ISSN: 0976-3031

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

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 11, Issue, 12 (D), pp. 40415-40422, December, 2020

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

A CORRELATION STUDY OF FINE NEEDLE ASPIRATION CYTOLOGY AND HISTOPATHOLOGY OF BREAST LESIONS: AN EXTENSIVE STUDY OF 636 CASES

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DOI: http://dx.doi.org/10.24327/ijrsr.2020.1112.8691

ARTICLE INFO

Article History:

Received 4th September, 2020
Received in revised form 25th October, 2020
Accepted 23rd November, 2020
Published online 28th December, 2020

Key Words:

Breast lump, Fibroadenoma, Fibrocystic disease, Fine Needle Aspiration Cytology (FNAC), Malignancy

ABSTRACT

Context: Breast carcinoma is the most common malignant neoplasm.Fine needle aspiration cytology (FNAC) of breast lumps is an important part of triple assessment (clinical examination, imaging, and FNAC) of palpable breast lumps.

Aims:

1. To test the utility of F.N.A.C. in clinically suspected breast lesions
2. To classify benign and malignant lesions and subclassify them whenever possible on cytological grounds.
3. To establish the sensitivity and specificity of F.N.A.C. in diagnosing breast lesions.
4. To know the efficiency of cytological examination by correlating it with histopathological examination

Settings and Design: *A prospective study.*

Methods and Material: FNAC was done in all cases with palpable breast lump and wherever enlarged lymph node was found. The slides were fixed in methanol and staining was done with H & E stain, Giemsa stain, PAP stain and AFB wherever suspected for TB.

The cytological diagnosis was classified as benign, suspicious of malignancy & malignant. Later was subjected to excisional biopsy or mastectomy and histopathological confirmation. Later diagnostic accuracy of cytology reporting was compared with that of histopathology.

Statistical analysis used: sensitivity, specificity and positive/ negative predictive values were determined.

Results: Cytological and histological correlation found in 172 cases (97.72%) out of 176 cases. Accuracy of the present study as: sensitivity 95.75%, specificity 98.45%,positive predictive value 95.75%, negative predictive value 98.45% and efficiency 97.72%.

Conclusions: FNAC serves as a rapid, highly sensitive, economical, and reliable tool for the diagnosis of palpable breast lesions and serves as an important diagnostic modality in early detection of suspicious cases.

INTRODUCTION

Breast carcinoma is the most common malignant neoplasm and the leading cause of death from cancer in women, with more than 1million cases occurring worldwide annually.[1]Most of the breast lesions produce palpable lumps associated/ unassociated with other symptoms. Fine needle aspiration cytology (FNAC) of breast lumps is an important part of triple assessment (clinical examination, imaging, & FNAC) of palpable breast lumps.[2,3] FNAC is sensitive, simple, cost-effective, less traumatic and rapid method with a high sensitivity and high predictive value for a positive diagnosis of malignancy.This project focuses on evaluation of utility of FNAC in breast lesions and will classify them wherever possible as per their neoplastic or non-neoplastic characteristics and correlating the findings of FNAC with histopathology of breast lesions.

SUBJECTS AND METHODS

Aim to conduct present study is:

1. To test the utility of F.N.A.C. in clinically suspected breast lesions.
2. To classify benign and malignant lesions and sub classify them whenever possible on cytological grounds.

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3. To establish the sensitivity and specificity of F.N.A.C. in diagnosing breast lesions.
4. To know the efficiency of cytological examination by correlating it with histopathologic examination.

Research project was initiated & data was collected over a period of 18 months from 1st January 2014 to 30th June 2015 with inclusion and exclusion criteria as mentioned below and was analyzed over next 3 months.

Study population source: Patients came in OPD and admitted under Dept. of Surgery with palpable breast lesions

Sample size: 636 patients

Inclusion criteria: Patients presenting with palpable breast lesions

Exclusion criteria: The patients who are not willing for the procedure

In outpatient department, a detailed history and thorough physical examination of the patient having palpable breast lump was carried out and entered in the proforma. The patient was informed about the procedure and informed consent was obtained from the patient before subjecting to fine needle aspiration cytology of the breast lump. The standard procedure was followed, making use of a 10ml syringe bearing a 22-gauze needle (external diameter of 0.6mm) or rarely even 23 and 24 gauze needles. The mass was located clinically and fixed in position with free hand. The skin over the puncture site was sterilized with spirit swab.

The needle was placed over the skin and its direction was determined before it was introduced in the mass in one swift motion. This minimized the discomfort to the patient. Once the tumor was engaged (as indicated by the resistance to the needle) full vacuum was applied, while the needle was moved back and forth in the mass with short strokes. The hub of the syringe was observed for appearance of any specimen. When this appeared, the syringe piston was slowly released and allowed to return to the neutral position. The needle was then withdrawn from the mass.

The needle was temporarily removed from the apparatus, and the syringe was filled with air by pulling back the plunger. The needle was reattached. The specimen was expressed on to one end of glass slides-as is done when preparing a blood smear. The material was then drawn into a smear with a spreader/another glass slide. It was then immersed in a fixative 95% methyl alcohol. The slides were stained with H&E or Papanicolaou stain and AFB wherever suspected for TB.(Fig no. 3). The interpretation of the slide was done by the cytopathologist. Data was recorded regarding the age of the patient, site of involvement, size of the lesion, cytological diagnosis and presence of metastasis in case of malignancies.

The smears were screened under low and high magnification and diagnosis was made as: Benign, Suspicious of malignancy, Malignant. The patients were informed about the cytological diagnosis. The appropriate surgical procedures were performed depending on cytopathology report. Cases were further followed up for histopathology. The surgical specimen (of excised mass/ tissue) was sent for the histopathological confirmation of the diagnosis. Tissues send for histopathology section in the form of biopsy, lumpectomy and MRM specimen. Tissue obtained was fixed in 10 % formalin. After fixation gross examination was done which included size, shape, color, consistency and cut surface. The gross histopathological tissue sampling was done as per the standard procedures. Tissue processing was done using automatic tissue processor.

Sections were dehydrated in alcohol, cleared in xylol and embedded in paraffin wax. Three to five micron thick sections were cut from each paraffin block and stained with haematoxyline and eosin.[4]The sections were examined and microscopic findings were noted.

RESULTS

Distribution of Lesions in Present Study

Total 636 aspirates were done during study period of one and half years, from all patients presented with complaints related to breast, to surgical O.PD and then referred to our O.P.D. Age of the patients were in the range of 14 years to 80 years. Out of 636, 594 were female patients and 42 were male patients Aspirates were examined and reported according to the standard criteria into different categories. Thus maximum no. of cases were of benign lesions 336[52.83%] out of total breast leasions, These lesions were further sub typed.(as shown in Table 1)

Table 1 Showing cytological categories of total aspirates from breast [n=636].

Sr. No.	Lesion Category	No. of cases	Percentage (%)
1	Inflammatory(Non-neoplastic)	196	30.81
2	Benign Neoplastic	336	52.83
3	Atypical or intermediate	20	3.14
4	Suspicious of Malignancy	8	1.25
5	Malignant	76	11.94
	TOTAL	636	100%

Age Incidence

Age group 21-30 years shows highest incidence with 256(40.25%) cases of which 250(39.30%) are benign and 6(0.95%) are malignant indicating high incidence of benign tumor in this age group. Least affected group is 71-80 years, in this present study with 4 cases (3 benign and 1 malignant).(as shown in Table 2)

Table 2 Showing Frequency Distribution of Different Categories of Lesion According To Age Group [n=636]

Sr.no.	Category	Cytological diagnosis	11-20 yrs.	21-30 yrs.	31-40 yrs.	41-50 yrs.	51-60 yrs.	61-70 yrs.	71-80 yrs.	No. of cases	Percentage (%)
1	Inflammatory (Non-neoplastic)	Acute Mastitis	4	17	5	3	0	1	0	30	4.71
		Granulomatous Mastitis (Fig no. 1)	2	8	9	3	0	0	0	22	3.45
		Galactocele	2	28	0	0	0	0	0	30	4.71
		Fibrocystic disease	14	53	25	11	5	1	0	109	17.13
		Duct ectasia	0	2	3	0	0	0	0	5	0.78
2	Benign Neoplastic	Fibroadenoma (Fig no. 4)	110	121	37	15	0	2	0	285	44.82
		Lactating adenoma	2	4	0	0	0	0	0	6	0.95
		Phyllodes -benign	0	3	2	0	0	0	0	5	0.78
		Gynecomastia	12	12	4	5	3	3	1	40	6.28
3	Atypical or intermediate	Atypical ductal Hyperplasia	0	0	5	10	5	0	0	20	3.14
4	Suspicious of Malignancy	Suspicious of Malignancy	0	2	2	0	2	0	2	8	1.25
5	Malignant	I.D.C.(N.O.S.) (Fig no.6)	0	6	12	18	22	10	0	68	10.69
		Medullary (Fig no. 8)	0	0	2	1	0	2	1	6	0.95
		Lobular	0	0	0	0	0	1	0	1	0.15
		Mucinous	0	0	0	0	1	0	0	1	0.15
		TOTAL	146	256	106	66	38	20	4	636	100

Sex incidence

Table 3 Showing sex wise distribution. [n=636]

Sr. No.	Sex	Benign	Malignant	Total
1	Female	520(81.76%)	74(11.63%)	594(93.39%)
2	Male	40(07.24%)	02(0.31%)	42(6.60%)
	Total	560(88.05%)	76(11.94%)	636(100%)

only 40 patients were male out of total 560 benign lesions And only 2 patient were male out of total 76 malignant neoplasms. Hence female to male ratio in case of malignant neoplasm was 37:1..(as shown in Table 3)

Histopathological Correlation

Table 4 Showing no. of cases followed up with histopathological examination

Sr. No.	Cytological Category	No. of cases on cytology	No. of cases with Histopathological examination	Percentage (%)
1	Benign non neoplastic	216	23	10.64
2	Benign neoplasm	336	106	31.54
3	Suspicious	8	3	37.50
4	Malignant lesions	76	44	57.89
	Total	636	176	27.67

Thus of the total 636 cases, 176 (27.67%) were followed up with histopathological examination. Out of 76 malignant lesions 44 (57.89%) were with histopatological exam. Remaining cases were lost on follow up..(as shown in Table 4)

Of the 552 cases of benign report by fine needle aspiration cytology,127 were confirmed by histopathology. Two cytology smears which were diagnosed as benign were later found to be malignant on histopathology. These are false negative cases. These false negative cases occurred due to inability to aspirate the representative site of the lesion, while performing FNAC. Also two cytology smear which was diagnosed as suspicious of malignancy was later found to be benign on histopathology. These are false positive cases. Of the 76 cases of malignancy report by fine needle aspiration cytology, 46 were confirmed by histopathology. Three cytology smears were reported as suspicious for malignancy. Out of three suspicious smears one case was further confirmed as malignant on histopathology and other two cases were later found to be benign on histopathology.(as shown in Table 5)

Table 5 Showing cytohistological correlation of 176 cases

Sr. No.	Cytological Diagnosis	No. of cases	Histopathological Diagnosis													
			Benign										Malignant			
			F.C.D.	GNM	A.M.	F.A.	L.A.	B.PHT	G.M.	A.D.H.	I.D.C.	M.C.B.	L.C.B.	METP	M.PHT	MUC.
1	Benign															
a)	Non-neoplastic	23														
i	F.C.D.	10	10													
ii	GNM	7		7												
Iii	A.M.	2			2											
iv	A.D.H.									4						
b)	Neoplastic	106														
i	F.A.	90				90										
ii	L.A.	2					2									
Iii	B.PHT	4						2							2*	
Iv	G.M.	10							10							
2	Suspicious	3				2"					1					
3	Malignant	44														
i	I.D.C.-NOS	36									34	1		1	-	
ii	M.C.B.	6										6				
Iii	L.C.B.	1											1			
Iv	MUC.	1														1
	TOTAL	176				129								47		

Statistical Analysis

Table 6 Showing statistical analysis of the results

Sr. no.	Test Result	True diagnosis		Total	Percentage (%)
		Positive	Negative		
1	Carcinoma	45^a	2^b	47	26.71
2	No carcinoma	2^C	127^d	129	73.29
	Total			176	100.00

Sensitivity =

$$\frac{\text{True positive}}{\text{True positive + False negative}} = \frac{a}{a+c} \times 100$$

$$= \frac{45}{45+2} \times 100$$

$$= \mathbf{95.75\ \%}$$

Specificity =

$$\frac{\text{True negative}}{\text{True negative + false positive}} = \frac{d}{d+b} \times 100$$

$$= \frac{127}{127+2} \times 100$$

$$= 98.45\%$$

Positive predictive value =

$$\frac{\text{True positive}}{\text{True positive + False positive}} = \frac{a}{a+b} \times 100$$

$$= \frac{45}{45+2} \times 100$$

$$= 95.75\%$$

Negative predictive value =

$$\frac{\text{True negative}}{\text{True negative + false negative}} = \frac{d}{d+c} \times 100$$

$$= \frac{127}{127+2} \times 100$$

$$= 98.45\ \%$$

$$\text{Efficiency} = \frac{[T.N + T.P]}{[T.P + F.P + T.N + F.N]} = \frac{127+45}{45+2+127+2} \times 100$$

$$= \frac{172}{176} \times 100$$

$$= 97.72\%$$

Thus in the present given study the sensitivity, specificity , positive predictive value, negative predictive value and efficiency of malignant cases is 95.75%, 98.45%, 95.75%, 98.45%, 97.72% respectively.

Microscopic Observations

Granulomatous mastitis: In the present study Granulomatous mastitis comprises of 7 cases.

Cytopathology: Granulomatous mastitis is characterized by a cellular aspirate demonstrating conspicuous numbers of lymphocytes, plasma cells and granulomas with epitheloid and multinucleated giant cells. Isolated clusters of fibroblast and reactive ductal epithelial cells are also present. Occasionally necrosis may be seen. (Fig no.1) The cellular material from such aspirates should be carefully examined for the presence of acid-fast bacilli. The ziel-Nielson staining was done for presence of acid fast bacilli of tuberculosis. Out of 22 cases diagnosed on cytology only 2 cases shows AFB positivity on ziel-Nielson staining (Fig no.3)

Histopathology: Microscopically it is characterized by presence of caseation in an epithelioid granuloma with Langhans giant cell and demonstrating acid fast bacilli in the

draining lymph nodes. However, acid fast bacilli can only be demonstrated in about 25-40% of caseating lesions. (Fig no.2)

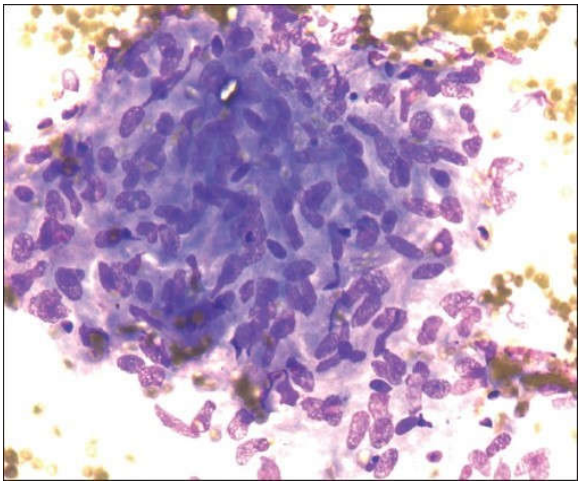

Figure 1

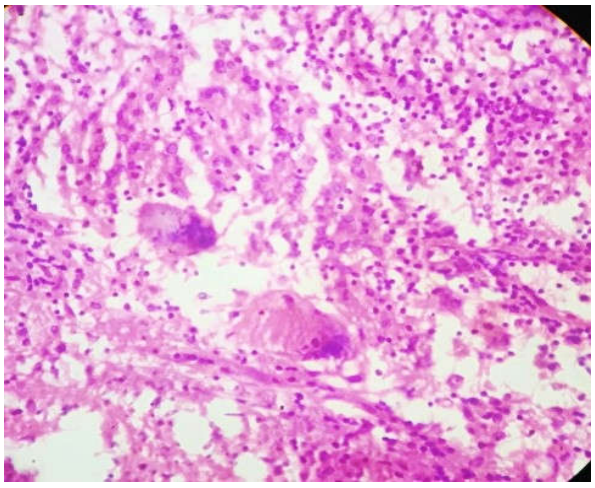

Figure 2

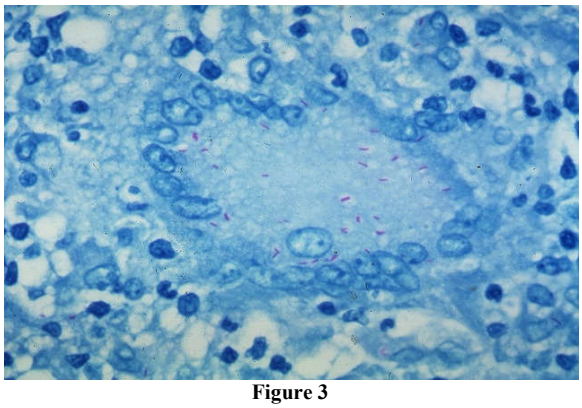

Figure 3

Fibroadenoma

Of the total 176 cases of mammary gland lesions confirmed on histopathology 92 cases were of fibroadenoma.

Cytopathology

FNAC smears show monolayered sheet of tightly cohesive ductal cells and many scattered bare bipolar nuclei. (Fig no.4)

Histopathology

Histopathologically, it shows 2 patterns:

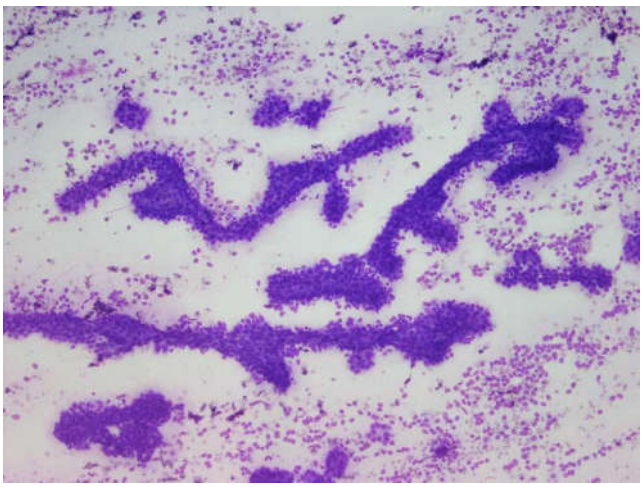

Figure 4

Pericanalicular pattern

It shows round tubular dilated ducts lined by inner cuboidal epithelium and outer myoepithelial cell layer. These ducts are surrounded by abundant fibromyxoid connective tissue stroma.

Intracanalicular pattern

It shows multiple ducts lined by inner cuboidal epithelium and outer myoepithelial cell layer. At places ducts are compressed to slit like spaces due to abundant proliferation of dense fibromyxoidstroma. (Fig no.5)

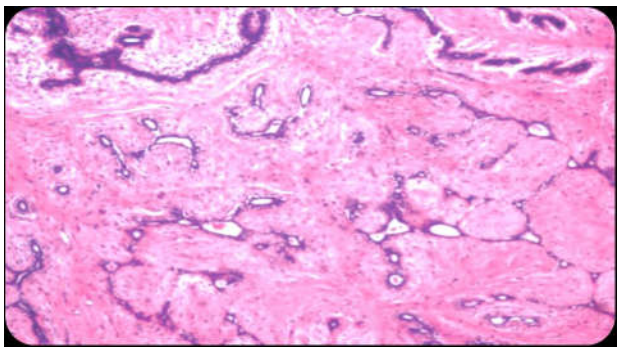

Figure 5

All the cases diagnosed on cytopathology correlated well with the histopathological findings of fibroadenoma.

Infiltrating duct carcinoma

Infiltrating duct carcinoma was the predominant lesion of mammary gland accounting for 34 cases as confirmed on histopathology.

Cytopathology

On gross the aspirate was whitish, granular and in some cases it was hemorrhagic. On cytopathology, the smears were cellular and the cells were arrange in loosely cohesive groups and isolated forms. The cells were large showing pleomorphism and anisonucleosis. The nuclei were hyperchromatic with coarse chromatin and prominent nuclei. The cytoplasm was scanty. (Fig no.6)

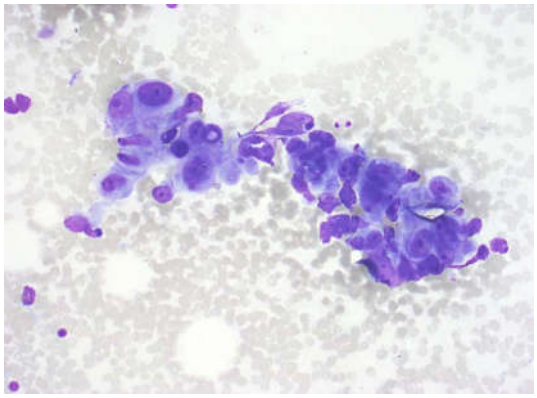

Figure 6

Histopathology

On histopathology, it shows tumor mass composed of sheets, cords and trabeculae of round to oval tumor mass with large hyperchromatic nuclei and scanty eosinophilic cytoplasm. Sheets of tumor cells separated by scanty fibrocollagenousstroma infiltrated by chronic inflammatory cells were seen. (Fig no.7) There was a good correlation between cytopathological and histopathological diagnosis.

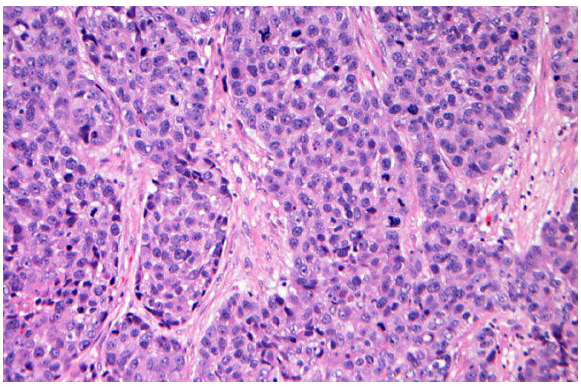

Figure 7

Medullary carcinoma

7 cases were of medullary carcinoma of mammary gland in the present study.

Cytopathology

In medullary carcinoma the aspirate was whitish and granular and showed pleomorphic tumor cells with hyperchromatic nuclei. The nucleus to cytoplasm ratio was reversed. Background showed few lymphocytes. (Fig no.8)

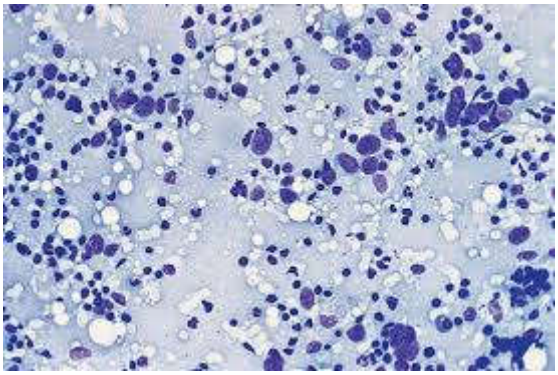

Figure 8

Histopathology

Microscopic findings of biopsy specimen shows tumor mass composed of pleomorphic tumor cells with large vesicular nuclei, prominent nucleoli and acidophilic cytoplasm. Cells are arranged in sheets and syncytial pattern. Sheets of tumor cells are separated by fibrocollagenousstroma which is densely infiltrated by lymphocytes and plasma cells. (Fig no.9)

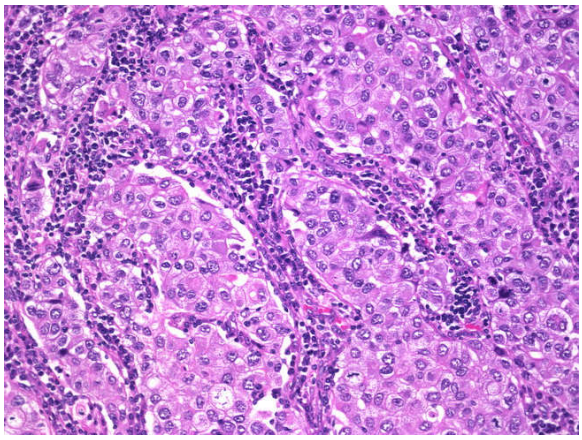

Figure 9

DISCUSSION

Breast is an easily accessible site for fine needle aspiration cytology. There is an increasing tendency to seek to confirm the diagnosis of the breast cancer at first consultation by some form of needle biopsy technique. The present series confirms the accuracy and clinical utility of fine needle aspiration cytology in the investigation of the patient with benign and malignant breast disease.

Distribution of Lesions

In present study, out of 636 cases, 86.79% were classified as benign lesions, 11.94% cases were classified as malignant lesions and 1.25% cases were classified as suspicious for malignancy on cytology.

Table 7 Showing comparison of percentage of breast lesions on cytology between different studies

Sr. No.	Author	Total no. of cases	% of Benign lesions	% of Malignant lesions	% of Suspicious lesions
1	Rocha and Nadkarni[5](1997)	837	76.58	11.82	3.10
2	George *et al*[6](1997)	1472	68.10	12.30	3.3
3	Ishita *et al*[7] (2003)	125	68.00	20.00	1.60
4	Pradhan[8](2008)	2246	81.92	15.49	2.58
5	Mahajan N.A.*et al* [9](2013)	106	64.15	22.64	6.60
6	ValaM *et al*[10](2014)	35	71.42	28.57	0
7	Paramesh&Saha *et al*[11](2015)	100	61	38	0
8	Present study 2015	636	86.79	11.94	1.25

Thus percentage of benign lesions in this study correlates with that of Rocha and Nadkarni[5] (1997) and Pradhan[8] (2008)

Percentage of malignant lesions correlates with Rocha and Nadkarni[5] (1997), George *et al*[6] (1997). Percentage of suspicious lesions correlates with Ishita *et al*[7] (2003). (as shown in Table 7)

Age Distribution

Table 8 Showing comparison of most common age group affected in benign and malignant lesions between different studies

Sr. No.	Author	Benign [years]	Malignant [years]
1	Baptist *et al*[12](1973)	21-30	41-50
2	Haque *et al*[13](1980)	21-30	41-50
3	Rocha and Nadkarni[5](1997)	31-40	51-75
4	Ishita *et al*[7](2003)	21-30	41-50
5	ValaM *et al*[10](2014)	15-30	40-60
6	Paramesh&Saha *et al*[11](2015)	31-40	41-50
7	Present study 2015	21-30	51-60

Thus most common age group affected in benign lesions correlates with that of Baptist *et al*[12](1973) Haque *et al*[13](1980) and Ishita *et al*[7](2003) and the most common age group affected in malignant lesions correlates with that of ValaM *et al*[10](2014) and Rocha and Nadkarni[5](1997). (as shown in Table 8)

Cyto-Histo Correlation

Table 9 Showing comparison of percentage of cytohistological correlation between different studies

Sr. No.	Author	% of cytohistological correlation	
		Benign	Malignant
1	Rocha and Nadkarni[5](1997)	98.97	97.82
2	Ishita *et al*[7](2003)	96.66	100.00
3	Pinto and singh[14](2004)	82.40	-
4	Present study 2015	98.45	95.75

Thus percentage of cytohistological correlation for benign lesions in this study correlates with Rocha and Nadkarni[5] (1997) and Ishita *et al*[7] (2003). (as shown in Table 9)

Statistical Analysis

Table 10 Showing comparison of statistical analysis of results between different studies

Sr. No.	Author	No.of total Cases	Corre-lated cases	T. P.	T. N.	F. P.	F. N.	S E N	S P E	Eff.
1	Rocha and Nadkarni[5](1997)	837	600	106	494	9	7	93.8	98.21	97.4
2	Ishita *et al*[7](2003)	125	60	25	32	1	2	92.1	97.06	95.2
3	Naggada[15](2007)					1.9	2.9	95.7	98.7	97.7
4	Mahajan N.A.*et al* [8](2013)	106	106	30	74	1	1	96.6	98.66	98.11
5	Paramesh&Saha *et al*[10](2015)	100	55	21	33	1	0	95.4	100	
6	Present study 2015	636	176	127	45	2	2	95.7	98.45	97.7

Thus in the final statistical analysis of the results, sensitivity and specificity correlates with that of Naggada[15] (2007), Mahajan N.A.*et al* [9](2013). (as shown in Table 10)

Explanation of causes of occurrence of F.P. and F.N. diagnosis

2 cases of F.P. - was case reported as suspicious of malignancy on cytological examination but reported as 'Fibroadenoma' on histopathology. It is given in the literature that-Fibroademona is the most common cause of false suspicious and FP. diagnosis on F.N.A.C. of breast. As prominent nuclear atypia shown by a proportion of the epithelial cells is a common phenomenon in fibroadenoma in premenopausal and women on hormone replacement therapy. Nevertheless if prominent atypia is found in F.N.A.C. smears, a recommendation of excision is advisable.[16]

2 cases of F.N. were of phyllodes tumors, where Benign phyllodes tumor on cytology but reported as malignant phyllodes on histopathology. Occasionally, only the epithelial component of a phyllodes tumor is represented in the smears and there may be atypia suggesting epithelial malignancy. Focal malignant transformation can be missed by FNAC in large tumors.

CONCLUSION

F.N.A.C. is a simple, rapid, cost effective procedure to diagnose breast lesions. It is not associated with any drawback of harm to patients or any complications of the procedure. It is valuable in diagnosing pure benign lesions and avoids surgical intervention in benign lesion, also reduces the anxiety on part of the patient. Diagnostic accuracy of the procedure for malignant lesions is well established. More over FNAC can be repeated in cases of suspicious diagnosis or in adequate smear, further cases can be followed with biopsy for further confirmation. Along with this, few pitfalls such as -False positive and false negative cases must be kept in mind so as to avoid harm to the life of the patients both clinically and psychologically. Cytological subtyping pitfalls are present but at the end they are of not much harm to life of the patients. As malignancy confirms on cytology only subtyping requires histopathology in few cases. Taking into consideration all advantages and disadvantages of F.N.A.C. in diagnosing breast lesion F.N.A.C. remains important tool for early diagnosis of malignancy, especially in remote places of developing countries like India in all aspect.

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How to cite this article:

Pandit Sudarshan S and Dwivedy Shweta.2020, A Correlation Study of Fine Needle Aspiration Cytology And Histopathology of Breast Lesions: An Extensive Study of 636 Cases. *Int J Recent Sci Res.* 11(12), pp. 40415-40422.
DOI: http://dx.doi.org/10.24327/ijrsr.2020.1112.8691

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