	1	0	5	4	
Total	66	35	6	2	0	10	13	

For Cell block diagnosed 14 cases of lymph node lesions, histopathological & clinical diagnosis was available in 11 cases. 5 malignant and 6 benign cases confirmed by histopathological & clinical diagnosis.

For Cell block diagnosed 20 cases of thyroid lesions, histopathological diagnosis was available in 14 cases. 4 malignant and 9 benign cases confirmed by histopathological diagnosis, while 1 case were false negative on cell block.

For Cell block smear diagnosed 22 cases of Breast lesions, histopathological & clinical diagnosis was available in 18 cases. 12 malignant and 5 benign cases confirmed by histopathological & clinical diagnosis, while 1 case was false negative on cell block.

Thus total 56 cases were diagnosed on cell block. In 35 cases, the cell block smear diagnosis was confirmed by histopathologically. There were 2 false-negative aspirates. Clinical follow-up was used to evaluate the cytological diagnosis in 6 cases. In 6 cases, the cell block smear diagnosis was clinically confirmed. In 13 cases, patients were lost to follow up.

Table 19 Comparison of Cell Block with Histopathological & Clinical Diagnosis

Cell Block	Histopathological & Clinical Diagnosis	
	Malignant	Benign
Malignant	21	0
Benign	2	20

Thus for Cell block diagnosed total 56 cases of lymph node, thyroid and breast lesions, histopathological & clinical diagnosis was available in 43 cases. 21 malignant and 20 benign cases confirmed by histopathological & clinical diagnosis, while 2 cases were false negative on cell block.

Table 20 Statistical parameters of Cell Block

Statistic Parameter	Cell block	95% Confidence Interval
Accuracy (%)	95.34	89.05-101.6
Sensitivity (%)	91.30	73.20-97.58
Specificity (%)	100	83.89-100.0

Thus in lymph node, thyroid and breast lesions accuracy, sensitivity, specificity, positive predictive value, negative predictive value of cell block smear was as follows. The overall predictive values of cell block positivity and negativity were 100% and 90.9% respectively.

Table 21 Combined Cell Block and FNAC smear diagnosis

Site of FNAC	No. of cases	Combined Cell Block and FNAC smear Diagnosis					
		Confirmed		Disproven		Not proven	
		Histologic	Clinical	Histologic	Clinical	In sufficient evidence on Cell Block and FNAC	Lost to follow up
Lymph node	16	7	5	0	0	1	3
Thyroid	23	15	0	1	0	1	6
Breast	27	20	1	1	0	1	4
Total	66	42	6	2	0	3	13

For combined FNAC smear and Cell block diagnosed 15 cases of lymph node lesions, histopathological & clinical diagnosis was available in 12 cases. 6 malignant and 6 benign cases confirmed by histopathological & clinical diagnosis.

For combined FNAC smear and Cell block diagnosed 22 cases of thyroid lesions, histopathological diagnosis was available in 16 cases. 5 malignant and 10 benign cases confirmed by histopathological diagnosis, while 1 case was false negative on combined FNAC smear and cell block.

For combined FNAC smear and Cell block diagnosed 26 cases of Breast lesions, histopathological & clinical diagnosis was available in 22 cases. 12 malignant and 9 benign cases confirmed by histopathological & clinical diagnosis, while 1 case was false negative on combined FNAC smear and Cell block.

Thus total 63 cases of which 30 malignant and 33 benign were diagnosed when both FNAC smear and cell block were combined. In 42 cases, the cell block smear diagnosis was confirmed by histopathology. There were 2 false-negative aspirates. Clinical follow-up was used to evaluate the cytological diagnosis in 6 cases. In 6 cases, the FNAC smear diagnosis was clinically confirmed. In 13 cases, patients were lost to follow up.

Table 22 Comparison of combined FNAC & Cell Block with Histopathological & Clinical Diagnosis

Combined FNAC & Cell Block	Histopathological & Clinical Diagnosis	
	Malignant	Benign
Malignant	23	0
Benign	2	25

Thus for combined FNAC smear and Cell block diagnosed total 63 cases of lymph node, thyroid and breast lesions, histopathological & clinical diagnosis was available in 50 cases. 23 malignant and 25 benign cases confirmed by histopathological & clinical diagnosis, while 2 cases were false negative on combined FNAC smear and cell block.

Table 23 Statistical parameters of combined FNAC & Cell Block

Statistic Parameter	FNAC smear & Cell block	95% Confidence interval
Accuracy (%)	96.0	90.57-101.4
Sensitivity (%)	92.0	81.37-97.78
Specificity (%)	100	86.68-100.0

Thus in lymph node, thyroid and breast lesions accuracy, sensitivity, specificity, positive predictive value, negative predictive value of combined FNAC smear and cell block was as above. The overall predictive values of cytological positivity and negativity were 100% and 92.6% respectively.

Table 24 Comparative statistical parameters

Statistic Parameter	FNAC smear	Cell Block smear	Combined FNAC and Cell Block smear
Accuracy (%)	93.8	95.3	96.0
Sensitivity (%)	87.5	91.3	92.0
Specificity (%)	100	100	100

Thus the comparative statistical parameters of FNAC smears, Cell Block smears and combined FNAC and Cell Block smears are as above.

DISCUSSION

The present study was conducted during period December 2012 to November 2014 in department of Pathology to understand significance of fine needle aspiration cytology and cell block, to access cyto-morphological features of FNAC smear and slide of cell block technique and to access utility of FNAC smear and cell block technique in cyto-diagnosis of neck swellings and breast lesions.

In present study the cell block sections showed clearly recognizable normal and abnormal cells with minimal shrinkage. Many a times we obtained a tissue piece which on processing given good tissue morphology and architectures like cell ball and papillae and three dimensional clusters, nuclear and cytoplasmic details, and individual cell characteristics. Cell-block mimics the biopsy in good identification.

Table 25 Percentage increase additional valuable information by cell block

Sr. No.	Study	Percentage increase additional valuable information by cell block (%)
1	Rajib <i>et al</i> (2012)	68.4
2	Kung and Yuen (1989)	44.4
3	Rofagha <i>et al</i> (1984)	55.0
4	Sanchez and Selvaggi (2006)	31.0
5	Diana <i>et al</i> (2014)	28.5
6	Kern and Haber (1986)	27.2
7	Present study	31.8

Out of 63 diagnosed cases morphology and architecture was preserved in 34 cases on FNAC smears. Morphology and architecture was poor or absent in 29 cases on FNAC smears. Cell block provided additional morphological and architectural information in 20 (31.8%) cases and aided diagnosis in 3 (4.8%) cases. Cell block provided same morphological and architectural information in 30 (47.6%) cases. Cell block

provided no additional morphological and architectural information in 13 (20.6%) cases.

Also, in the study done by Diana *et al*¹⁶ (2014), Cell block provided additional information which was helpful for a more accurate diagnosis in 28.5% of the cases; the diagnosis remained the same in 55.3% and did not give any additional information in 16.2% cases. Thus their results correlate with our study.

Rofagha *et al*¹⁷ (1984) reviewed 286 adequate fine needle aspiration specimens. A total of 85 cases of these had both smear and cell block specimens. Of the 85 cell block specimens that had accompanying smears, 46 (55%) showed that the smear diagnosis had improved after the cell block was reviewed. In the remaining 39 cases (45%), there was no significant improvement in the smear diagnosis after the cell block specimen was reviewed.

Kern and Haber¹⁸ (1986) in their study out of 1375 aspirates 393 had simultaneous cell block preparations and FNAC smear. Out of 393 cases 103 aspirates (27.22%) of all cases could be diagnosed or definitely classified only on the basis of the examination of particles in the cell button. They found that the diagnosis of papillary adenocarcinoma of thyroid could be given on smears but the presence of distinct neoplastic papillary structures in the FNA cell button is confirmatory and by itself diagnostic.

Kung and Yuen¹⁹ (1989) studied distinction between colloid nodules and follicular neoplasm of thyroid using cell blocks. Hyperplastic papillae of colloid nodules and intact dilated follicles were easily recognized on cell blocks alone on 11 cases, on both cell blocks and smear, in 7 cases and on smears alone in 2 cases. Among the 15 follicular neoplasms, dilated or micro follicles were seen in only 5 cases. Histopathological examination or the architecture is essential for separating colloid nodule and thyroiditis and such architecture can be seen in cell blocks. In their study they found that architecture of the tissue was preserved in cell blocks and cellular morphology on sections was familiar to the histopathologist.

Sanchez and Selvaggi²⁰ (2006) conducted a study to determine utility of cell blocks in diagnosis of thyroid lesions. They found that the cell block were contributory in 31% cases.

Rajib *et al*²¹ (2012) found that by adding cell block technique 12.5% inadequate direct smear could yield a diagnosis. Cell blocks also provided additional valuable information in 68.4% cases. The two procedures can be performed easily in single sitting. Combined efforts of two procedures have beneficial effect in diagnosis.

Table 26 Percentage Improvement in diagnosis by cell block

Sr. No.	Study	Improvement in diagnosis by cell block (%)
1	Raafat and Abdelmonem (2013)	12.5
2	Rajib <i>et al</i> (2012)	12.5
3	Basnet and Talwar (2012)	8.16
4	Nathan <i>et al</i> (2000)	6.6
5	Fredrick <i>et al</i> (2012)	6.0
6	Present study	4.8

In present study when both FNAC smear and cell block were combined diagnosis was improved in 4.8% cases. Raafat and

Abdelmonem²² (2013) in their study found that by adding cell block technique 12.5% inadequate direct smear could yield a diagnosis and combined use direct smear of and cell block increases diagnostic efficacy up to 95.0%. Rajib *et al*²¹ (2012) in their study found that when direct smear was supplemented by cell block 12.5% cases became adequate for diagnosis and diagnostic efficacy increased from 85.0% to 95.7%. Basnet and Talwar⁸ (2012) in their study found that diagnosis was improved in 8.16% cases. Diagnosis improved in 6.6% cases in the study of Nathan *et al*¹² (1998) and in 6% cases in the study of Fredrick *et al*²³ (1997).

Table 27 Diagnostic material present in FNAC Smears

Sr. No.	Study	Diagnostic material present in FNAC Smears (%)
1	Diana <i>et al</i> (2014)	96.4
2	Nathan <i>et al</i> (2000)	84.4
3	Rajib <i>et al</i> (2012)	83.1
4	Libo <i>et al</i> (2008)	40.0
5	Present study	92.4

In present study out of 66 randomly taken FNACs diagnostic material obtained in 61 smears and in 56 cell blocks. In the present study diagnostic material in FNAC smears was present in 92.42% cases. In study of Diana *et al*¹⁶ (2014) diagnostic material in FNAC smears was present in 96.4% cases. In study of Nathan *et al*¹² (2000) the diagnostic material in FNAC smears was present in 84.4% cases. In study of Rajib *et al*²¹ (2012) the diagnostic material in FNAC smears was present in 83.1% cases. In study of Libo *et al*²⁴ (2008) the diagnostic material in hemorrhagic thyroid FNAC smears was present in 40.00 % cases.

Table 28 Diagnostic material present in cell blocks

Sr. No.	Study	Diagnostic material present in cell blocks (%)
1	Oslen <i>et al</i> (1986)	98.13
2	Diana <i>et al</i> (2014)	93.30
3	Libo <i>et al</i> (2008)	92.98
4	Fredrick <i>et al</i> (1997)	92.00
5	Sanchez and Selvaggi (2006)	90.20
5	Zitoet <i>al</i> (1995)	87.94
7	Kung and Yuen(1989)	85.91
8	Rajib <i>et al</i> (2012)	75.00
9	Nathan <i>et al</i> (2000)	73.30
10	Present study	84.85

In the present study diagnostic material in cell blocks was present in 84.85% cases. Also, in the study done by Kung and Yuen¹⁸ they found that in lymph node, thyroid and breast specimen diagnostic material in cell blocks was present in 85.91% cases. Thus their results correlate with our study.

Table 29 Diagnostic material present on combination of FNAC Smears and Cell blocks

Study	Diagnostic material present on combination of FNAC Smears and Cell blocks (%)
Rajib <i>et al</i> (2012)	95.6
Present study	95.5

In present study on combination of FNAC smears and cell blocks the diagnostic material was present in 95.5% cases. Also, in the recent study done by Rajib *et al*²¹ (2012), in which on combination of FNAC smears and cell blocks the diagnostic material was present in 95.6% cases. Thus their results correlate with our study.

Table 30 Percentage of Malignant cases

Sr. No.	Study	Malignant cases (%)
1	Oslen et al (1986)	63.00
2	Zito et al (1995)	41.00
3	Nathan et al(2000)	31.41
4	Raafat and Abdelmonem(2013)	16.25
5	Rajib(2012)	11.90
6	Present study	45.45

In present study FNAC smear and cell block smear were evaluated separately for diagnosis. Diagnosis of malignancy was given in 45.45% cases. In his study Zito et al⁹ (1995) had given 41% cases as malignant by cell block. Thus their results correlate with our study.

Table 31 Percentage of Benign cases

Sr. No.	Study	Benign cases (%)
1	Raafat and Abdelmonem(2013)	83.75
2	Nathan et al(2000)	68.38
3	Rajib et al(2012)	63.10
4	Zito et al (1995)	50.45
5	Oslen et al (1986)	37.00
6	Present study	50.0

Diagnosis of benign lesion in our study was 50.0%. In his study Zito et al⁹ (1995) had given 50.45% cases as benign by cell block. Thus their results correlate with our study.

Table 32 Percentage Absolute concordance

Sr. No.	Study	Absolute concordance between two techniques (%)
1	Diana et al(2014)	81.60
2	Basnet and Talwar(2012)	87.75
3	Rajib et al(2012)	90.00
4	Fredrick et al(1997)	92.00
5	Raafat and Abdelmonem(2013)	96.25
6	Present study	84.12

In present study out of 63 diagnosed cases positive correlation was found in 53 cases and non-correlation in 10 cases i.e. in 15.88% cases. Out of these 53 correlated cases, 27 cases were benign and 26 cases were malignant. Out of 10 non-correlated cases, 6 cases were benign and 4 cases were malignant.

Out of 33 benign cases positive correlation for benign lesion was found in 27 (81.82%) cases. Non-correlation for benign lesions was found in 6 (18.18%) cases. Out of these 6 cases, 1 case was hemorrhagic on FNAC smear and benign on cell block, while other 5 cases were benign on FNAC smear and acellular on cell blocks.

Out of 30 malignant cases positive correlation for malignant lesions was found in 26 (86.67%) cases. Non-correlation for malignant lesions was found in 4 (13.33%) cases. Out of these 4 cases, 1 case was hemorrhagic on FNAC smear and malignant on cell block, 1 case was benign on FNAC smear and suspicious on cell block which was later confirmed malignant on histopathology, while other 2 cases were malignant on FNAC smear and acellular on cell blocks.

Absolute concordance was found in 84.12% between both techniques. The strength of agreement was good and the kappa index was within acceptable limits ($\kappa=0.683$). Of the 10 discordant cases, 9 were explained by sampling (7 of them

sample non diagnostic on the cell block but diagnostic on the FNAC smear, and of them 2 sample nondiagnostic on the FNAC smear but diagnostic on the cell block). The remaining 1 case was benign on FNAC smear and suspicious on cell block, on follow up the histology was positive for malignancy.

Also, in the recent study done by Diana et al¹⁶, absolute concordance was 81.6% and the kappa index was within acceptable limits ($\kappa=0.56$) between cell block and FNAC smear and the strength of agreement was moderate. Thus their results correlate with our study.

Table 33 Sensitivity of FNAC smear

Sr. No.	Study	Sensitivity of FNAC smear (%)
1	Raafat and Abdelmonem(2013)	91.6
2	Nathan et ai(2002)	84.8
3	Rajib et al(2012)	84.6
4.	Present study	87.5

FNAC smear diagnosed total 61cases of lymph node, thyroid and breast lesions, histopathological & clinical diagnosis was available in 48 cases. 21 malignant and 24 benign cases confirmed by histopathological & clinical diagnosis, while 3 cases were false negative on FNAC smear. Thus accuracy, sensitivity and specificity of FNAC smear was 93.8%, 87.5% and 100% respectively. The overall predictive values of FNAC smear positivity and negativity was 100% and 88.9% respectively.

In study of Rajib²¹ et al (2012) cytological results of FNAC of breast lesions were compared to the cell-block histopathological picture. The accuracy of FNAC in comparison with histopathological results of cell-blocks was 98.0%, sensitivity was 84.62% and specificity was 100%. In study of Nathan⁶ et al (2000) sensitivity of FNAC smear was 84.8%.

Table 34 Sensitivity of Cell Block

Sr. No.	Study	Sensitivity of Cell Block (%)
1	Rofagha et al (1984)	100
2	Oslen et al (1986)	95.0
3	Zito et al (1995)	95.0
4	Basnet and Talwar(2012)	94.6
5	Nathan et al(2000)	73.3
6	Present study	91.3

Cell block diagnosed total 56 cases of lymph node, thyroid and breast lesions, histopathological & clinical diagnosis was available in 43 cases. 21 malignant and 20 benign cases confirmed by histopathological & clinical diagnosis, while 2 cases were false negative on cell block. Thus accuracy, sensitivity and specificity of FNAC smear was 95.34%, 91.30% and 100% respectively. The overall predictive values of FNAC smear positivity and negativity was 100% and 90.9% respectively.

Rofagha et al¹⁷ (1984) reviewed 286 adequate fine needle aspiration specimens of which 98 had simultaneous cell block preparations. Tissue specimens for 40 (41%) of the 98 cell block specimens were present and there was 100% correlation between the cell block and the tissue diagnosis, including that the cell block diagnosis was as definitive as was the conventional biopsy in these cases. Thus accuracy, sensitivity and specificity of cell block was 100%. Also in the study of

Oslen *et al*²⁵ (1986) the cell block accuracy, sensitivity and specificity was 100% in thyroid aspirates and overall sensitivity was 95% and specificity was 100%. In the study of Zito *et al*⁹ (1995), of the 333 patients 135(40% cases) were positive and 168 (50% cases) were negative for malignancy. The statistical evaluation based only on the histologically confirmed 67 cases showed an overall cell block accuracy was 97% with a sensitivity of 95% and specificity of 100%. In study of Nathan⁶ *et al* (2000) sensitivity of cell block was 73.3%. In study of Basnet and Talwar⁸(2012) cytological results of FNAC aspirates of 49 malignant cases were compared to the histopathological picture. In lymph node, thyroid and breast lesion the accuracy and specificity of FNAC smear was 94.6%.

Table 35 Sensitivity of combined FNAC smear and Cell Block

Sr. No.	Study	Sensitivity (%)
1	Basnet and Talwar(2012)	100
2	Rofagha <i>et al</i> (1984)	100
3	Oslen <i>et al</i> (1986)	95.0
4	Zito <i>et al</i> (1995)	95.0
5	Raafat <i>et al</i> (2014)	94.0
6	Nathan <i>et al</i> (2000)	89.4
7	Present study	92.0

Total 63 cases were diagnosed when both FNAC smear and cell block were combined. For 44 of the cases, biopsy or surgically removed tissue was available for confirmation of diagnosis. In 42 cases, the cell block smear diagnosis was confirmed by histopathology. There were 2 false-negative aspirates. Clinical follow-up was used to evaluate the cytological diagnosis in 6 cases. In 6 cases, the cytological diagnosis was clinically confirmed. In 13 cases, patients were lost to follow up. Thus out of 63 cases final diagnosis of 50 cases was available. On combination of FNAC smear and cell block the accuracy of present study was 96.0% with sensitivity 92.0 % and specificity 100%.

CONCLUSION

The present study entitled “Significance of study of fine needle aspiration cytology and cell block preparation in neck swellings and breast lesions” was conducted during period December 2012 to November 2014 in Department of Pathology.

The findings of present study are summarized as follows

1. The commonest age group of patients was 31-40 years.
2. In the present study diagnostic material in FNAC smears was present in 92.42% cases and in cell blocks was present in 84.85% cases.
3. On combination of FNAC smears and cell blocks the diagnostic material was present in 95.5% cases.
4. Morphology and architecture was preserved better in cell blocks. Cell block provided additional morphological and architectural information in 31.8% cases and aided diagnosis in 4.8% cases.
5. Diagnosis of malignancy was given in 45.45% cases. Most common site of malignant cases was breast.
6. Diagnosis of benign was given in 50% cases. Most common site of benign cases was thyroid.
7. Positive correlation between cell block and FNAC smear for malignant and benign lesions was seen in 86.67% and 81.82% cases respectively.
8. In 10 cases no correlation between cell block and FNAC

smear was found.

9. Absolute concordance was found in 84.12% between both techniques and the strength of agreement was good.
10. The accuracy of FNAC smear was 93.8% with sensitivity 87.5%and specificity 100%. The overall predictive value of FNAC smear positivity and negativity was 100% and 88.9% respectively.
11. The accuracy of cell block was 95.34% with sensitivity 91.30%andspecificity 100%. The overall predictive value of cell block positivity and negativity was 100% and 90.9% respectively.
12. On combination of FNAC smear and cell block the accuracy of present study was 96.0% with sensitivity92.0 % andspecificity100%. The overall predictive value of present study positivity and negativity was 100% and 92.6% respectively.

Conclusion drawn from the present study are

1. FNACs after smear preparation leaves behind some residue that may contain valuable tissue fragments which can be processed by cell block method.
2. The two techniques can be performed easily using single aspirate from single sitting.
3. The method is simple to perform and no expertise is required to handle the specimen.
4. The cell block method using Nathan’s fixative gives good results.
5. Tissue architecture is well appreciated in cell blocks.
6. Cell blocks can be used for special stains.
7. Cell blocks can be used for immunohistochemistry which can be carried out on sections and can diagnose certain tumors without requiring surgical interventions.
8. Combined use of FNAC smear and cell-block can be useful for establishing a more definitive cytopathologic diagnosis.
9. As cell blocks were prepared from remnant of FNAC aspirates, cellularity is the major drawback.
10. The disadvantage with cellblock technique is delay in the in the diagnosis when compared to FNAC smear and sometimes risk of losing material during processing.

References

1. Ministry of Health and Family Welfare Government of India. Manuals for Training in Cancer Control Manual for Cytology. November 2005.
2. Kumar H, Chandanwale SS, Gore CR, Buch AC, *et al*. Role of fine needle aspiration cytology in assessment of cervical lymphadenopathy. *Medical Journal of Dr. D.Y. Patil University* 2013; 6(4):400-404.
3. Kaur G, Sivakumar S. Comparison of Unsatisfactory Aspirates in Fine Needle Aspiration Performed by Surgical Medical Officers and Pathologists. *Journal of Cytology* 2007;24(2): 82-84.
4. The Papanicolaou Society of Cytopathology Task Force on Standards of Practice. Guidelines of the Papanicolaou Society of Cytopathology for Fine-Needle Aspiration Procedure and Reporting. *Diagnostic Cytopathology* 1997;17(4): 239-247.

5. Mittra P, Bharti R, Pandey MK. Role of Fine Needle Aspiration Cytology in Head and Neck Lesions of Paediatric Age Group. *Journal of Clinical and Diagnostic Research* 2013;7(6): 1055-1058.
6. Muddegowda PH, Lingegowda JB, Kurpad R, Konapur PG, et al. The value of systematic pattern analysis in FNAC of breast lesions: 225 cases with cytohistological correlation. *JCytol* 2011;28(1): 13-19.
7. Grace McKee. The role of fine needle aspiration cytology in the diagnosis of thyroid lesions. *J R Soc Med* 1998;91(33): 28-32.
8. Basnet S, Talwar OP. Role of cell block preparation in neoplastic lesions. *Journal of Pathology of Nepal* 2012;2: 272-276.
9. Zito FA, Gadaleta CD, Salvatore C, Filotico R, et al. A modified cell block technique for fine needle aspiration cytology. *ActaCytol* 1995; 39(1):93-99.
10. Hecht SA, McCormack M. Comparison of three cell block techniques for detection of low frequency abnormal cells. *Pathology and Laboratory Medicine International* 2013;5:1-7.
11. Yun Gong. Breast Cancer: Pathology, Cytology, and Core Needle Biopsy Methods for Diagnosis, In: M.K. Shetty (ed.), *Breast and Gynecological Cancers: An Integrated Approach for Screening and Early Diagnosis in Developing Countries*. New York Springer Science+Business Media; 2013. p19-37.
12. Nathan NA, Narayan E, Smith MM, Horn MJ. Cell Block CytologyI Improved Preparation and Its Efficacy in Diagnostic Cytology. *Am J Clin Pathol* 2000; 114:599-606.
13. Bhatia P, Dey P, Uppal R, Shifa R, et al. Cell blocks from scrapping of cytology smear comparison with conventional cell block. *Acta Cytol*2008;52: 329-333.
14. Gamble M, Bancroft JD. Theory and practice of histological techniques. The hematoxylin and eosin. 16th edn. Philadelphia: Churchill livingstone Elsevier;2008.
15. Khan S, Omar T, Michelow P. Effectiveness of the cell block technique in diagnostic cytopathology. *Journal of Cytology* 2012 ; 29(3):177-182.
16. Castro-Villabón D, Avello Y, Ruiz N, Rodríguez-Urrego PA. Implementation of routine thromboplastin-plasma cell block technique in the evaluation of non-gynecologic specimens: A methodologic comparison with conventional cytology. *Journal of Microscopy and Ultrastructure* 2014;2:177-181.
17. Rofagha SK, Toole RV, Leming MF. Role of the cell block in fine needle aspiration. *Acta Cytol* 1984;28: 630-631.
18. Kern WH, Haber H.Fine needle aspiration minibiopsies. *Acta Cytol* 1986;30: 403-408.
19. Kung ITM, Yuen RWS. Fine needle aspiration of thyroid. Distinction between colloid nodules and follicular neoplasm using cell blocks and 21-gauge needles. *Acta Cytol* 1989;33: 53-60.
20. Sanchez N, Selvaggi SM. Utility of cell blocks in the diagnosis of thyroid aspirates. *Diagn Cytopathol*. 2006; 34(2): 89-92.
21. Rajib RC, Majid N, Rahman MR, Baqui MN, et al. Role of cell block preparation in thyroid cytology a cross sectional study. *JAFMC Bangladesh* 2012;8(1):30-34.
22. Hegazy RA,, Hegazy AA. FNAC and Cell-block Study of Thyroid Lesions. *Universal Journal of Medical Science* 2013;1(1): 1-8.
23. Frederick M, Bridget, Ann D. A review of 50 consecutive cytology cell block preparations in a large general hospital. *J Clin Pathol* 1997;50:985-990.
24. Qui Libo, Crapanzano JP, Saqi JP, Vidhun R, et al. Cell block alone as an ideal preparatory method for hemorrhagic thyroid nodule aspirates procured without onsite cytologists. *Acta Cytol* 2008; 52: 139-144.
25. Oslen NJ, Gogle HK, Willams WL, Mettler FA Jr.. Processing of aspiration cytology samples: An Alternative method. *Acta Cytol* 1986; 30: 409-412.

How to cite this article:

Pravin M.Meshram et al.2018, Significance of Study of Fine Needle Aspiration Cytology And Cell Block Preparation In Neck Swellings And Breast Lesions. *Int J Recent Sci Res*. 9(2), pp. 24543-24552.
DOI: <http://dx.doi.org/10.24327/ijrsr.2018.0902.1680>
