

## Interpretation Of Microbiopsies In Cytological Smears And Histopathological Correlation

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### ABSTRACT

**Background:** Fine Needle Aspiration Cytology (FNAC) is a quick and inexpensive technique in which the smears prepared, contain well preserved tissue fragments known as tissue microbiopsies, which provide information on tissue architecture and contribute to the tumor diagnosis.

**Aim:** To study significance of tissue microbiopsies in fine needle aspiration cytology and its histopathological correlation.

**Methods:** A prospective study conducted in the Department of Pathology. 186 cases were examined between February 2023 to January 2024. 160 cases that had well preserved tissue fragments were included in the study.

**Results:** Of the 160 cases 78% cases of FNAC were done conventionally. 22% of the cases with deep seated lesions along with all thyroid lesions were done under USG guided and CT guided. The organs with highest yield of microbiopsies were lymph nodes 50; (31.2%), followed by breast 46; (28.7%), thyroid 30; (18.8%), soft tissue 20; (12.5%), salivary gland 6; (3.8%), lung 2; (1.25%), liver 2; (1.25%), oral cavity 2; (1.25%), ovary 1; (0.6%) and bone 1; (0.6%). Out of 160 cases, 85 cases (53.1%) were malignant, 43 benign (26.9%) and 32 cases (20%) were inflammatory lesions. Among 85 malignant tumors, 70 (82.3%) cases were primary and 15 (17.7%) cases were metastatic tumors.

**Conclusion:** FNAC smears containing tissue microbiopsies are helpful in diagnosing benign, inflammatory and malignant lesions. It also helps in predicting possible primary sites in case of metastatic tumors which would be difficult by cytology alone. Therefore, technique can be employed to enhance the diagnostic accuracy of FNAC, without increasing any financial burden on the patients.

**Keywords:** FNAC, microbiopsies, histopathology, tissue fragment.

### 1. INTRODUCTION

Fine Needle Aspiration Cytology (FNAC) is a quick and inexpensive highly useful investigation in the diagnosis of clinically suspected malignant lesions as well as benign and inflammatory lesions. Lesions located superficially are aspirated by palpation. Radiological imaging modalities like ultrasonography and computed tomography guide fine needles to lesions not palpable or deep-seated lesions<sup>1,2</sup>.

Orell SR et al, are of the opinion that, if the treatment of the lesion involves neoadjuvant preoperative chemo or radiotherapy, then cytodiagnosis must be equivalent to a histological tissue diagnosis, i.e. providing grade for malignancies

and histogenetic tumour type<sup>1</sup>. Microbiopsies are defined as well preserved viable tissue fragments obtained on cytology smears<sup>3,4</sup>. They likely provide information on the tissue architecture and aid in the diagnosis. The aim is to study significance of tissue microbiopsies in fine needle aspiration cytology and its histopathological correlation.

## 2. MATERIALS AND METHODS

A total of 186 cases with clinically palpable swellings and suspected malignant lesions underwent FNAC.

Needle aspiration of the palpable masses were done using 22-23 gauge disposable needles and 10 mL disposable syringes except in cases of bone lesions where 18 gauge needles were used. The smears prepared were fixed in 95% ethyl alcohol and stained with hematoxylin and eosin stain as well as May Grünwald Giemsa<sup>5</sup>.

Histopathological correlation with the results of FNAC was done whenever available. The histopathological diagnosis was taken as the gold standard against which the cytological diagnosis was compared. All FNAC cases with palpable masses and suspected malignant lesions which had tissue fragments were included in the study. Smears with no tissue fragments and low cellularity were excluded from the study.

Data was entered in Excel spread sheet and analysed using SPSS software (version 20). Descriptive statistics were carried out. Qualitative data was expressed as cases and percentage. Correlation between FNAC (microbiopsies) and histopathology was analyzed using Chi-square test. p-value (<0.05) was considered statistically significant.

## 3. RESULTS

The study population was in the age range of 15-80 years with median age being 54.3 years. There was a slight male preponderance with the male to female ratio being 1.7:1.

Out of the 186 fine needle aspiration cytology smears, 160 had well preserved tissue fragments (microbiopsies) which aided in the diagnosis.

The organs with highest yield of micro-biopsies were lymph nodes 50 (31.2%); followed by breast 46 (28.7%); thyroid 30 (18.8%); soft tissue 20 (12.5%); salivary gland 6 (3.8%); lung 2 (1.25%); liver 2 (1.25%); oral cavity 2 (1.25%); ovary 1 (0.6%) and bone 1 (0.6%).

Out of total 160 cases, 85 cases (53.1%) were malignant, 43 cases (26.9%) were benign and 32 cases (20%) were inflammatory. Among 85 malignant tumors, 70 (82.3%) cases were primary and 15 (17.7%) cases were metastatic tumors. Among 15 metastatic tumors, 14 (93.3%) cases showed tumor deposit in Lymph nodes and 1 (6.7%) showed tumor deposit in liver.

### **FNA cases interpreted as positive for malignant cells, n=85:**

Among 85 malignant tumors, 70 (82.3%) cases were primary and 15 (17.7%) cases were metastatic tumors.

Among 70 primary malignant tumors, 30 cases were of Invasive ductal carcinoma of breast (43%), followed by Papillary carcinoma of thyroid in 16 cases (23%), Hodgkin lymphoma in 3 cases (4.2%), Non-Hodgkin lymphoma in 5 cases (7.1%), Adenocarcinoma of lung in 2 cases (2.8%), Hepatocellular carcinoma in 1 case (1.4%), Soft tissue sarcoma in 6 cases (8.6%), Adenoid cystic carcinoma of salivary gland in 2 cases (2.8%), Carcinoma ex pleomorphic adenoma in 1 case (1.4%), Squamous cell carcinoma of oral cavity in 2 cases (2.8%), High grade serous carcinoma of ovary and Osteosarcoma 1 case each (1.4%).

Among 15 metastatic tumors, 14 (93.3%) cases were secondary tumor deposit in Lymph nodes and 1 (6.7%) was tumor deposit in liver.

### **FNA Cases interpreted as negative for malignant cells, n=75 (Benign and Inflammatory lesions with no atypical or malignant cells):**

Among 75 remaining cases including both benign and inflammatory lesions, Fibroadenoma in 7 cases (9.3%), Usual ductal hyperplasia in 3 cases (4.0%), Acute mastitis in 3 cases (4.0%), Benign phyllodes in 3 cases (4.0%), Colloid goiter/ Adenomatous goiter in 9 cases (12%), Hashimoto thyroiditis/ lymphocytic thyroiditis in 5 cases (6.7%), Reactive lymphadenitis in 14 cases (18.7%), Granulomatous lymphadenitis in 9 cases (12%), Necrotizing lymphadenitis in 5 cases (6.7%), Lipoma in 10 cases (13.3%), Spindle cell lesions in 4 cases (5.3%), Pleomorphic adenoma in 1 case (1.3%), Sialadenitis in 1 case (1.3%) and Warthin tumor in 1 case (1.3%).

Comparison of Benign and Inflammatory lesions with Malignant lesions based on microbiopsies is done [Table 1].

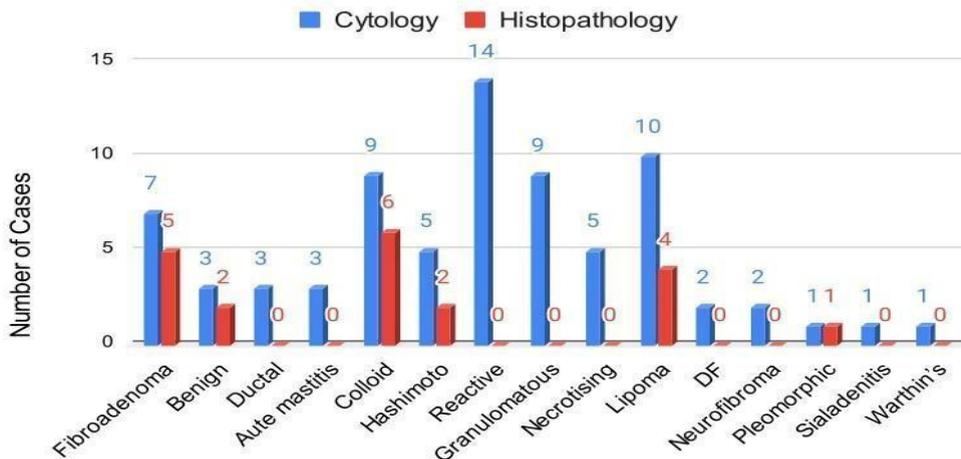
**Table 1: Comparison Between Benign, Inflammatory And Malignant Lesions Based On Microbiopsies.**

		Benign&Inflammatory lesions	Malignant	
		Number (n)	Primary Number (n)	Metastatic Number (n)
Conventional procedure	Lymph nodes	28	8	14
	Breast	16	30	0
	Soft tissue	14	6	0
	Salivary gland	3	3	0
	Oral cavity	0	2	0
	Bone	0	1	0
Image guided procedure	Thyroid	14	16	0
	Ovary	0	1	0
	Lung	0	2	0
	Liver	0	1	1

**FNA Cases with Cytological-Histological Correlation (n=100):**

Histopathological details were available in 100 cases. Out of 70 cases of primary malignant tumors in FNAC, 67 cases turned up for histopathological examination. Out of 16 cases of secondary deposits, 13 cases underwent histopathology examination. And out of 75 cases including both benign and inflammatory lesions, only 20 cases underwent histopathological examination. When compared, the histopathological diagnoses were same as those of the cytological diagnoses in all 100 cases. Cytological-Histological correlation of benign and inflammatory lesions is shown in Graph 1.

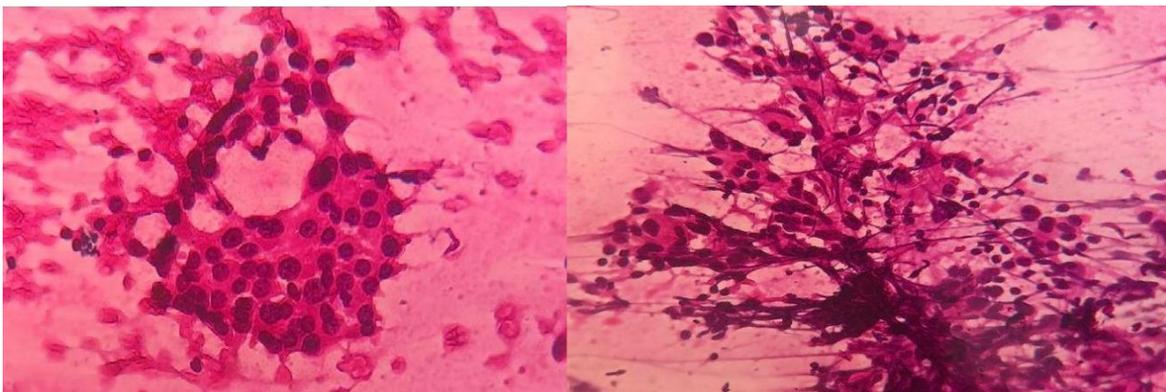
**Graph 1: Cytological-Histological Correlation of Benign lesions:**



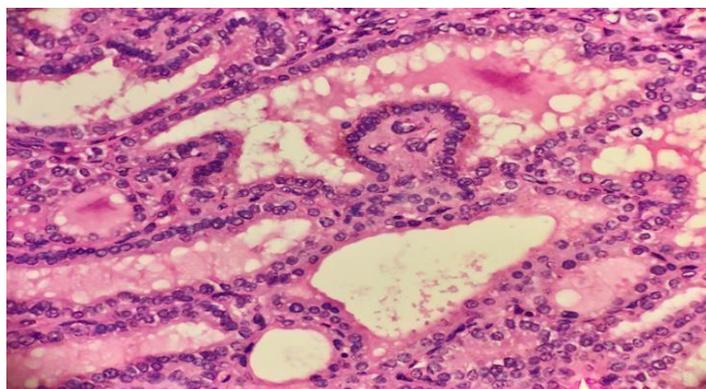
**Cytological-Histological Correlation of Malignant lesions (n=70):**

Out of 160 patients who had undergone FNAC, 70 cases were primary malignant tumors and out of which 67 cases had turned up for histopathological examination. Of all 30 cases diagnosed as Invasive ductal carcinoma in cytology were concluded as the same in HPE. All 16 cases of Papillary thyroid carcinoma in cytology were affirmed with the histopathological diagnoses. All 3 cases of Hodgkin lymphoma in cytology were concluded as the same in HPE and out of 5 cases of Non Hodgkin lymphoma in cytology, 2 were concluded as the same in HPE and rest 3 cases were not received for HPE. All 2 cases of Adenocarcinoma of lung, 1 case of Hepatocellular carcinoma, 6 cases of Soft tissue sarcoma, 2 cases of Adenoid cystic carcinoma, 1 case of Carcinoma ex pleomorphic adenoma, 2 cases of Squamous cell carcinoma of buccal mucosa, 1 case of High grade serous carcinoma of ovary and 1 case of Osteosarcoma had turned out to be the same in HPE.

FNA from a swelling in front of neck showed tissue fragments with malignant cells arranged in papillary pattern having vascular core. Tumor cells showed a distinct anatomical border, formed by columnar cells with nuclear crowding, nuclear grooving and overlapping with intranuclear vacuoles which was cytologically concluded as Papillary thyroid carcinoma (Fig. 1A). Subsequent histopathological examination showed malignant cells arranged in papillary pattern showing cytological nuclear features confirmed concordance with the cytological diagnosis (Fig.1B)<sup>6,7</sup>.

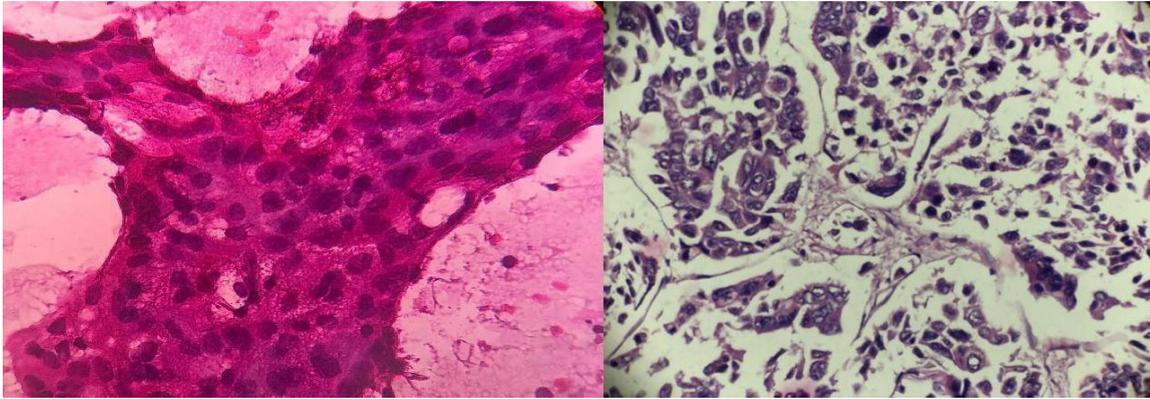


**Fig. 1A: Papillary thyroid carcinoma (Cytology), H&E under 400X (Left) and 100X (Right)**



**Fig. 1B: Papillary thyroid carcinoma (HPE), H&E under 400X**

FNA from breast lump showed tissue fragments comprised of tumor cells arranged in sheets, clusters and singles. Individual cells were large, polygonal with round to oval vesicular nuclei having prominent nucleoli and moderate amount of eosinophilic cytoplasm<sup>6</sup>. Subsequent HPE showed tumor cells arranged in glandular pattern and in nests showing pleomorphism with cytological atypia, concluded as Infiltrating Duct Carcinoma which affirmed with cytological diagnosis (Fig. 2A & 2B)<sup>7</sup>.



**Fig. 2A: Cytology image Infiltrating Ductal Carcinoma of breast (Cytology), H&E under 400X (Left)**

**Fig. 2B: Infiltrating Ductal Carcinoma of breast (HPE), H&E under 400X (Right)**

USG guided FNAC of an ovarian mass showed multilayered finger like papillae comprised of malignant glandular cell aggregates with psammoma bodies<sup>4</sup>, suggested as High grade serous carcinoma. This cytological diagnosis of ovary was found to be concordant with the histopathological diagnosis.

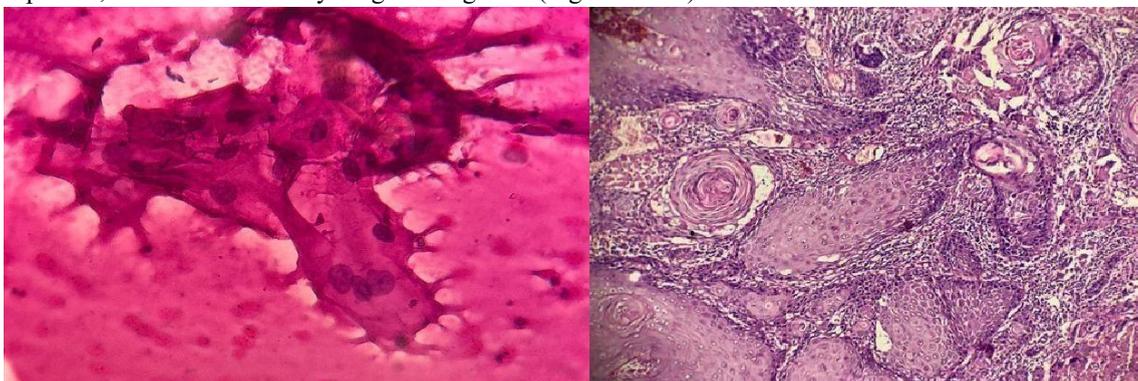
A case of a lung mass aspirate under USG guidance showed tissue fragments comprised of tumor cells arranged in glandular pattern, suggested as Adenocarcinoma. Subsequent histopathological examination showed malignant cuboidal epithelial cells arranged in glandular pattern, affirmed with the cytological diagnosis<sup>7</sup>.

FNAC of thigh swelling showed hypercellular smears comprised of large uniform cells arranged in discohesive clusters with round to oval vesicular nuclei showing prominent nucleoli. Areas of multinucleated tumor cells also noted, suggested as Soft tissue sarcoma. It was found to be concordant with the histopathology which was further typed as Rhabdomyosarcoma<sup>6,7</sup>.

A case of swelling over parotid region showed round to oval cells with hyperchromatic nuclei and coarse chromatin, dispersed and adhering to a large hyaline globule, cytologically suggested as Adenoid cystic carcinoma of parotid gland. This cytological diagnosis was found to be concordant with the histopathological diagnosis<sup>6</sup>.

Aspiration of lymph node in case of cervical lymphadenopathy, showed atypical mononuclear cells with scattered binucleated and few multilobated Reed-Sternberg cells. Background showed lymphocytes, eosinophils and histiocytes, suggested as Hodgkin lymphoma<sup>6</sup>, showed concordance with the histopathological diagnosis.

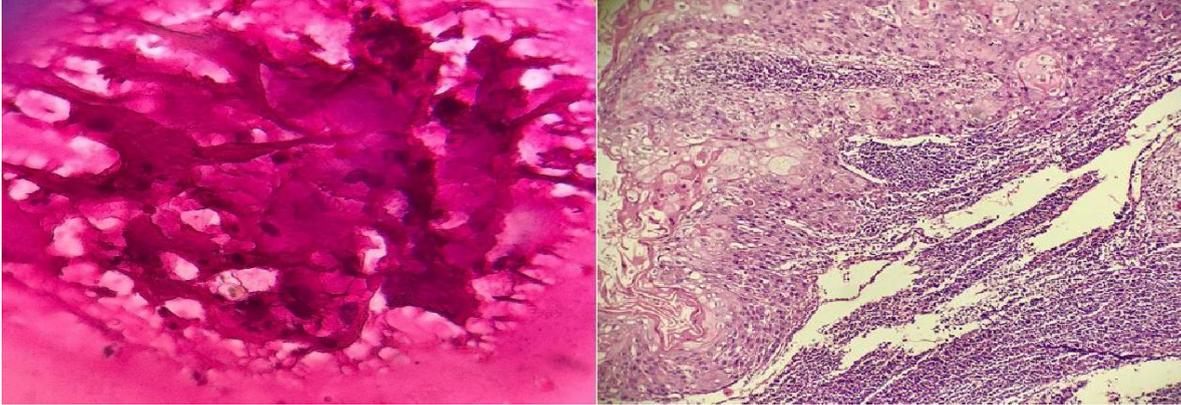
A case of ulcerative swelling in buccal mucosa showed dispersed keratinizing malignant cells and necrotic debris which was cytologically diagnosed as Squamous cell carcinoma<sup>6</sup>. Subsequent HPE showed keratinizing tumor cells arranged in sheets and nest with vesicular nuclei having prominent nucleoli and moderate amount of eosinophilic cytoplasm along with keratin pearls<sup>7</sup>, affirmed with the cytological diagnosis (Fig. 3A &3B).



**Fig. 3A: Squamous Cell Carcinoma (Cytology), H&E under 400X (Left)**

**Fig. 3B: Squamous Cell Carcinoma (HPE), H&E under 100X (Right)**

An aspirate from cervical lymph node showed malignant keratinizing squamous cells, concluded as secondary deposit of Squamous cell carcinoma which showed concordance with its HPE diagnosis<sup>8</sup>. (Fig. 4A & 4B).

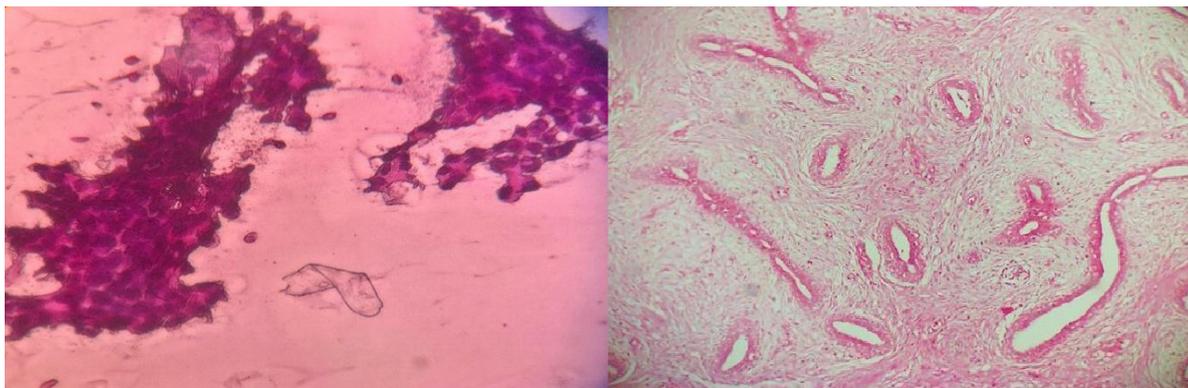


**Fig. 4A: Secondary deposit of Squamous Cell Carcinoma- Lymph node (Cytology), H&E under 400X (Left)**

**Fig. 4B: Secondary deposit of Squamous Cell Carcinoma- Lymph node (HPE), H&E under 100X (Right)**

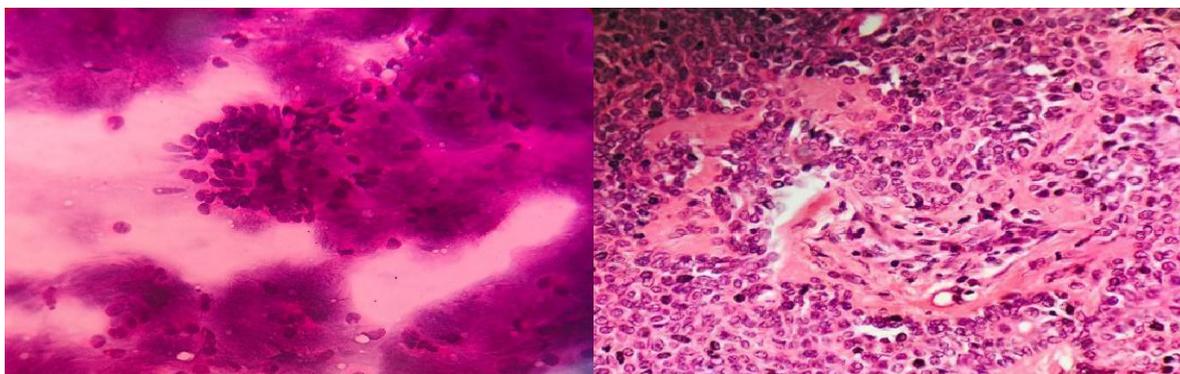
Another aspirate from axillary lymph node showed tumor cells in clusters and sheets with cytological atypia, concluding as metastatic deposit probably of Invasive carcinoma of breast. Subsequent HPE of lymph node showed tumor deposits, hence affirmed with the cytology.

Other cytologically diagnosed cases (from microbiopsies) like Fibroadenoma of breast, Pleomorphic adenoma of salivary gland, Multinodular goiter, Lipoma etc were also correlated with HPE showing concordance (100%). (Fig. 5A&B, 6A&B, 7A&B, 8A&B).



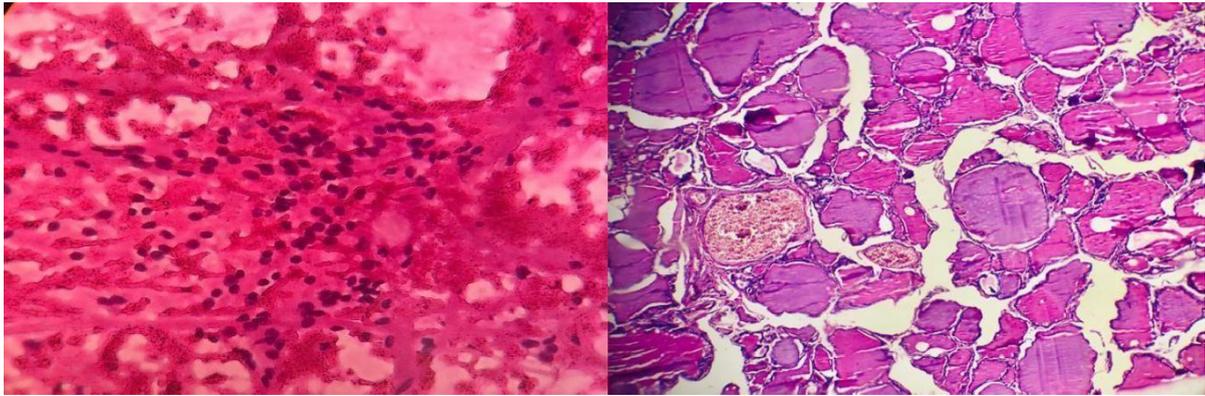
**Fig. 5A: Fibroadenoma (Cytology), H&E under 400X (Left)**

**Fig. 5B: Fibroadenoma (HPE), H&E under 100X (Right)**



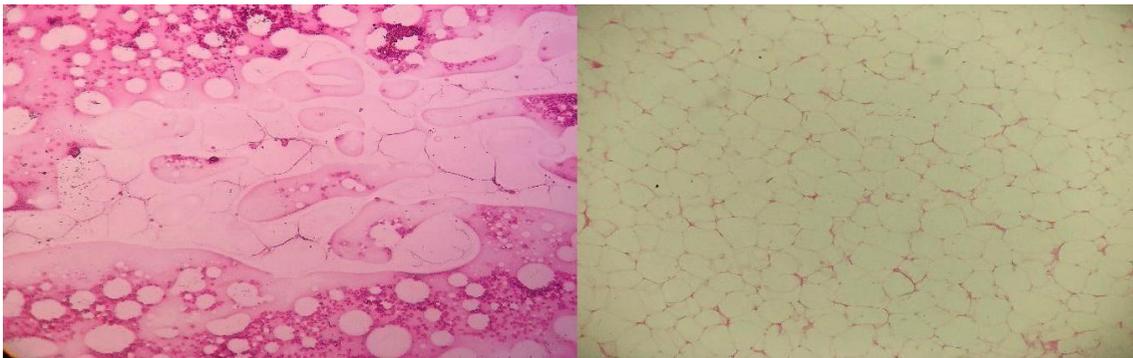
**Fig. 6A: Pleomorphic Adenoma (Cytology), H&E under 400X (Left)**

**Fig. 6B: Pleomorphic Aenoma (HPE), H&E under 400X (Right)**



**Fig. 7A: Colloid Goiter (Cytology), H&E under 400X (Left)**

**Fig. 7B: Colloid Goiter (HPE), H&E under 100X (Right)**



**Fig. 8A: Lipoma (Cytology), H&E under 100X (Left)**

**Fig. 8B: Lipoma (HPE), H&E under 100X (Right)**

### 3. DISCUSSION

Microbiopsies are difficult to be recognized while evaluating the smears in cytology in favour of other areas where cells are sparsely dispersed<sup>9</sup>. So careful examination of these tissue fragments thus provide important clues to the micro-histology of the lesions<sup>10</sup>.

Nosanchuk JS et al<sup>9</sup> and Verbeek DH et al<sup>2</sup> showed variety of processing techniques and evaluation of microbiopsies from the smears.

In this study, we applied our own procedure by examining all the smears containing tissue fragments and were considered microbiopsies.

Among these cases, many were followed up for histopathological examination and correlation between histopathological and cytological diagnoses were done, which was found to have p value <0.001 (i.e, statistically significant) [Table 2].

**Table 2: Correlation between Cytology and Histopathological Diagnoses:**

CYTOLOGY	HISTOPATHOLOGY			Total	Chi-Square, P-value
	Benign & Inflammatory lesions	Primary Malignant	Secondary Malignant		
Benign & Inflammatory (20)	20	0	0	20	
Primary Malignant (67)	0	67	0	67	35.340, <0.001
Secondary Malignant (13)	0	0	13	13	
<b>Total</b>				<b>100</b>	

Table 2: shows correlation between cytology and histopathology diagnoses with a p value of <0.001, which is statistically significant.

While assorting the different types of benign and inflammatory lesion diagnosed based on microbiopsies, Reactive lymphadenitis (18.7%) was found to be the most frequently diagnosed benign lesion in microbiopsies in our study, followed by Lipoma (13.3%), Granulomatous lymphadenitis and Colloid goiter/Adenomatous goiter (12%) each, Fibroadenoma (9.3%), Hashimoto thyroiditis/ lymphocytic thyroiditis and Necrotizing lymphadenitis (6.7%) each, Spindle cell lesions (5.3%), Usual ductal hyperplasia, Acute mastitis and Benign phyllodes (4.0%) each, Pleomorphic adenoma, Sialadenitis as well as Warthin tumor (1.3%) each.

While assorting the different types of malignancy diagnosed based on microbiopsies, Invasive ductal carcinoma of breast (43%) was found to be the most frequently diagnosed malignancy in our study, followed by Papillary carcinoma of thyroid (23%), Soft tissue sarcoma (8.6%), Non-Hodgkin lymphoma (7.1%), Hodgkin lymphoma (4.2%), Adenoid cystic carcinoma of Salivary duct (2.8%), Carcinoma ex pleomorphic adenoma (1.4%), Adenocarcinoma of lung and Squamous cell carcinoma of buccal mucosa (2.8%) each, Hepatocellular carcinoma, High grade serous carcinoma of ovary and Osteosarcoma each (1.4%).

In analysis of microbiopsies in breast, among benign and inflammatory lesions, showed Fibroadenoma in 7 cases (9.3%), Usual ductal hyperplasia 3 (4.0%), Acute mastitis 3 (4.0%) and Benign phyllodes 3 (4.0%). Our findings were correlated with Jaswanthini AR et al<sup>3</sup>, Saxena et al<sup>11</sup>, Ariga et al<sup>12</sup> and Iyer SP et al<sup>13</sup>. Whereas, among the malignant tumors, it showed Ductal carcinoma of breast was commonly reported constituting around 43% which again showed concordance with Jaswanthini AR et al<sup>3</sup>, Saxena et al<sup>11</sup>, Ariga et al<sup>12</sup> and Iyer SP et al<sup>13</sup>.

In analysis of microbiopsies in lymph node, among benign lesions and inflammatory lesions, showed Reactive lymphadenitis 14 (18.7%), Granulomatous lymphadenitis 9 (12%) and Necrotizing lymphadenitis 5 (6.7%). Whereas in Jaswanthini AR et al<sup>3</sup>, Granulomatous lymphadenitis was the most commonly diagnosed benign lymph node lesion in tissue microbiopsy.

Lymph nodes were the commonest sites for secondary tumor deposit with 14 cases (93.3%). Jaswanthini AR et al<sup>3</sup> and Mravunac et al<sup>14</sup> also found similar results in their studies. In lymph node metastatic tumors, primary site was mostly from oral cavity with Squamous cell carcinoma in our study which was again concordance with Jaswanthini AR et al<sup>3</sup> and Hirachand S et al<sup>15</sup>.

In our study, liver showed Hepatocellular carcinoma as primary in 1 (1.4%) whereas secondary deposit in 1 (6.7%) in concordance with Sherwani RK et al<sup>6</sup>.

Spencer JA et al<sup>16</sup> in his study showed how the diagnosis of ovarian malignancies was important preoperatively.

Diagnosing an ovarian neoplasm is a diagnostic challenge for the pathologists as it is the fluid that is mostly aspirated and then commenting if it is a benign or a malignant is quite difficult<sup>1</sup>. But if a tissue core is aspirated in USG guided FNAC, it would definitely help in providing the proper architecture of tumor and its cytomorphology, hence aiding a diagnosis.

In our study we had a case of ovarian mass, on which we did guided FNA and diagnosed as High grade serous carcinoma in cytology as well as in HPE.

In correlation of cytology and histopathology in 100 cases, we had Invasive ductal carcinoma of breast (30%), Papillary carcinoma of thyroid (16%), Hodgkin lymphoma (3%), Non-Hodgkin lymphoma (2%), Adenocarcinoma of lung (2%), Hepatocellular carcinoma of liver (1%), Soft tissue sarcoma (6%), Adenoid cystic carcinoma of salivary duct (2%), Carcinoma ex pleomorphic adenoma (1%), Squamous cell carcinoma of buccal mucosa (2%), High grade serous carcinoma of ovary (1%), Osteosarcoma (1%), metastatic Squamous cell carcinoma deposit in Lymph nodes (11%), Infiltrating ductal carcinoma deposit in lymph node (1%) and Renal cell carcinoma deposit in liver (1%). Fibroadenoma (5%), Benign phyllodes (2%), Colloid goiter /Adenomatous goiter (6%), Hashimoto thyroiditis/ lymphocytic thyroiditis (2%), Lipoma (4%) and pleomorphic adenoma (1%).

#### 4. LIMITATION OF THE STUDY

Small sample size and all the cases did not have histopathology correlation.

#### 5. CONCLUSION

The scope of FNAC as a diagnostic tool of benign as well as malignant lesions is increasing by time. Microbiopsies by FNAC, provide additional information on the tissue architecture, thus aiding in diagnosis with tumor typing. Hence whatever be the type of FNAC, whether conventional or guided (CT or USG), fine needle aspirate with tissue microbiopsy is an effective tool in evaluating and diagnosing suspected lumps or masses.

There are certain pitfalls however exist in conventionally assessed FNA smears as they do contain very little amount of tissue material, hence this relative absence of recognizable tissue architecture in FNA smears often makes diagnoses very difficult especially to recognize undifferentiated tumor is a carcinoma, sarcoma etc. In these cases, cytology can provide a differential diagnosis rather than a conclusive diagnosis. Hence, in this study, application of cytology in correlation with histopathology has helped in arriving at the definitive diagnosis in such cases.

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