



## Diagnosing Breast Lesions by Cell Pattern Analysis On FNAC

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### Abstract

**Introduction:** Fine needle aspiration cytology (FNAC) is a simple, safe, cost-effective, reliable, technique for preliminary evaluation of all the palpable breast lumps. FNAC helps in early diagnosis of malignancy so that appropriate management can be done for the patient.

**Aims:** To study the role of cell pattern analysis in diagnosing breast lesions and to evaluate diagnostic accuracy of breast FNAC.

**Material and methods:** The present study is a retrospective evaluation of 70 cases of breast lesions diagnosed on FNAC and their histopathological correlation over a period of two years in a Tertiary care center. Aspirates were grouped into six different categories depending on their predominant cellular pattern on FNAC. Cytological diagnosis was based on systematic pattern analysis which included: - Benign, Atypical, Suspicious and Malignant.

FNAC diagnosis given on pattern analysis was correlated with histopathological diagnosis. The sensitivity, specificity, Positive predictive value and Negative predictive value were calculated.

**Results:** This present study of breast lesions by cell pattern analysis on FNAC showed sensitivity of 95%, specificity of 100%, Positive predictive value 100%, Negative predictive value 98.03 % and diagnostic accuracy of 98.57%.

**Conclusion:** Cell pattern analysis in diagnosing breast lesions will play a significant and crucial role in reducing false negative diagnosis on FNAC

**Keywords:** Breast lesions, cell pattern analysis, FNAC, histopathology

### Introduction

Fine needle aspiration cytology (FNAC) was introduced by Martin and Ellis at Memorial Hospital USA in the year 1930. <sup>(1)</sup> FNAC is a simple, safe, cost-effective, reliable, technique for initial evaluation of all the palpable breast lumps. It is minimally invasive and equally sensitive as a biopsy. <sup>(2-4)</sup> The overall accuracy of FNAC in diagnosis of breast lesion in recent studies is reported to be around 99% <sup>(5)</sup>. FNAC helps in early diagnosis of malignancy so that appropriate management can be done for the patient.

Many studies are published on various method for diagnosing breast lesions. In present study emphasis is given on the systematic pattern analysis of the various breast lesions by broadly classifying them into three major categories i.e., cellular pattern, background and any other additional features if present. FNAC is very useful in diagnosis and accordingly further planning the management, it can also help in prognostication of the tumor factors like mitotic index, nuclear grading, hormone receptors status and DNA contents <sup>(6)</sup>.

Primary screening tests like self-breast examination has helped in growing the awareness in community and thus the number of cases presenting with breast lump has increased over a period of time making it possible for early diagnosis and treatment of the breast lesions<sup>(7)</sup>. Many of these lesions are benign<sup>(8)</sup>.

Molecular ancillary techniques like IHC, DNA pattern analysis can be performed on FNAC smears, and with the help of USG guidance ample amount can be aspirated from the significant (appropriate) lesion thus assisting to increase the diagnostic accuracy<sup>(9)</sup>.

FNAC helps to differentiate the malignant lesions from the benign, thus helping the surgeon to plan for an appropriate treatment regime in case of malignant lesions and follow a conservative approach for the benign lesions. Therefore, FNAC diagnosis based on pattern analysis and co-relation with radiological and clinical findings is useful in the preoperative assessment of various breast lesions.

The main aim was to study the role of cell pattern analysis in diagnosing breast lesions and evaluation of diagnostic accuracy of breast FNAC.

By identifying the various cellular patterns by cytologists will help to reduce the inter-observer variations in diagnosing breast lesions.

### Materials and Methods:

The present retrospective study included 70 females who presented with breast lump, having both cytological and histopathological correlation. The study was conducted over a period of 1.5 years from July 2018- December 2019 after getting ethical clearance from the Institutional Ethical Committee. Females below the age of 10 years and FNAC cases with inadequate material were excluded from this study.

All the FNAC were performed in the Cytology section of department of Pathology. Before FNAC, consent from the patient was obtained after explaining the procedure and its related complications. The detailed clinical history of patient and physical examination of both breasts i.e., size, number, mobility along with skin, nipple, areolar changes along with examination of both axillary regions for enlarged lymph node were performed.

FNAC procedure was done using 22-gauge needle attached to 10cc BD disposable syringe. Sample were smeared onto glass slides and fixed, wet fixed smears were stained with Papanicolaou stain, while smears which were air-dried stained with May-Grunwald-Giemsa stain.

All cystic lesions were aspirated completely and reassessed for any residual lump. If any residual lump was present re-aspiration was performed. The cystic fluid was centrifuged and the smears were prepared from the sediment.

Specimens received for histopathological examinations were grossed and tissue section were stained with Hematoxylin and Eosin (H and E).

All cytological smears were reviewed and evaluated for the different cellular patterns Biphasic, neutrophil rich, macrophage rich, spindle cells, round cell, pleomorphic, along with background and other cellular features by the cytopathologist who was blind to the original cytological diagnosis (Table 1).

The final cytological diagnosis was given in the following categories:

- 1) Benign
- 2) Atypical
- 3) Suspicious for malignancy
- 4) Malignant

The cytological diagnosis was co-related with the final histopathological diagnosis. The Specificity, Sensitivity, Positive predictive value, Negative predictive value and diagnostic accuracy were calculated after the tabulation of data.

### Results:

There were total 70 cases of breast lesions, the age ranged from 12 to 84 years; mean age 36.

Table no.2: Maximum cases were seen in the age group of 31- 40 (29 cases, 42.42 %), followed by age group of 21- 30 (10 cases,14.28 %). Benign breast lesions were commonly seen in the age group of 31- 40 years, whereas malignant lesions were seen in the age group of 31-50.

Left breast was more commonly involved with 38/70 cases (54.28 %), and the lump was predominantly seen in upper outer quadrant i.e., 40 cases (57.14%).

<b>Table 1: List of various patterns used in this study.</b>	
<b>Patterns</b>	<b>Observations</b>
<b>Cellular pattern:</b>	
Biphasic	<ul style="list-style-type: none"> <li>• Sheets and aggregates of cohesive small uniform epithelial cells</li> <li>• Elongated branching fragments of duct epithelial cells</li> <li>• Scattered small dark nuclei of myoepithelial cells</li> </ul>
Neutrophil-rich	<ul style="list-style-type: none"> <li>• Plenty of neutrophils, histiocytes</li> <li>• Biphasic cells</li> <li>• Giant cells</li> </ul>
Macrophage-rich	<ul style="list-style-type: none"> <li>• Plenty of cyst macrophages</li> <li>• Biphasic cells</li> <li>• Apocrine cells</li> </ul>
Spindle cell pattern	<ul style="list-style-type: none"> <li>• Atypical spindle cells</li> </ul>
Small round cell pattern	<ul style="list-style-type: none"> <li>• Monotonous small round cells with scanty cytoplasm</li> </ul>
Pleomorphic cell pattern	<ul style="list-style-type: none"> <li>• Cell-rich smears</li> <li>• Atypical cells – irregular clusters and single cells</li> <li>• No myoepithelial cells</li> <li>• No bare bipolar nuclei</li> </ul>
<b>Background:</b>	
Myxoid	<ul style="list-style-type: none"> <li>• Fragments of loosely fibromyxoid stroma which stain pink or magenta with MGG</li> </ul>
Hemorrhagic	<ul style="list-style-type: none"> <li>• Highly haemorrhagic</li> </ul>
Necrotic	<ul style="list-style-type: none"> <li>• Necrotic debris</li> </ul>
Proteinaceous	<ul style="list-style-type: none"> <li>• Protein rich background</li> </ul>
<b>Other features:</b>	
Bipolar nuclei	<ul style="list-style-type: none"> <li>• Variable numbers of single, bare, bipolar nuclei</li> </ul>
Apocrine cells	<ul style="list-style-type: none"> <li>• Sheets of duct epithelial cells with apocrine change (with abundant dense finely granular eosinophilic cytoplasm)</li> </ul>
Multinucleated giant cells	<ul style="list-style-type: none"> <li>• Multinucleated giant cells</li> </ul>
Dyshesion	<ul style="list-style-type: none"> <li>• Loss of cohesion</li> </ul>
Nuclear features	<ul style="list-style-type: none"> <li>• Regenerative epithelial atypia</li> <li>• Nuclei exhibiting increased N:C ratio, irregular nuclear membrane and chromatin irregularities.</li> </ul>

**Table 2: Age distribution according to the lesions based on pattern analysis.**

Age group	Benign	Atypical	Suspicious for malignancy	Malignant	Total
11 – 20	10	0	0	0	<b>10</b>
21 – 30	12	0	0	1	<b>13</b>
31 – 40	22	2	2	3	<b>29</b>
41 – 50	6	1	0	4	<b>11</b>
51 – 60	1	0	0	1	<b>2</b>
61 – 70	0	0	1	1	<b>2</b>
71 – 80	0	0	0	2	<b>2</b>
81 – 90	0	0	0	1	<b>1</b>
<b>Total</b>	<b>51</b>	<b>3</b>	<b>3</b>	<b>13</b>	<b>70</b>

**Table 3: Distribution of breast lesions based on cytological diagnosis**

Cytological diagnosis	Number of cases
Benign	51
Atypical	3
Suspicious for malignancy	3
Malignant	13

**Table 4: Distribution of cases based on their patterns with cyto-histological correlation**

Group	Cellular pattern	Background and other features	Cytological diagnosis	Histopathological diagnosis
I (43)	Biphasic	Myxoid background	Benign	Fibroadenoma (35) Fibroadenoma with fibrocystic change (4)
		Haemorrhagic background		Fibroadenoma (3) Sclerosing adenosis (1)

II (4)	Neutrophil rich	Necrosis and Reactive atypia Necrosis and Histiocytes	Benign	Acute mastitis (1) Granulomatous mastitis (3)
III (5)	Macrophage rich	Haemorrhagic background and nuclear features	Atypical	Atypical ductal hyperplasia (1)
		Cohesive and bipolar nuclei Proteinaceous, apocrine Apocrine cells	Benign	Fibroadenoma with cystic change (2) Fibrocystic disease (1) IDC (1)
IV (2)	Spindle cell pattern	Necrosis and pleomorphism	Malignant	Leiomyosarcoma (1)
		Haemorrhage and atypical spindle cells	Atypical	Undifferentiated pleomorphic sarcoma with multinucleated giant cells (1)
V (1)	Small round cell pattern	Nuclear features and haemorrhage	Malignant	IDC (1)
VI (15)	Pleomorphic cell pattern	Atypical cells, haemorrhage and necrosis	Suspicious	IDC (3)
		Cohesive group of atypical cells and necrotic debris	Atypical	DCIS (1)
		Haemorrhage, dyshesion and pleomorphic nuclear features Necrosis and atypical cells	Malignant	IDC (9) IDC (2)

**Table 5: Statistical comparison of present study with other studies**

Study	Sensitivity %	Specificity %	PPV %	NNV %	Accuracy %
<b>Present study</b>	<b>95</b>	<b>100</b>	<b>100</b>	<b>98.03</b>	<b>98.57</b>
Muddegowda PH et al <sup>(10)</sup> (2011)	<b>94.50</b>	98	95.80	<b>97.40</b>	97
Alema ON et al <sup>(11)</sup> (2012)	85.29	<b>100</b>	<b>100</b>	<b>98.79</b>	<b>98.70</b>
Hebbar AK et al <sup>(12)</sup> (2013)	93.10	<b>100</b>	<b>100</b>	90.47	<b>98</b>
Sankaye SB et al <sup>(13)</sup> (2014)	88.37	96.4	97.43	84.37	91.54
Farhath SSK et al <sup>(14)</sup> (2016)	83.33	<b>100</b>	<b>100</b>	<b>97.77</b>	-
Pandey A et al <sup>(15)</sup> (2017)	98.30	98.90	98.30	<b>98.90</b>	<b>98.70</b>

Edwin IA et al <sup>(16)</sup> (2017)	100	93.55	95.65	100	-
Banik T et al <sup>(17)</sup> (2018)	87.23	<b>100</b>	<b>100</b>	94.87	96.05
Saldanha P et al <sup>(5)</sup> (2020)	92.9	<b>100</b>	<b>100</b>	97.2	<b>99</b>

Table 3: Out of the total 70 cases: 51 were benign, 3 atypical, 3 suspicious and 13 malignant. Table 4:

On histopathological examination, the most common benign lesion was fibroadenoma (61.42%) and the most common malignant lesion was infiltrating duct carcinoma (22.85%).

The breast lesions were classified as per the patterns on FNAC and its diagnostic accuracy was evaluated.

Group I – 43 cases showed biphasic pattern and most common diagnosis was fibroadenoma and with one case of sclerosing adenosis.

Group II – Total four cases presented with neutrophilic-rich pattern. Most common diagnosis was granulomatous mastitis (three cases) which were reported as abscess on cytology.

Group III – Total five cases were reported with macrophage-rich pattern. The diagnosis included fibroadenoma with cystic change (two cases), fibrocystic disease (one case), atypical ductal hyperplasia (one case) and one cases of IDC which was reported as fibrocystic change on aspirate.

Group IV – Comprised of spindle cell pattern. We reported one case of Leiomyosarcoma and one case of undifferentiated pleomorphic sarcoma with multinucleated giant cells.

Group V – Single case with small round cell pattern was reported as IDC.

Group VI – This group comprised of pleomorphic cell pattern where all the 15 cases were diagnosed malignant on aspirate. The most common diagnosis was Infiltrating duct carcinoma.

The diagnostic accuracy of this study was 98.57% with sensitivity of 95%, specificity of 100%, positive predictive value 100% and negative predictive value of 98.03%.

### Discussion:

FNAC is a useful tool for preoperative evaluation of breast lumps. This simple and cost-effective method

helps in early diagnosis and further treatment of various breast lesion. Accurate diagnosis of breast lesions on FNAC depends upon adequacy of sample and correlation of cytomorphological features with clinical details of the patient. However, diagnostic efficacy of breast FNAC can be increased by studying various morphological attributes of aspirates which includes cellular patterns, cellular features and background material. Predominant pattern analysis helps a cytopathologist to narrow down differential diagnosis of breast lesions and its correlation with cellular features and background material helps to give final cytology diagnosis.

In present study, maximum cases were seen in the age group of 31-40 (29 cases/41.42%) wherein, 22 cases were categorized as benign, two cases as atypical, one case as suspicious, and two cases as malignant. The most common benign lesion in this study was fibroadenoma (62.85%), whereas commonest malignant lesion was infiltrating duct carcinoma (25.71%).

Amongst all the various patterns biphasic, neutrophil rich and macrophage rich patterns were commonly seen in benign lesions whereas, small round cell, pleomorphic and spindle cell pattern were commonly seen in malignant lesions.

Biphasic pattern: It includes bimodal population of cells comprising of epithelial and myoepithelial cells arranged in monolayered sheets, clusters and antler-horn pattern. The presence of myoepithelial cells indicates benign nature of the lesion<sup>(18)</sup>. In the present study 43 (61.42%) cases were showing biphasic pattern, out of which 39 cases (55.71%) predominantly showed myxoid background and four cases showed haemorrhagic background. On histopathological diagnosis 35 cases were diagnosed as fibroadenoma and four cases as fibroadenoma with fibrocystic disease. We also observed four cases with haemorrhagic background which were diagnosed as fibroadenoma (three cases) and sclerosing adenosis (one case).



These findings are well correlated with the observations made by Muddegowda PH *et al.*,<sup>(10)</sup> which showed biphasic pattern in 78 cases (34.66%) wherein, 76 cases presented with predominant myxoid background which were diagnosed as fibroadenoma (71 cases) and benign phyllodes (six cases) on histopathological examination.

The study done by Jibhkate SN *et al.*,<sup>(19)</sup> also showed similar findings where biphasic pattern was seen in 165 cases (59.78%) and myxoid or mucinous background was seen in 164 cases. On histopathological examination out of 156/164 cases were diagnosed as fibroadenoma.

**Neutrophilic-rich pattern:** Cytological features of this pattern includes plenty of neutrophils along with lymphocytes, plasma cells, macrophages and apocrine cells. Singly scattered and clusters of epithelial cells with varying degrees of reactive atypia are also seen. In this study, all the four cases presenting with this pattern were benign on cytology as well as on histopathology. One case which was diagnosed as acute suppurative mastitis on FNAC showed concordance with histopathological diagnosis. Three cases diagnosed as granulomatous mastitis were called abscess on FNAC. The study done by Muddegowda PH *et al.*,<sup>(10)</sup> also showed abscess and mastitis as common diagnoses for neutrophil-rich pattern.

The cytological features of granulomatous mastitis are necrosis, epithelioid histiocytes, granulomas, giant cells and abundant neutrophils in the background<sup>(20)</sup>. Diagnosis of Granulomatous mastitis is often misdiagnosed as breast abscess when there are plenty of neutrophils on a necrotic background. The closest differential diagnosis for non-specific granulomatous mastitis is tuberculous mastitis. As tuberculosis is more prevalent in India, it is always advised to do Ziehl-Neelsen staining for acid-fast bacilli in these cases<sup>(20,21)</sup>. For definitive diagnosis of idiopathic granulomatous mastitis (IGM) one may require confirmation by histopathological examination, negative microbiological investigation with clinical correlation. As IGM is a diagnosis of exclusion, all other causes of granulomatous inflammation need to be ruled out by performing special test like AFB (TB bacilli), PCR (TB/ atypical micro bacteria), PAS (fungus) and Gram stain (bacterial) etc.<sup>(19,20)</sup>.

**Macrophage-rich pattern:** This pattern was seen in fibroadenoma with cystic change and fibrocystic disease. One case which was diagnosed as fibrocystic disease on cytology was reported as IDC on histopathology. This may be due to FNAC performed from non-representative area<sup>(13)</sup>. Similar observation was seen by Banik T *et al.*,<sup>(17)</sup> in their study, where they reported two cases of fibrocystic disease on cytology which were diagnosed as IDC on histopathology.

**Spindle cell pattern:** The common differential diagnosis for spindle cell pattern includes malignant phyllodes tumor, fibrosarcoma, malignant fibrous histiocytoma, metaplastic carcinoma and undifferentiated pleomorphic sarcoma. This pattern is characterized by presence of atypical spindle cells with plenty of mitotic figures<sup>(10)</sup>.

We reported two cases of spindle cell pattern, one case was diagnosed as leiomyosarcoma and the other was diagnosed as undifferentiated pleomorphic sarcoma with multinucleated giant cells. Leiomyosarcoma on cytology showed hypercellularity, singly scattered and clusters of malignant spindle cells exhibiting marked nucleomegaly and prominent nucleoli with few cells having bizarre nuclei.

Primary breast sarcomas are rare neoplasms with incidence of <1% of breast malignancy<sup>(21)</sup>. Because of the overlapping cytomorphological features of different malignant neoplasms (malignant phyllodes tumor, metaplastic carcinoma, pleomorphic breast carcinoma and melanomas) and its rarity it is difficult to diagnose primary sarcoma of breast on FNAC<sup>(21)</sup>. In this study due to paucicellularity of smears in one case we offered a diagnosis of spindle cell lesion with atypical features which was diagnosed as Undifferentiated pleomorphic sarcoma with multinucleated giant cells.

Chakrabarti I *et al.*,<sup>(21)</sup> reported a cases of undifferentiated high grade pleomorphic sarcoma of breast in a 60-year-old female patient. This patient also presented with brain metastasis (cerebellar) which is an important factor of mortality and morbidity.

**Small round cell pattern:** Differential diagnosed in this pattern includes malignant lesions like tubular carcinoma, mucinous carcinoma, lobular carcinoma,

Images

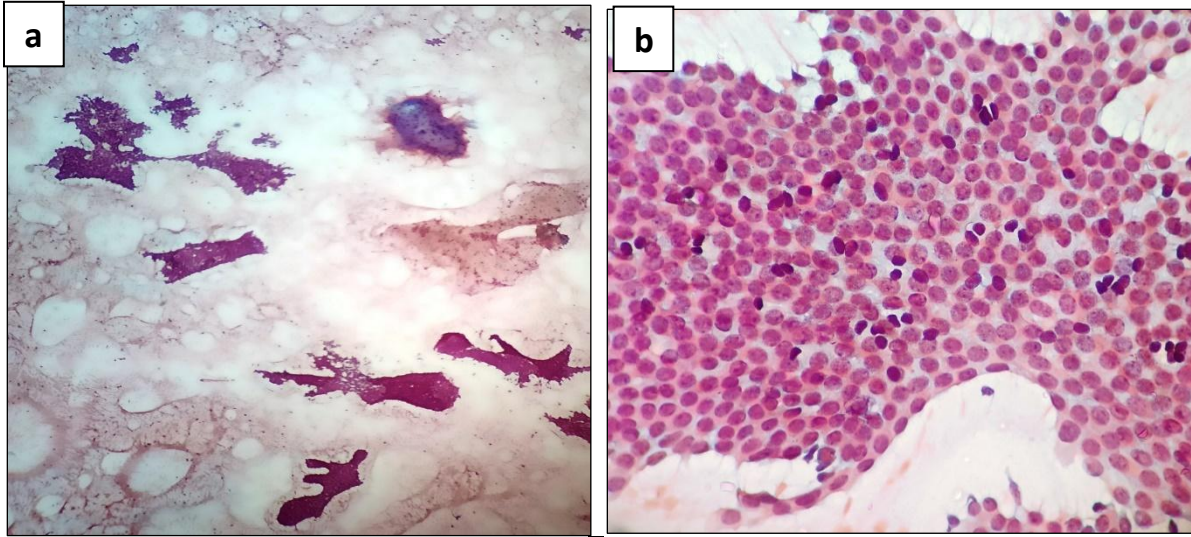


Figure no 1: Biphasic pattern a) PAP x100 b) PAP x400

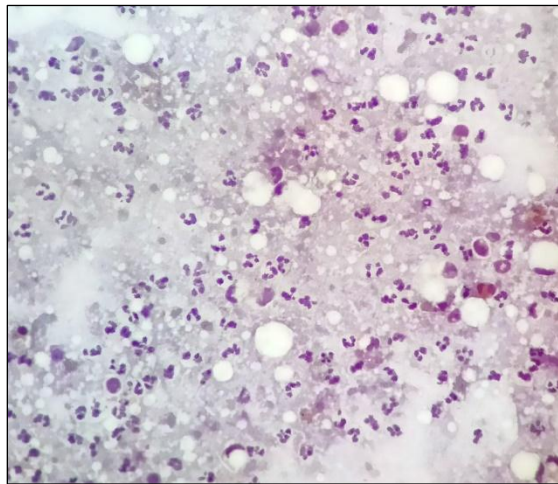


Figure no 2: Neutrophil rich pattern PAP x100

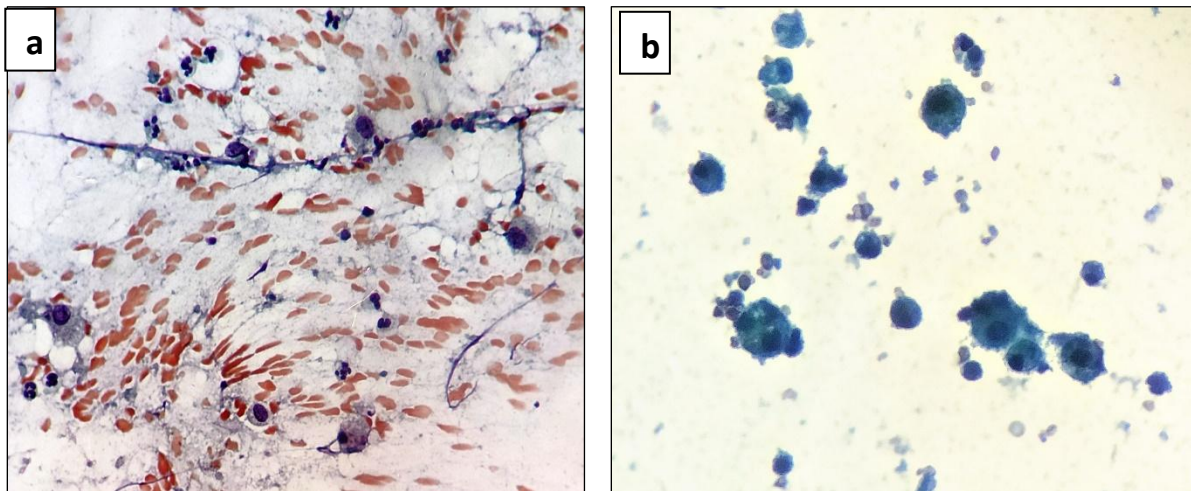


Figure no 3: Macrophage rich pattern a) PAP x100 b) MGG x400



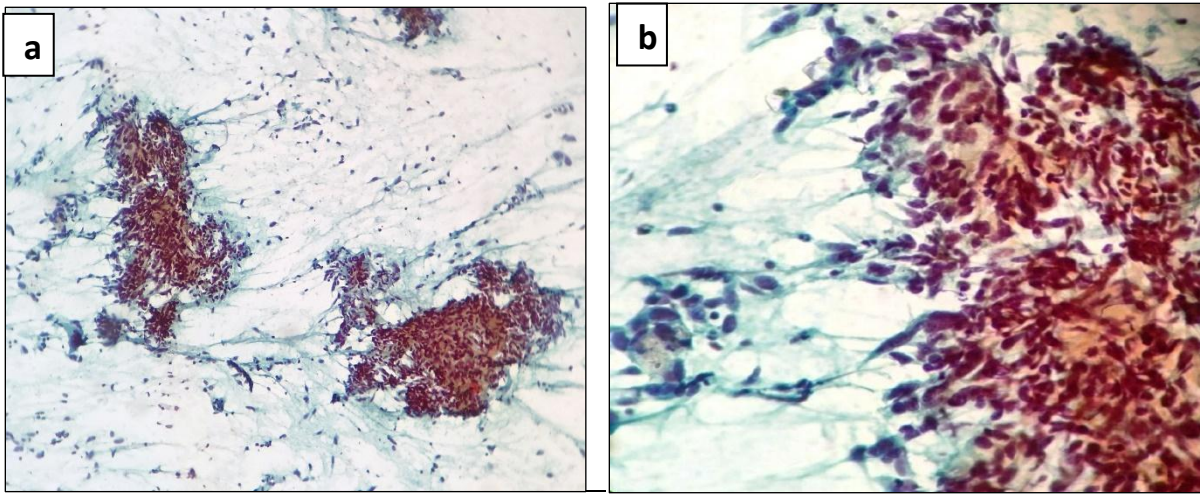


Figure no 4: Spindle cell rich pattern a) PAP x100 b) PAP x400

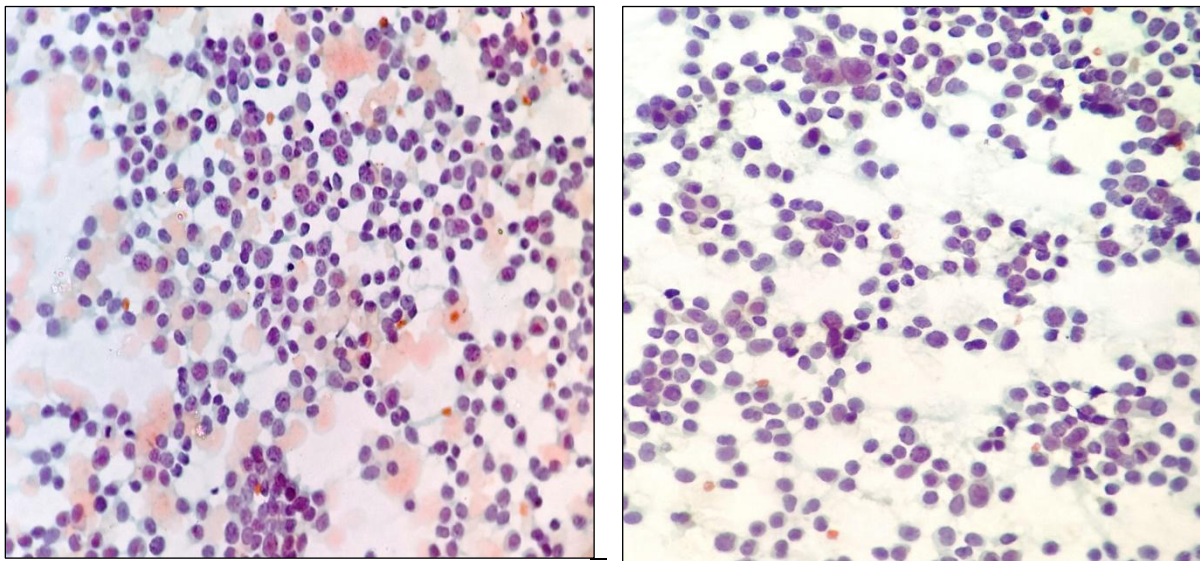


Figure no 5: Small round cell pattern PAP x100

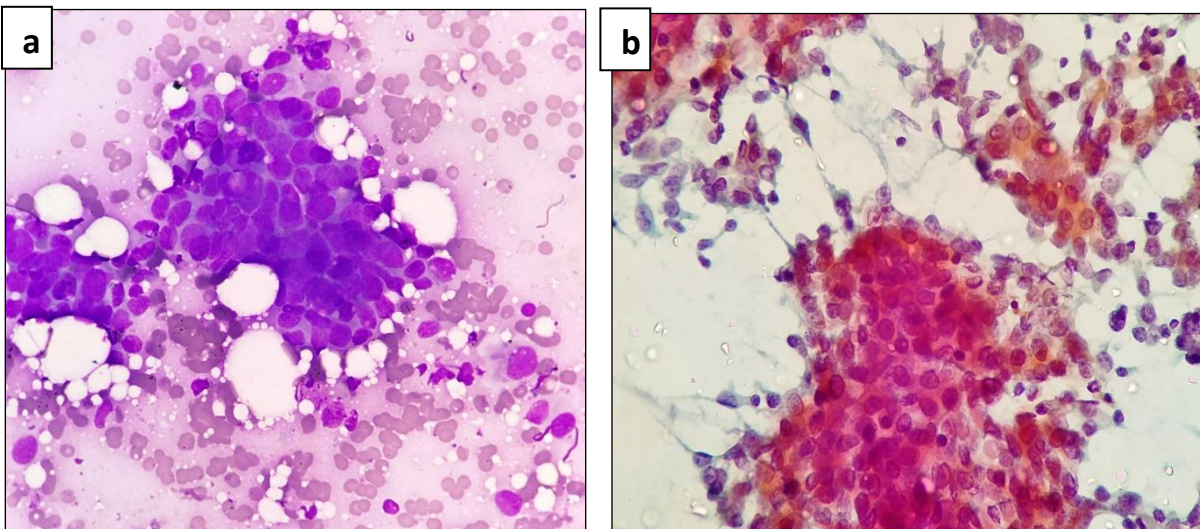


Figure no 6: Pleomorphic cell pattern a) MGG x100 b) PAP x400

adenoid cystic carcinoma, lymphoma and metastatic small round cell tumor, benign lesions like fibroadenoma, mucocele and adenosis.

A case of IDC was diagnosed as small round cell tumor on cytology. The cytological smears revealed hypercellularity, dyshesive cells with monomorphic nuclei.

**Pleomorphic pattern:** In this study we reported 15 cases with pleomorphic pattern where 14 cases were of infiltrating duct carcinoma and one case of DCIS. Pleomorphic pattern on cytology is usually diagnosed as malignant lesion, however benign lesions can also present with pleomorphism like fibroadenoma, fibrocystic disease and fat necrosis.

In our study the diagnostic accuracy was 98.57%, which was similar to the study done by Pandey A et al, and Alema ON et al, <sup>(11,15)</sup>. In other studies, the accuracy ranged from 91.54% to 97% (Table 5). The sensitivity and specificity were 95% and 100% respectively. Study done by Muddegowda PH et al, <sup>(10)</sup> showed similar sensitivity of 94.5 % and specificity of 98%. The studies done by Banik T et al., Saldanha P et al., Alema ON et al., and Farhath SSK et al., <sup>(5,11,14,17)</sup> all showed specificity of 100% which was similar to our study. The PPV and NPV were 100% and 98.03% respectively. These findings were similar to the findings observed by Alema ON et al., <sup>(11)</sup>. Other authors also showed 100% PPV in their studies <sup>(12,14,17)</sup>.

We observed Positive predictive value (PPV) of 100% which was concordance with the studies done by Farhath SSK et al., Alema ON et al., Hebbar AK et al., and Banik T et al., <sup>(11,12,14,17)</sup>. The Negative predictive value (NPV) 98.03% was similar to the observation found by Alema ON et al., and Pandey A et al., <sup>(11,15)</sup>.

### Conclusion:

FNAC is a simple, sensitive, less time consuming and cost-effective technique for evaluation of breast lesions with high specificity & accuracy. FNAC based on pattern analysis along with radiological and clinical examination is useful in the preoperative assessment of various breast lesions and will act as a valuable tool for surgeons in further management of the patients. FNAC in this study had accuracy, sensitivity & specificity rate of 98.57%, 95% & 100% respectively. Diligent examination of all the

cytological smears for various patterns, cellular features and background details will play a crucial role in minimizing false positive and false negative diagnosis on breast FNAC.

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