DIAGNOSTIC EFFICACY OF IMPRINT CYTOLOGY AND FROZEN SECTION OF BREAST LESIONS

By

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DOCTOR OF MEDICINE
IN
PATHOLOGY

Under the guidance of

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April 2015

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Date: Signature of the Candidate

Place: Kolar **Dr. CHAITHRA H**

Dedicated with

REVERENCE

to

My Parents

LIST OF ABBREVIATIONS

Sl No	Abbreviation	Expansion
1	TIC	Touch Imprint Cytology
2	FS	Frozen section
3	SS	Sensitivity
4	SP	Specificity
5	PPV	Positive predictive value
6	NPV	Negative predictive value
7	ACC	Accuracy
8	FP	False Positive
9	FN	False Negative
10	FA	Fibroadenoma
11	FC	Fibrocystic disease
12	IDC	Infiltrating Ductal Carcinoma
13	IDP	Intraductal Papilloma
14	ILC	Invasive Lobular carcinoma
15	DC	Ductal carcinoma
16	DCIS	Ductal carcinoma in situ
17	GF	Gross features
18	HPE	Histopathological examination
19	ER&PR	Estrogen and progesterone receptor
20.	OCT	Optimal cutting temperature compound
21	TSP	Touch smear preparation

ABSTRACT

Objective:

To perform imprint cytology of operable breast masses and evaluate its accuracy in relation to histopathological diagnosis after H&E staining and to perform frozen section on operable breast masses and evaluate its accuracy after histopathological diagnosis after H&E staining.Final correlation between frozen section and imprint cytology together with gold standard histopathology diagnosis.

Materials& Methods:

This study was conducted in R.L. Jalappa Hospital and Research Centre over a period of 2 year study total 82 patients surgically resected specimens were examined by both imprint cytology and frozen section and compared with histopathological sections which was considered as gold standard. Both morphological features of imprint and frozen section were compared with histopathological feature. Rest of the tissue is fixed in 10% formalin and processed in Leica Histokinete. Processed tissue is embedded in paraffin wax and blocks are made.

Results:

All 82 cases were subjected to Imprint cytology and frozen section and compared with HPE. Out of 82 cases 4 takens were inadequate so these cases were excluded for statistical analysis .Total 78 cases subjected to imprint cytology which diagnosed all 43 benign lesions correctly and out of 35 malignant lesions 29 were diagnosed correctly with 6 FN results and no FP results were seen. Imprint cytology showed sensitivity, specificity and accuracy of 100%,82.86% and 92.31%

Frozen section diagnosed all 43 benign lesions correctly and out of 35 malignant lesions 33 were correctly diagnosed with 2 FN results and no FP results were seen

Frozen section showed sensitivity, specificity and accuracy of 100%,94.29% and 97%

Correlation of both imprint and frozen section alone showed 45 benign lesions and out of 33 malignant lesions 29 were correctly diagnosed with4 FN results and no FP results were seen. Imprint cytology and frozen section combined efficacy showed sensitivity, specificity and accuracy of 91.84%, 100% and 94.87%.

Final correlation of both imprint cytology and frozen section with HPE showed significant association.

Conclusion:

FS is superior to imprint cytology when compared with gold standard HPE in intraoperative diagnosis of breast mass lesions. When the FS equipment is lacking, imprint cytology could be a reliable alternative with limited technical, financial provided that an experienced cytopathologist is available. Imprint cytology can be used as an adjuvant to FS in the intraoperative consultations.

CONTENTS

INTRODUCTION

Breast is one of the important organ in human being and in female, they represent motherhood, infant nutrition and sex. Breast lesions commonly affect female and form a spectrum. This spectrum consists of both benign and malignant tumors.

Breast carcinoma is the second most common malignant tumor and one of the leading cause of death in women.[1] The incidence of breast cancer is rising in the world especially in developing countries such as India .It accounts more than 1, 000, 000 cases occurring worldwide annually. [1]

According to the National Cancer Registry Programme report on time trends in cancer incidences rates (1982-2005) of Indian Council of Medical Research (ICMR), the estimated breast cancer cases in India in 2010 is 90,659. India's National Health Profile 2010 predicted that by 2020, breast cancer will overtake cervical cancer.[2] The incidence of breast cancer in Kolar district is around 6.41%.[3]

Breast cancer increases the anxiety, cosmetic concern, loss of symbol of femininity, fear of death due to cancer and decision of therapy becomes a challenge.[4] This has led to evolution of many diagnostic procedures for breast lesions apart from clinical examination they are[4]

1. Mammography
2. FNAC
3. Core needle biopsy
4. Incisional biopsy – for large tumors
5. Excisional biopsy for tumors < 2cms
6. Imprint cytology

7. Frozen section

A palpable breast lump is a common diagnostic problem to both attending clinician and pathologist. Aim of diagnostic procedure is should be simple, reliable, reproducible, less traumatic, cost effective, less time consuming and its diagnostic accuracy. Mammography helps in detection of malignant neoplasms non invasively.[4] Approximately 20% of palpable tumor remains undetected.[4] Excisional or incisional biopsy of breast lumps also a diagnostic procedure which is time consuming.

The triple assessment consisting of clinical evaluation, mammography and fine needle aspiration cytology has been routinely practiced and is an alternative to conventional open biopsy in the pre-operative diagnosis of breast lumps .[5] It is simple, reliable, reproducible, less traumatic, cost effective , less time consuming and its diagnostic accuracy has been reported to reach 100%.[5]

FNAC is challenging diagnostic tool which is performed preoperatively with studies mentioning its efficacy. Frozen section is another diagnostic procedure which is usually done intraoperatively and also considered as therapeutic decision making procedure.[6] Imprint cytology another diagnostic tool which is used intraoperatively and is considered better than FNAC.[6] To increase diagnostic accuracy the combined use of imprint cytology and frozen sections are recommended.[6]

The choice of diagnostic procedure varies according to the person evaluating the case. Present study is undertaken to find out the diagnostic accuracy of imprint cytology(IC) and frozen section(FS) which varies from centre to centre .

OBJECTIVES OF THE STUDY

1. To perform imprint cytology of operable breast masses and evaluate its accuracy in relation to histopathological diagnosis after H&E staining.
2. To perform frozen section on operable breast masses and evaluate its accuracy after histopathological diagnosis after H&E staining.
3. Final correlation between frozen section and imprint cytology together with gold standard histopathology diagnosis.

The Normal breast[7,8,]

Anatomy [7,8,]

Breast is a modified apocrine gland. It lies between the second and sixth ribs in the vertical axis and between the sternal edge to mid-axillary line in horizontal axis. The supero-lateral quadrant is prolonged to form axillary tail of Spence & reaches up to the apex of axilla. The paired mammary glands rest on the pectoralis muscle on the upper chest wall, it consists of about 15-25 independent glandular units called breast lobe or mammary lobes each almost pyramidal in shape & having an apex at areola. Each of these lobes represents the morphofunctional unit of the organ consisting of compound tubuloacinar glands.

These lobes are embedded in a mass of adipose tissue and are separated by connective tissue of breast. A single large duct, the lactiferous duct drains each lobe via separate opening on the surface. Each breast lobe is sub-divided into a variable number of lobules. The lobule with terminal duct is called "terminal duct lobular unit". The overlying skin is lined by keratinized squamous epithelium which continues into ducts and then changes in double layered epithelium which rests on a continuous basement membrane. The luminal cuboidal or columnar cells which produce milk rest on the flat discontinuous myoepithelial cells which help in milk ejection. The interlobular stroma consists of dense fibrous connective tissue with adipose tissue & intralobular stroma consists of myxomatous stroma with scattered lymphocytes. Areola contains sebaceous glands which open via lactiferous ducts or directly on the surface. Male breast: remains rudimentary throughout life. It is formed by small ducts without lobules or alveoli.

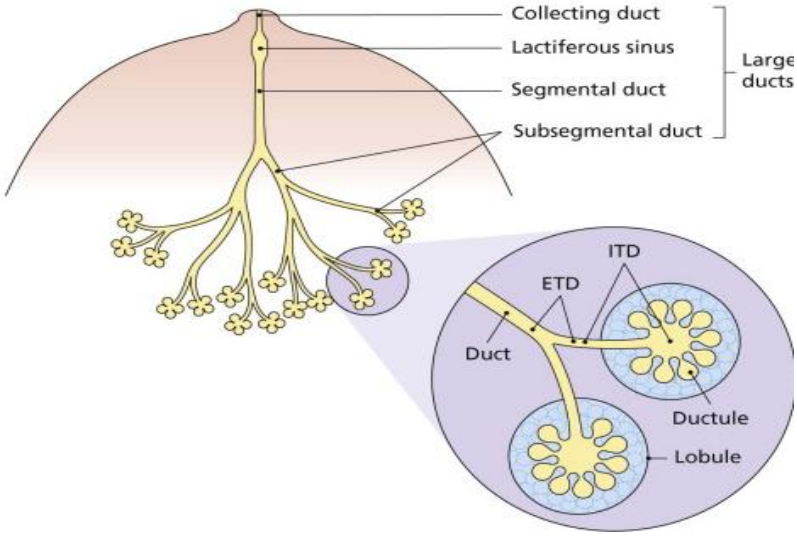


Fig 1.Diagramatic representation of structure of a female breast

Embryology[8,9,10]

Breast develops from mammary bud. After its development no major changes are seen till puberty.

Physiology[7,8]

In reproductive age of women breast shows following changes with maturation. With rise in estrogen the ductal and ductular cells proliferate and continue throughout the menstrual cycle .under the influence of progesterone during secretory phase the terminal duct proliferates and there is vacuolization and increased mitotic activity of basal epithelial cells. Stromal cells proliferate with stromal edema.When menstruation occurs the above changes regress due to fall in hormonal levels .Also seen atrophy of connective tissue , disappearance of stromal edema and overall shrinkage in size of ducts and glands.

The breast assumes its complete morphological maturation and functional activity with pregnancy. Numerous secretory glands pouch out from each bud to form grape like clusters. By the end of pregnancy breast is composed of glands separated by relatively scanty stroma.

Following lactation the glands once again regress and atrophy. The ducts shrink and the total breast size diminishes . The lobules may disappear in a aged female leaving only ducts resembling male breast .

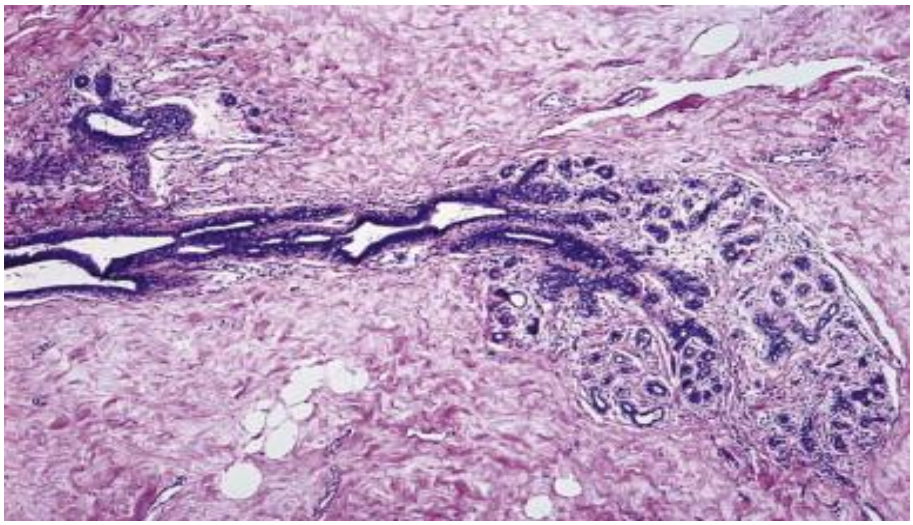


Fig 2 : Terminal duct lobular unit of of normal female breast

THE WORLD HEALTH ORGANIZATION (WHO) [1]

Table 1: CLASSIFICATION OF TUMORS OF THE BREAST (2003)

Epithelial Tumors

Invasive ductal carcinoma ,not otherwise specified (NOS)

Mixed type carcinoma

Pleomorphic carcinoma

Carcinoma with osteoclastic giant cells

Carcinoma with choriocarcinomatous features

Carcinoma with melanotic features

Invasive lobular carcinoma

Tubular carcinoma

Invasive cribriform carcinoma

Medullary carcinoma

Mucinous carcinoma and other tumors with abundant mucin

Mucinous carcinoma

Cystadenocarcinoma

Columnar cell mucinous carcinoma

Signet ring cell carcinoma

Neuroendocrine tumors

Solid neuroendocrine carcinoma

Atypical carcinoid tumor

Small cell/oat cell carcinoma

Large cell neuroendocrine carcinoma

Invasive papillary carcinoma

Invasive micropapillary carcinoma

Apocrine carcinoma

Metaplastic carcinoma

Pure epithelial metaplastic carcinoma

Squamous cell carcinoma

Adenocarcinoma with spindle cell metaplasia

Adenosquamous carcinoma

Mucoepidermoid carcinoma

Mixed epithelial/mesenchymal metaplastic carcinomas

Lipid-rich carcinoma

Secretory carcinoma

Oncocytic carcinoma

Adenoid cystic carcinoma

Acinic cell carcinoma

Glycogen-rich clear cell carcinoma

Sebaceous carcinoma

Inflammatory carcinoma

Precursor lesions

Lobular Neoplasia

Lobular carcinoma In situ (LCIS)

Intraductal proliferative lesions

Usual ductal hyperplasia

Flat epithelial hyperplasia

Atypical ductal hyperplasia

Ductal carcinoma insitu(DCIS)

Microinvasive carcinoma

Intraductal papillary neoplasms

Central papilloma

Peripheral papilloma

Atypical papilloma

Intraductalpapillarycarcinoma

Intracystic papillary carcinoma

Benign epithelial proliferations

Adenosis , including variants

Sclerosingadenosis

Apocrine adenosis

Blunt duct adenosis

Microglandularadenosis

Adenomyoepithelialadenosis

Radial scar / complex sclerosing lesion

Adenomas

Tubular adenoma

Lactating adenoma

Apocrine adenoma

Pleomorphic adenoma

Ductal adenoma

Myoepithelial lesions

Myoepitheliosis

Adenomyoepithelialadenosis

Adenomyoepithelioma

Malignant myoepithelioma

Mesenchymal Tumors

Haemangioma

Angiomatosis

Hemangiopericytoma

Pseudoangiomatous stromal hyperplasia(PASH)

Myofibroblastoma

Fibromatosis(aggressive)

Inflammatory myofibroblastic tumor

Lipoma

Angiolipoma

Granular cell tumor

Neurofibroma

Schwannoma

Angiosarcoma

Liposarcoma

Rhabdomyosarcoma

Osteosarcoma

Leiomyoma

Leiomyosarcoma

Fibroepithelial tumors

Fibroadenoma

Phyllodes tumor

Benign

Borderline

Malignant

Periductal stromal sarcoma, low grade

Mammary hamartoma

Tumors of Nipple

Nipple adenoma

Syringomatous adenoma

Pagets disease of the nipple

Malignant lymphoma

Diffuse large B cell lymphoma

Burkitt lymphoma

Extranodal marginal zone B –cell lymphoma of MALT type

Follicular lymphoma

Metastatic tumor

Tumors of male breast

Gynecomastia

Carcinoma:

In situ Invasive

Histopathological type:

Granulomatous Mastitis [8]

Clinically Appears after pregnancy. Usually presents about 2 years postpartum, may be seen any years later.Clinically mimics carcinoma.Gross features show firm to hard breast mass, sually located peripherally mass often has a nodular architecture and measures up to 8 cm. Microscopy shows Granulomatous inflammation in and around breast lobules (granulomatous lobulitis) Inflammatory reaction in lobules consisting of granulomas, multinucleated giant cells, plasma cells, and eosinophils.Fat necrosis and small abscess formation occasionally present.

Fibrocystic Disease[8]

Clinical affects primarily premenopausal women (third to fifth decades) can be bilateral and multifocal.Gross features of tumor can be Irregular, rubbery, fibrotic breast tissue. Macroscopic cysts containing clear or turbid fluid often seen. Blue-domed cysts may be present. Microscopy shows variable sized cysts lined by flattened or cuboidal epithelial cells. Stroma shows Dense periductal and perilobular fibrosis,apocrine metaplasia. Cysts lined by large, polygonal cells with abundant granular, eosinophilic cytoplasm and small, hyperchromatic nuclei. Most common diagnosis made after lumpectomy (50% of all surgical procedures involving the breast)

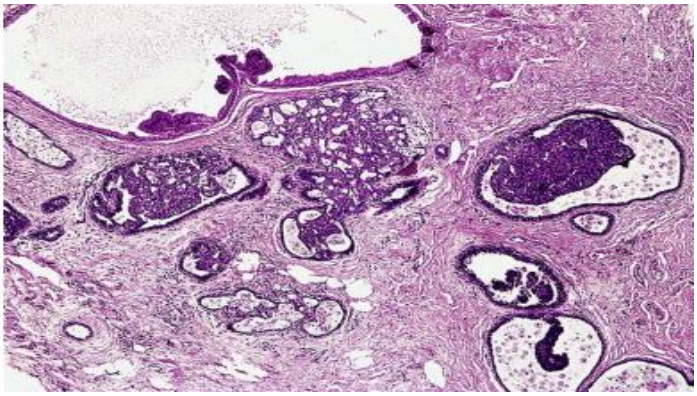


Fig 3. Fibrocystic changes including cystic dilatation, apocrine metaplasia, florid ductal hyperplasia and fibrosis.

Fibroadenoma[8]

Clinically most common breast tumor in adolescents and young women. Often develops in transplant recipients receiving cyclosporine. Presents as discrete, non tender, palpable breast Mass. Gross Pathology shows tan-white to gray, firm to rubbery, round to oval mass.Well circumscribed and typically encapsulated. No infiltration into surrounding fatty breast tissue. Most fibroadenomas measure up to 3 cm. Tumor arises from lobules and stroma of the terminal duct–lobular unit. Typically presents with well-defined capsule .Characteristic features include a collagenous stroma and distorted, slitlike, elongated ducts. Variable degree of stromal cellularity. Fibrocystic changes (apocrine metaplasia, adenosis, ductal epithelial hyperplasia) are common associated findings .Benign multinucleated giant cells may be seen.

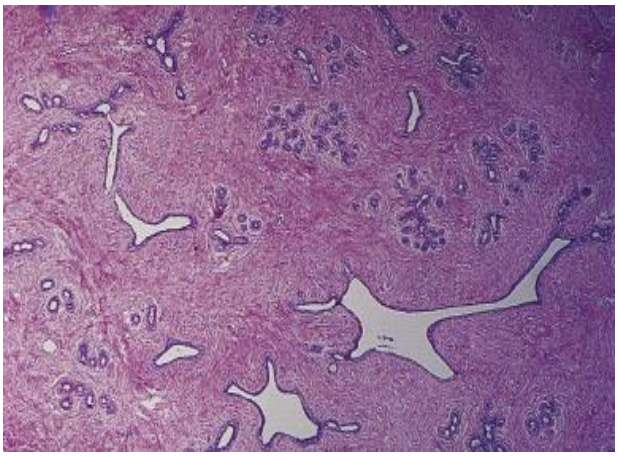


Fig 4: Microscopic appearance of Fibro adenoma

Phyllodes Tumor[8]

Clinically presents about 1% of all breast tumors**.**Usually affects adults (fifth and sixth decades). Gross Pathology shows variable sized, discrete, gray-tan mass with firm in consistency. Variegated, lobulated cut surface; cleft formation may be seen. Areas of necrosis and hemorrhage (more common in malignant lesions).Microscopy Composed of both mesenchymal and epithelial elements. It can be well circumscribed or microscopically invasive. Epithelial

component comprising elongated, leaflike epithelial proliferation (similar to that seen in fibroadenoma)squamous metaplasia of ductal epithelium. Mesenchymal component shows Increased stromal cellularity typically in periductal regions .Cellular atypia or increased mitotic activity may be seen Metaplastic bone, cartilage, fat, or muscle can be present in mesenchymal component (more frequent in malignant tumors)

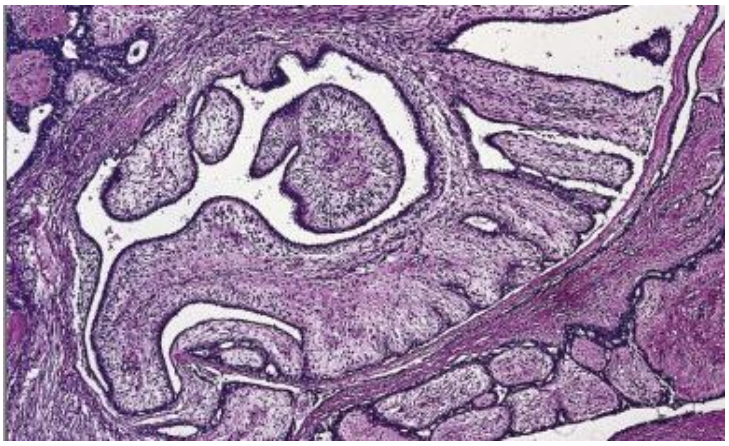


Fig 5: Low-grade phylloides tumor, showing cleft-like spaces and concentration of tumor cells beneath the epithelium.

Intraductal papilloma[8]

Usually presents in their fifth or sixth decade. It is of 2 types solitary and multiple papillomas Gross Pathology shows large papillomas visible in the lumen of a dilated or cystic duct. Palpable lesions typically measure 2 to 3 cm, but cystic lesions can be larger than 10 cm. Microscopically Shows organized papillary proliferation of ductal epithelium on a frond-forming fibrovascular core or stroma. Any degree of epithelial hyperplasia of the usual type may be seen. Fusion of papillae often results in glandlike spaces or solid areas.Presence of a myoepithelial cell layer in papillae and around glandular spaces, although it can be focally absent. Papillomas may show apocrine, squamous, mucinous, clear cell, or sebaceous metaplasia.

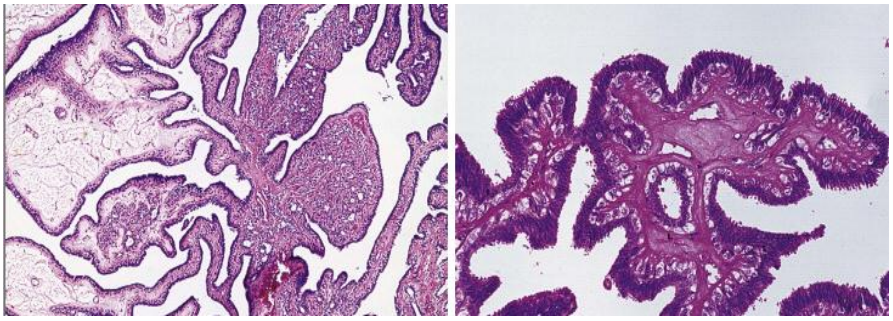


Fig6 :Intraductal papilloma..Low-power showing complex arborizing architecture .High-power view showing dual cell composition, with a well-defined row of myoepithelial cell

INVASIVE DUCTAL CARCINOMA (NOS)[1,8]

It is the largest group of invasive breast cancer and is a heterogeneous group of tumours. It does not exhibit specific histological type such as lobular or tubular carcinoma.The tumor is firm in consistency and cut surface shows yellow-gray with trabeculae radiating through the surrounding parenchyma into the fat. Areas of necrosis, hemorrhage, and cystic degeneration may present in larger neoplasms. The tumor arranged in diffuse sheets, nests, cords, or as individual cells, Glandular/tubular differentiation are seen . The tumor cells are large pleomorphic nuclei and prominent nucleoli are present and mitotic figures are more numerous. Areas of necrosis occur in approximately 60% of cases. Foci of squamous metaplasia, apocrine metaplasia, or clear cell changes may be seen. The amount of stroma may be densely fibrotic to cellular (‘desmoplastic’).

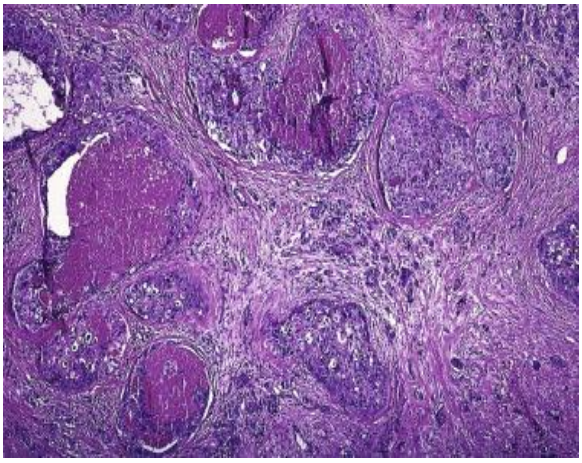


Fig 7 Invasive ductal carcinoma

INFILTRATING DUCTAL CARCINOMA[1,8]

Infiltrating ductal carcinoma is a tumor which belongs to the largest group of primary breast carcinoma .These tumors vary in size from 1cm to 10cm , firm to hard in consistency.[1]Microscopically tumor cells are arranged in cords, clusters and trabecular pattern. Nuclei may be regular ,uniform or highly pleomorphic with prominent nucleoli. Cytoplasm is often abundant and eosinophilic ,focal necrosis can be seen.[1,11]Prognosis is slightly worse with 35 – 50 % of 10 year survival compare to breast carcinoma as a whole with a 55 % 10 year survival.

INVASIVE LOBULAR CARCINOMA[1,8] Clinically presents as bilateral or multifocal lesion, most commonly seen around 50 years of age group .Infiltrating lobular carcinoma is characterized by presence of small and uniform tumor cells which are arranged in single linear cords (Indian file pattern) that invade the stroma. Neoplastic cells have round to notched ovoid nuclei with thin rim of cytoplasm with occasional intracytoplasmic lumen.[1] Prognosis for classical type of lobular carcinoma has favorable outcome and poor prognosis for pleomorphic lobular carcinoma.[1]

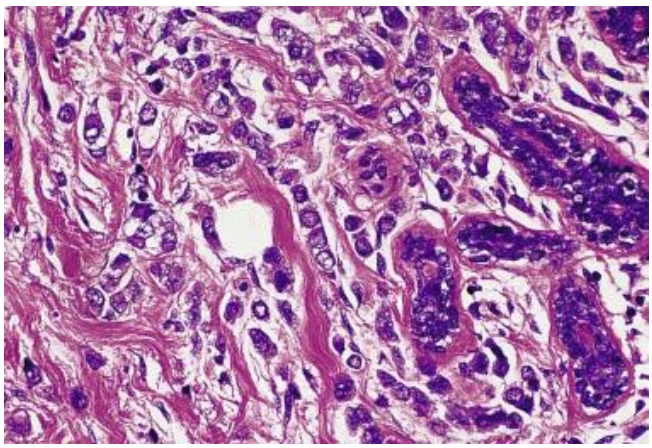


Fig 8: Invasive lobular carcinoma. The tumor cells are small and uniform with round nuclei and grow in an Indian file fashion

TUBULAR CARCINOMA[1,8,9]

These are special type of breast carcinoma with excellent prognosis, occurs in elderly patients more than 50 years. These tumors vary in size between 0.2 cm to 2 cm. They are composed of well differentiated tubular structures with open lumina lined by single layer of epithelial cells .These tubules are oval or rounded and angulated with cellular desmoplastic stroma. [1,11]

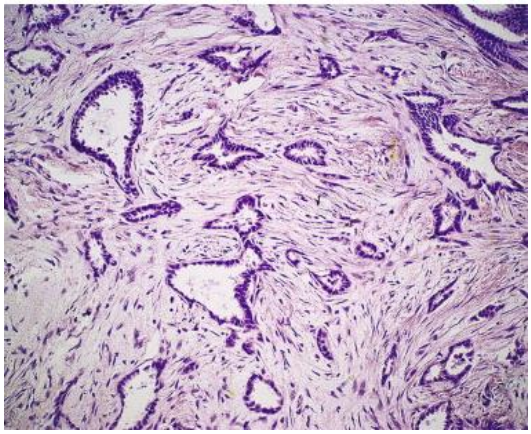


Fig 9 Tubular carcinoma of breast. The angulated shape of the glands and the cellular stroma are characteristic of this lesion.

INVASIVE CRIBRIFORM CARCINOMA[1,8,9]

Invasive cribriform is a rare form of breast malignancy , with excellent prognosis that grows in a cribriform pattern , where tumor cells are arranged as invasive islands often angulated , in which well defined spaces are formed by arches of cells .These cells are small and show mild pleomorphism. [1,11]

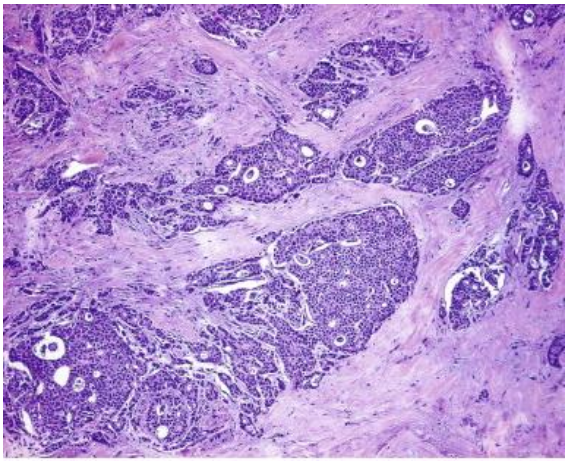


Fig10: Invasive cribriform carcinoma. Some of the nodules have a predominantly solid appearance.

MEDULLARY CARCINOMA[1,8]

Medullary carcinoma accounts between 1 and 7% of all breast carcinomas. These tumors are well circumscribed, composed of tumor cells arranged in syncytial pattern in 75 % of tumor masses, usually four or five cell thickness with no glandular or tubular structures .These tumor cells are round to pleomorphic with vesicular chromatin and prominent 1 to 2 nucleoli. These tumor cells are separated by lymphoplasmacytic stromal infiltrate with pushing margins.[1,11] It has better prognosis than Infiltrative ductal carcinoma.[1]

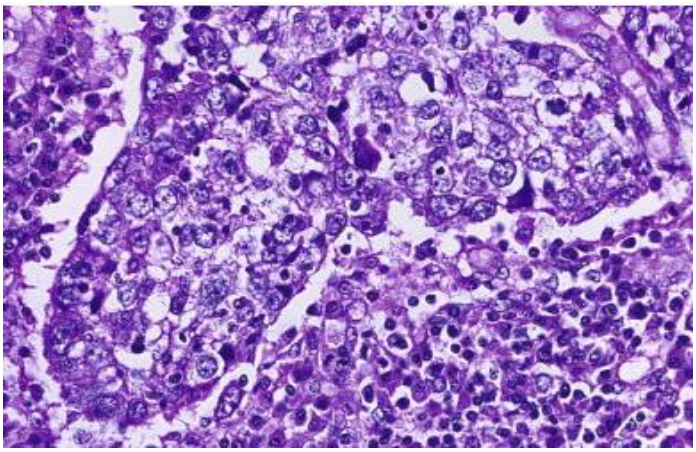


Fig11 : Medullary carcinoma. The large tumor cells grow in a 'syncytial' fashion and are sharply separated from the surrounding stroma, which is heavily infiltrated by lymphocytes and plasma cells.

MUCINOUS (COLLOID)CARCINOMA[1,8]

It is a variety of carcinomas in the breast which is characterized by production of abundant extracellular and intracellular mucin. It accounts for about 2% of all breast carcinom and usually occurs in post-menopausal women. These tumors are well circumscribed and cut section shows glistening gelatinous appearance. They are characterized by proliferation of clusters of small uniform tumor cells floating in mucinous lakes with delicate fibrous septa divide the mucous lakes into compartments. [1] Pure mucinous carcinomas have a favorable prognosis .

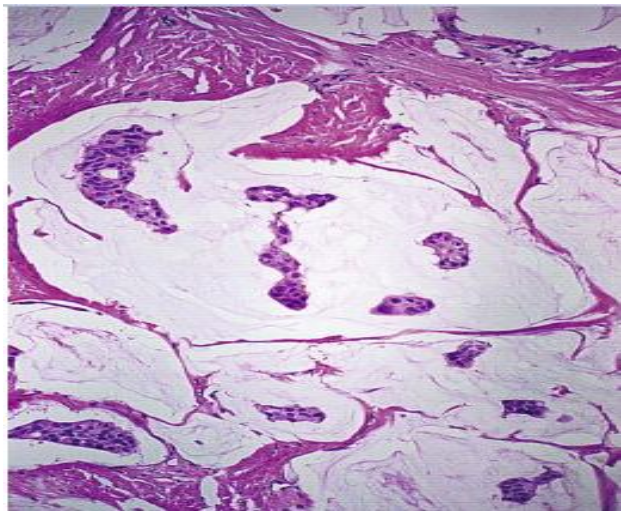


Fig12: Mucinous carcinoma of the breast. Clusters of well-differentiated tumor cells are seen floating in a sea of mucin.

NEUROENDOCRINE TUMORS [1,8,9]

Primary neuroendocrine tumors of breast exhibit morphological features which are similar to neuroendocrine tumors of gastrointestinal tract and lung. They express neuroendocrine markers in more than 50% of the tumors, and present as expansile or infiltrative lesion. These tumors are arranged in solid sheets or alveolar pattern separated by delicate fibrous septa, cells are small round cells with salt and pepper chromatin with scanty cytoplasm.

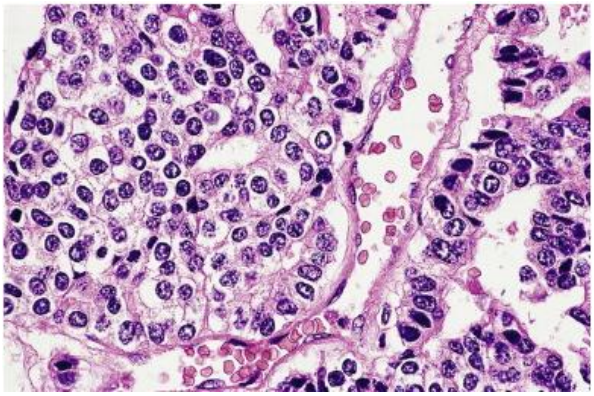


Fig 13: Breast carcinoma with neuroendocrine differentiation

INVASIVE PAPILLARY CARCINOMA. [1,8]

Papillary carcinomas of the breast include both invasive and in situ papillary lesions. These tumors are clinically small and circumscribed in majority of the cases with relatively good prognosis. These tumors shows delicate blunt papillae and also shows focal solid areas, nuclei are intermediate grade with amphophilic cytoplasm with scant stroma.[1]

METAPLASTIC CARCINOMA.[1,8,9]

It is a heterogeneous group of neoplasms . Characterized by an admixture of adenocarcinoma with dominant areas of spindle cell, squamous or mesenchymal differentiation. It can be broadly classified into pure epithelial and mixed epithelial with mesenchymal components. They are more aggressive tumors than infiltrative ductal carcinoma.

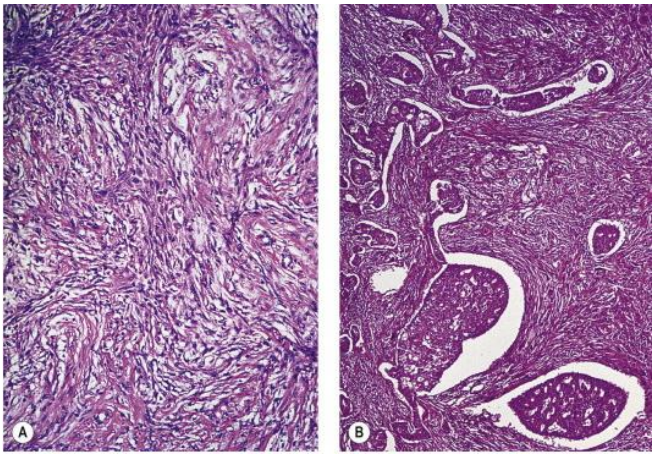


Fig14: Metaplastic carcinoma, biphasic type carcinomatous and sarcoma-like components

APOCRINE CARCINOMA.[1,8,9]

It is a type of carcinoma showing cytological and immunohistochemical features of apocrine cells in >90% of the tumour cells. The incidence of apocrine carcinoma depends on the method of detection. Based on light microscopy alone it is only 0.3-4%. apocrine duct carcinoma compared with non apocrine duct carcinoma re v e a l. Apocrine carcinom predominantly composed of apocrine type of epithelium in more than 90% of the tumor cells. Tumor cells are large, having abundant acidophilic granular cytoplasm which contains golden brown granules that are strongly positive for PAS. Nuclei are vesicular with prominent nucleoli and glandular differentiation is usually found with apocrine snouts. Survival analysis of 72 cases of invasive [1]

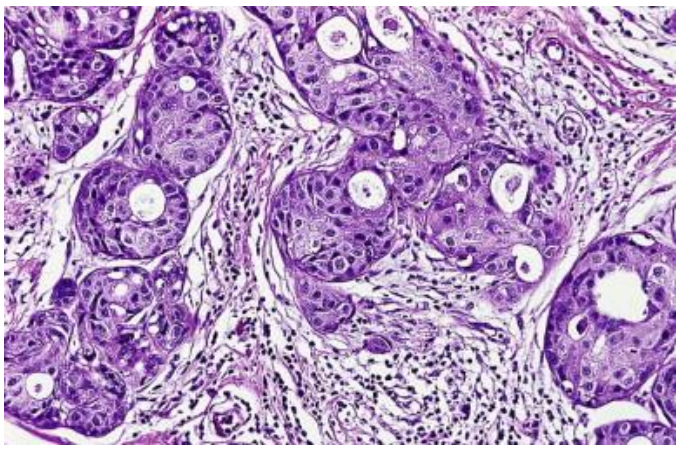


Fig15 : Apocrine carcinoma

STAGING :[1]

Table 2: TNM Classification of carcinomas of the breast

TNM Clinical Classification[1]

TX - Primary tumour cannot be assessed
T0 - No evidence of primary tumour
Tis - Carcinoma in situ
Tis (DCIS) Ductal carcinoma in situ
Tis (LCIS) Lobular carcinoma in situ
Tis (Paget) Paget disease of the nipple with no tumour
T1 : Tumour 2 cm or less in greatest dimension
T1 mic : Microinvasion 0.1 cm or less in greatest dimension
T1a : More than 0.1 cm but not more than 0.5 cm in greatest dimension
T1b : More than 0.5 cm but not more than 1 cm in greatest dimension
T1c : More than 1 cm but not more than 2 cm in greatest dimension
T2 : Tumour more than 2 cm but not more than 5 cm in greatest dimension
T3 : Tumour more than 5 cm in greatest dimension
T4 : Tumour of any size with direct extension tochest wall or skin
Only as described in T4a to T4d
T4a : Extension to chest wall
T4b : Edema (including peaud'orange), ulceration of the skin of the breast or satellite skin nodules confined to the same breast
T4c - Both 4a and 4b, above

T4d - Inflammatory carcinoma

N – Regional Lymph Nodes

NX – Regional lymphnodes cannot be assessed

N0 – No regional lymph nodes metastasis

N1 – Metastasis in movable ipsilateral axillary lymphnode(s)

N2 – Metastasis in fixed ipsilateral axillary lymph node(s)or in clinically apparent ipsilateral internal mammary lymphnode (s) in the absence of clinically evident axillary lymphnode metastasis

N2a –Metastasis in axillary lymphnode(s) fixed to one another or to other structures

N2b – Metastasis only in clinically apparent internal mammary lymphnode (s) and in the absence of clinically evident axillary lymphnode metastasis

N3 – Metastasis in ipsilateral infraclavicular lymph node(s) with or without axillary lymphnode involvement or in clinically apparent ipsilateral internal mammary lymphnode (s) in the presence of clinically evident axillary lymphnode metastasis or metastasis in ipsilateral supraclavicular lymph node(s) with or without axillary or internal mammary lymphnode involvement

N3a –Metastasis in infraclavicular lymph node (s)

N3b - Metastasis in internal mammary and axillary lymph nodes

N3c - Metastasis in supraclavicular lymph node (s)

Metastasis – There are two metastatic classification values (M0 or M1)which depend on the presence or absence of breast cancer cells in locations other than the breast and lymphnodes (Distant metastasis eg :-Bone , Brain , Lung)but it includes supraclavicular lymphnode.

MX - Distant metastasis cannot be assessed

M0 - No distant metastasis

M1 - Distant metastasis

Table 3: pTNM pathological classification[1]

pN – Regional Lymph Nodes
PNx - Regional lymph nodes cannot be assessed
pN0- No regional lymph node metastasis
pN1mi - Micrometastasis (larger than 0.2 mm, but none larger than 2 mm in greatest dimension)
pN1 - Metastasis in 1–3 ipsilateral axillary lymph node(s), and/or in internal mammary nodes with microscopic metastasis detected by sentinel lymph node dissection but not clinically apparent
pN1a -Metastasis in 1–3 axillary lymphnodes, including atleast one larger than 2 mm in greatest dimension
pN1b - Metastasis in internal mammary lymph nodes with microscopic metastasis detected by sentinel lymph node dissection but not clinically apparent
pN1c - Metastasis in 1–3 axillary lymph nodes and internal mammary lymph nodes with microscopic metastasis detected by sentinel lymph node dissection but not clinically apparent

pN2 Metastasis in 4–9 ipsilateral axillary lymph nodes, or in clinically apparent ipsilateral internal mammary lymph node in the absence of axillary lymph node metastasis

pN2a - Metastasis in 4–9 axillary lymph nodes, including atleast one that is larger than 2 mm

pN2b - Metastasis in clinically apparent internal mammary lymph node(s), in the absence of axillary lymph node metastasis

pN3-Metastasis in 10 or more ipsilateral axillary lymph nodes; or in infraclavicular lymph nodes; or in clinically apparent ipsilateral internal mammary lymph nodes in the presence of 1 or more positive axillary lymph nodes; or in more than 3 axillary lymph nodes with clinically negative, microscopic metastasis in internal mammary lymph nodes; or in ipsilateral supraclavicular lymph nodes

pN3a - Metastasis in 10 or more axillary lymph nodes (at least one larger than 2 mm) ormetastasis in infraclavicular lymph nodes

pN3b- Metastasis in clinically apparent internal mammary lymph node in the presence of 1 or more positive axillary lymph node; or metastasis in more than 3 axillary lymph nodes and in internal mammary lymph nodes with microscopic metastasis detected by sentinel lymph node dissection but not clinically apparent.

pN3c - Metastasis in supraclavicular lymph node

Microscopic grading of breast carcinoma

Nottingham modification of the Bloom–Richardson system

Tubule formation

1 point: Tubular formations in >75% of the tumor

2 points: Tubular formations in 10–75% of the tumor

3 points: Tubular formations in <10% of the tumor

Note: For scoring tubule formations, the overall appearance of the tumor has to be taken into consideration.

Nuclear pleomorphism

1 point: Nuclei with minimal variation in size and shape

2 points: Nuclei with moderate variation in size and shape

3 points: Nuclei with marked variation in size and shape

Note: The tumor areas having cells with greatest atypia should be evaluated.

Mitotic count

1, 2, or 3 points, according to Table 20.5

Note: Mitotic figures are to be counted only at the periphery of the tumor. Counting should begin in the most mitotically active area; 10 high-power fields (APF) are to be counted in the same area (but not necessarily contiguous). The fields should be filled with as much tumor as possible; poorly preserved areas are to be avoided. Cells in the prophase should be ignored.

PROGNOSTIC FACTORS

1. Age : Younger than 50 years – best or better prognosis.[1,8]
2. The risk of breast cancer increases with number of affected first degree relatives.[1,8]
3. Lymphnodemetastasis: Axillary lymphnode status is the most important prognostic factor for invasive carcinoma in absence of distant metastasis.[1,8]
4. Tumor size: Tumor size is one of the most powerful predictors oftumor behavior in breast cancer. The risk of axillary lymphnode metastasis increases with the size of primary tumor. Both lymphnode metastasis and tumor size are independent prognostic factors.[1,8]
5. Histopathological type: Morphological variants of invasive ductal carcinoma with a more favorable prognosis are tubular, cribriform, medullary, pure mucinous, papillary and secretory carcinoma.A variant of lobular carcinoma associated with bad prognosis is signet ring carcinoma. Tumors which are aggressive than ordinary ductal carcinoma are squamous cell carcinoma and metaplastic carcinoma.[1,8]
6. Histological grade: Most commonly used grading system is Nottingham Histological score (Scarf Bloom Richardson) .Survival for patients with well differentiated (Grade 1) carcinomas gradually declines to 70% at 24 years. Most deaths are seen in poorly differentiated (Grade 3) carcinomas which occur in first 10 years.Grade 2 (moderately differentiated) carcinomas have slightly better survival than grade 3.[1,8]
7. Lymphovascular invasion : Strongly associated with presence of lymphnode metastasis .It is a poor prognostic factor for overall survival and risk factor for local recurrence.[8,10]
8. ER and PR receptors : 80% of carcinomas that are ER and PR positive respond to hormonal treatment.[1,8]
9. Her 2 neu overexpression is associated with poor survival.[1,8]

Cytology :

Duct cells:[10,11]

- usually in tight clusters,sheets and in singles
- moderate variation in size
- cytoplasm is sparse blue
- nucleus is oval and shows coarse chromatin

Bare nuclei[10,11]

- they are know as naked nuclei
- as the name implies they do not have any cytoplasm
- nuclei are arranged in pairs or single
- oval to round in shape with chromatin smooth, homogenous
- presence of bare nuclei suggests the lesion is benign
- origin is debated to be either from myoepithelial cells or from intralobular connective tissue.

Apocrine cells[10,11]

- cells are in clusters, sheets and in singles
- nucleus is oval to round inshape with loose chromatin
- they are large with abundant cytoplasm and may show granulesstains pink with eosin and blue with MGG

Apocrine cells are duct cells which undergo apocrine metaplasia . they are found normally in axilla. the presence of apocrine cells suggests that the lesion is benign.

Histiocytes [10,11]

- Cells are in single
- Variation in cell size and cell border is poorly defined
- Cells have variable amount of pale blue cytoplasm and often vacuolated
- Nuclei is round to oval with pale nuclear chromatin

Other cells :

- Fat cells, blood cells and squamous cells are other cells which are normally seen.

Various features of breast lesions are described in cytology are

Fibrocystic change [10,11]

- Low to moderate cellularity
- Cohesive sheets of benign ductal cells arranged in honeycomb pattern
- Usual epithelial fragments are seen
- single bare bipolar or oval nuclei may be scattered
- Background of variable amounts of cyst fluid, macrophages, and apocrine metaplastic cells.

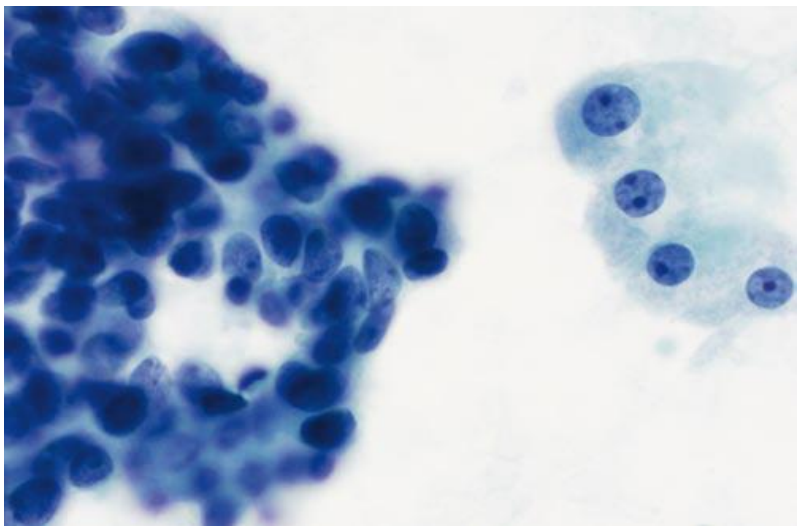


Fig15: Benign ductal epithelium in fibrocystic changes (FCC).

Fat necrosis [10,11]

- Fragments of normal and degenerating adipose tissue seen.
- A dirty background of granular debris
- Fat droplets and fragments of adipose tissue,
- Foamy macrophages, multinucleated giant cells and adipocytes with bubbly cytoplasm,
- Chronic inflammatory cells,
- Absence of epithelial cells.

Epithelial hyperplasia usual type [10,11]

- Moderate cellularity
- Bimodal pattern , flat groups of uniform cells in honey comb pattern
- Over crowding and overlapping of nuclei
- Mild atypia present
- Bipolar cells
- Stromal fragments
- Background clear, foam cells or apocrine cells

Atypical type [10,11]

- Increased crowding and overlapping of cells.
- Bipolar nuclei (myoepithelial cells) present
- High cellularity
- Cells with benign and atypical features
- Loss of polarity
- Nuclear hyperchromasia with presence of distinct nucleoli
- Background clear and sometimes necrotic

- Atypical ductal hyperplasia and ductal carcinoma in situ cannot be distinguished by FNAC.

Fibroadenoma [10,11]

- Moderate to high cellularity
- Sheets of cohesive epithelial cells;
- Cells arranged in bimodal pattern containing epithelial and stromal fragments,
- Large, branching sheets of bland epithelial cells,
- Numerous single, bare bipolar/oval nuclei,
- Fragments of fibromyxoid stroma.
- Occasional myxoid change seen
- Few or no foam cells or apocrine cells

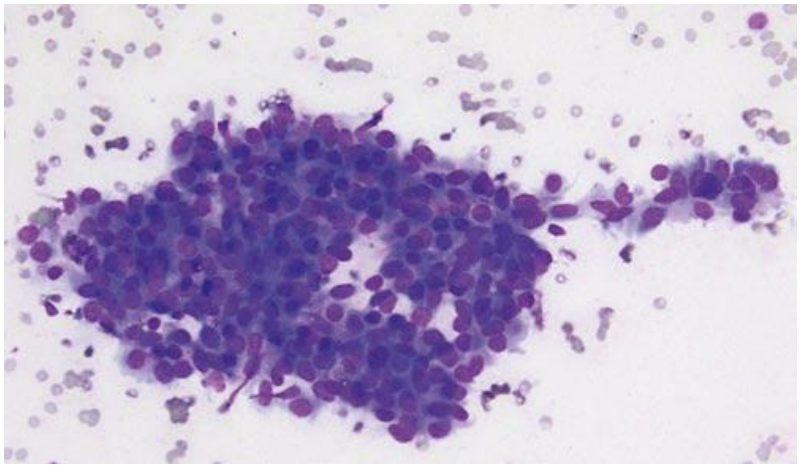


Fig 16: Clusters of tightly cohesive cells with minimal nuclear atypicality are characteristic of fibroadenomas .

Phyllodes tumor [10,11]

Cellular smears

Biphasic population of epithelial and stromal cells

Hypercellular stromal fragments consisting of spindle shaped cells present singly and enmeshed in metachromatically staining stroma

Stromal cell atypia is a feature of malignant phyllodes tumor

Epithelial hyperplasia can be present;

Numerous bipolar naked nuclei and

Occasional cases indistinguishable from fibroadenoma.

Papillary lesions [10,11]

- Branching epithelial sheets and finger-like fragments,
- Strands of dense fibrovascular stroma
- Dispersed epithelial cells with mild nuclear atypia,
- Rows of palisaded columnar epithelial cells,
- Macrophages and variable amounts of cyst fluid,
- Bare bipolar nuclei may be sparse

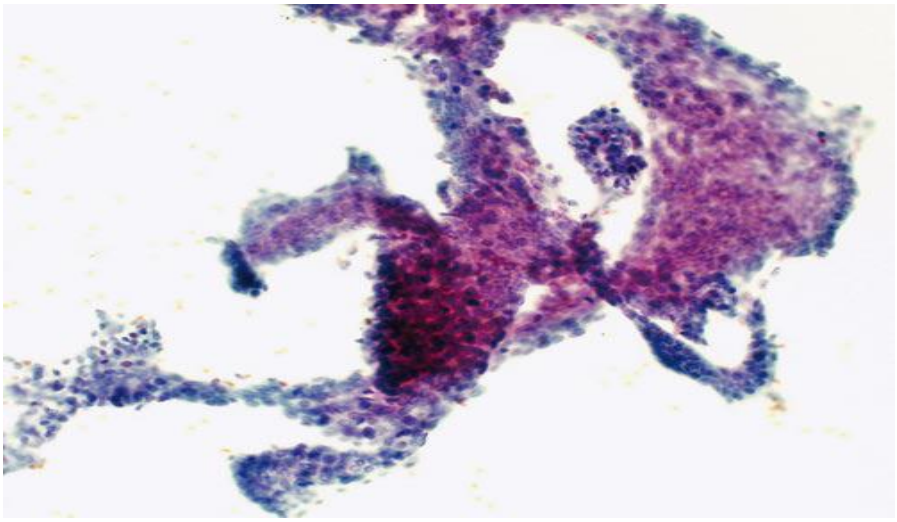


Fig 17 : Papillary neoplasm.

Infiltrating ductal carcinoma of no special type (NOS)[10,11]

- Moderately to high cellular smears
- Loosely cohesive and individual scattered malignant cells;
- Malignant epithelial cells arranged in three-dimensional clusters, syncytial groupings, and occasional acinar patterns;
- No bipolar naked nuclei
- Tumor diathesis may be present.
- Single epithelial cells with intact cytoplasm
- Moderate to severe nuclear atypia with enlarged, pleomorphism, irregular nuclear membrane and chromatin.
- Fibroblasts and fragments of collagen associated with the atypical cells.
- No myoepithelial cells seen.

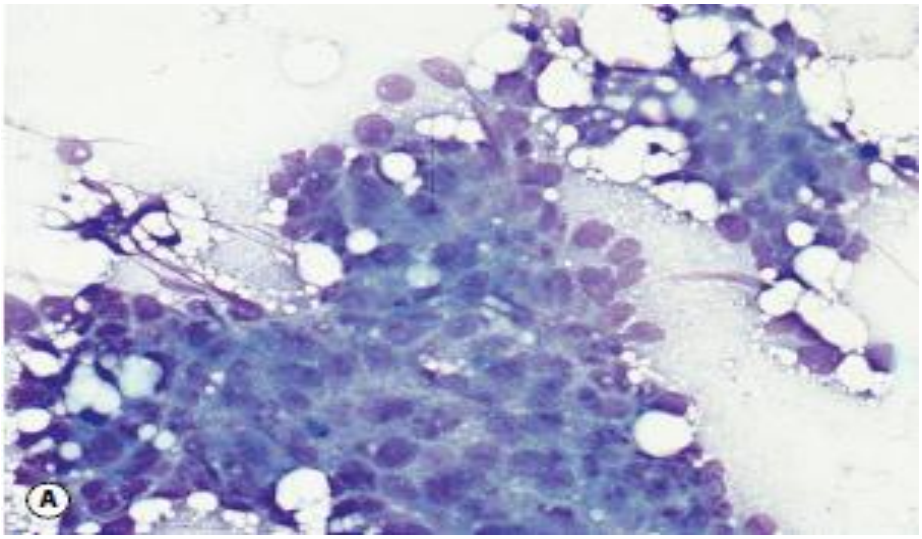


Fig 18 : Ductal carcinoma

Low-grade DCIS[10,11]

- cribriform, solid or micropapillary, noninvasive intracystic
- Epithelial cells mainly cohesive, forming large sheets, often with 'holes' or papillary fragments
- Arranged in cribriform, solid or micropapillary
- Bare bipolar nuclei absent,
- Variable, mild to moderate epithelial atypia,
- Necrotic debris, often calcium granules,Macrophages.

Lobular carcinoma[10,11]

- A variable or poor cell yield,
- Cells in single and small clusters,
- Single files characteristic
- Scanty cytoplasm

- Small hyperchromatic nuclei of uniform size,
- Irregularity of nuclear shape with angular, triangular, indented or budding nuclei,
- Intracytoplasmic lumina/mucin vacuoles/signet ring cells,
- Few naked bipolar nuclei,
- Thick, eosinophilic background with crushed material interspersed between fatty vacuoles and small fibrous stromal fragments

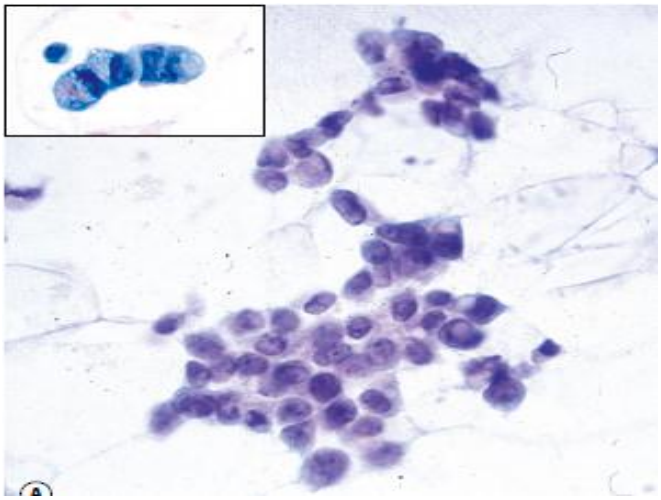


Fig19: Lobular carcinoma

Medullary carcinoma [10,11]

- High cellular smears
- Loose syncytial aggregates and single cells
- Bizarre tumor cells with pleomorphic high-grade nuclei, having prominent nucleoli and occasional stripped tumor nuclei
- Many lymphocytes and plasma cells.

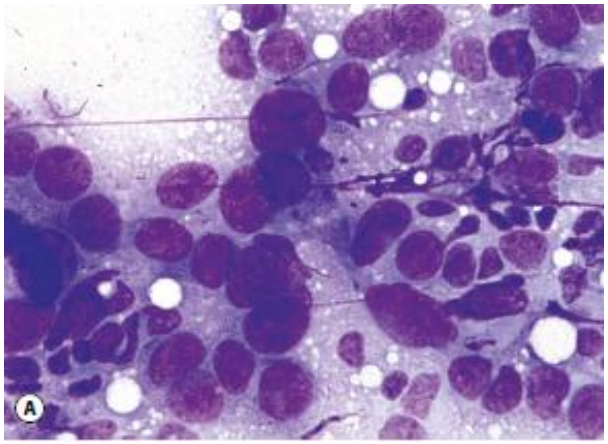


Fig 20: Medullary carcinoma

Mucinous (colloid) carcinoma [10,11]

- Abundant background mucin
- Atypical cells in small solid aggregates and single intact epithelial cells,
- Mild to moderate nuclear atypia,
- Benign epithelial cells and bipolar nuclei absent,
- Chicken wire blood vessels.

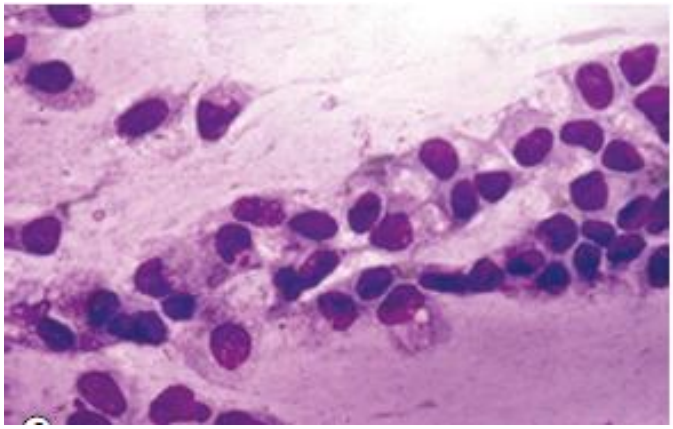


FIG 21 : Mucinous carcinoma

Tubular carcinoma [10,11]

- Moderate cellular smears
- Cells in cohesive clusters,
- Epithelial fragments with an angular or tubular shape,
- uniform, mild to moderate atypical epithelial cells,
- Absence or paucity of single bipolar nuclei of benign type,
- Fibroblastic cells;
- fragments of fibromyxoid

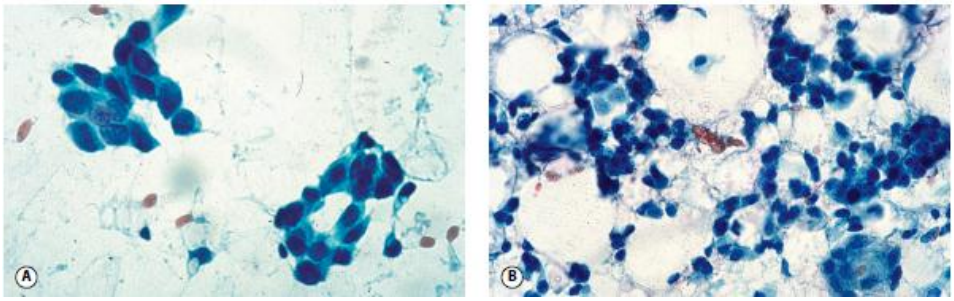


Fig 22 : Tubular carcinoma

High nuclear-grade DCIS[10,11]

- Usually cell-rich smears,
- Arranged in solid or comedo growth pattern
- Neoplastic cells in sheets, irregular aggregates and single,
- Large, pleomorphic cells showing obvious malignant nuclear features,
- Necrotic debris, granular calcium, lymphocytes and vacuolated macrophages.

REVIEW OF LITERATURE:

Increasing incidence of cancer in various organs have increased the scope for quick and accurate diagnostic method.[12]Fine needle aspiration cytology (FNAC),Papanicolau stain (PAP) smear, Imprint cytology, frozen section (FS), scrape cytology or combination of these methods are commonly used diagnostic methods.[12]

Imprint cytology :

Imprint cytology is technique which is accurate, simple, rapid, cost-effective ,do not require any special instrument and less time consuming and gives rapid tissue diagnosis.[12,13,15] It is a touch preparation in which tissue is touched on the slide and it leaves behind its imprint in the form of cells on glass slide.[13] Imprint cytology was first introduced by Dudgeon and Patrick in 1927, they examined fresh tissue by the wet film method on breast.[14]

This also used for the examination of individual cells and preserves the histological pattern.[15] The accuracy of the imprint cytology has been increased over the years both in breast pathology and in other body sites ,the average accuracy of (90-94%) in the past has reached (97- 98%) recent years.[13]

Table 4: showing sensitivity, specificity and accuracy of other studies

Authors	No.of cases	Sensitivity	Specificity	Accuracy	deferals
Scopa[16] 1990	82	100%	100%	100%	4(4.9%)
Khanna[17] 1991	86	98.4%	100%	98.8%	6(6.9%)
Veneti[18] 1996	351	97.1%	99.4%	98.3%	7(1.9%)
Albert [19]2000	173	96.5%	90%	95.4%	12(6.9%)
Bolkainy[20] 2008	122	92.2%	93.3%	92.5%	8(6.5%)
Khudier [15] 2009	107	96.3%	100%	98.9%	4(3.9%)

- Literature shows following points to improve the accuracy of imprint.[21,22,23]

1. The tissue surface should be flat for taking smears of imprint.
2. No portion of fat should protrude from the edges as this may lead to smudge the imprints.
3. If first imprint smear contains excess of tissue fluid and blood and the subsequent imprints gives better cytological results.

To obtain imprint nearest to one cell thickness, the amount of pressure applied at the time of imprinting should be varied.[21,22,23] Benign lesions usually requires more pressure in order to obtain sufficient cells for diagnosis while malignant tumors get imprinted more easily. [2] Imprinted piece or tissue should be flat and no fat should be left extruding from the surface.[24]

The study done by Tribe (1965) showed that gross examination of breast lump helps to distinguish between benign and malignant tumors in 95.1% cases.[21] Suen et al, (1978) examined 473 breast lesions and reported that grossly malignant lesions requires imprint cytology which provides rapid intraoperative diagnosis. [23]Singh et al., (1982) showed that imprint diagnosis gives 100% results when combined with clinical examination and gross appearance. [22] Scucchi 1997 described the advantages of imprint cytology with 2250 cases comparing with frozen section[32] and they are[25]

1. It is rapid with same accuracy rate with frozen section.
2. It has excellent preservation of cellular details devoid of freezing artifact
3. It helps in identifying focal macroscopically undetectable neoplatic lesions in larger tissue fragments.
4. Determines adequacy of small surgical samples for definitive histological examination.
5. Ability of wider sampling
6. It helps in sparing tissue for special investigation such as receptor studies,electron microscopy,immunocytochemistry and other biological studies.

Study done by Bell et al described that out of 137 patients, 141 lumpectomy specimens were obtained and imprint were taken from margins.The sensitivity was 80% and specificity was 85% and the over all accuracy was 85%. They concluded that imprint cytology is an accurate , simple,rapid and cost effective method for determining the margin status of breast conservation therapy specimen.[42]

Ramraje S et al, they examined 90 patients of breast lumps over a period of 2 years. Imprint smears were taken. On imprint cytology, out of 90 cases, 81 (90%) were diagnosed correctly and they described Imprint cytology along with clinical features and gross appearance of the excised mass can give an accurate diagnosis.[2]

Creager et al; they reviewed retrospectively the intraoperative imprint cytology margins of 141 lumpectomy specimens from 137 patients between May 1997 to 2001.They evaluated 758 separate margins. The sensitivity was 80%, the specificity was 85%, the positive predictive value was 40%, the negative predictive value was 97%, and the overall accuracy was 85%. Imprint cytology is an accurate, simple, rapid, and cost-effective method for determining the margin status of breast conservation therapy specimens intraoperatively.[27]

Mehar et al described the utility of imprint cytology on 35 benign lesions, 32(91.4%) were diagnosed correctly and 03(8.6) were false negative and on 65 malignant lesions 58 (89.2%) were diagnosed correctly, 07 (10.8%) were false negative. Finally concluded that imprint smear can be utilized as adjuvant to histological diagnosis and provides rapid diagnosis.[13]

Khudier et al showed Imprint cytology was obtained from (110) specimens of (107) patients with various breast lesions sensitivity and specificity of imprint cytology for both benign and malignant breast lesions were 96.3% and 100% respectively and over all accuracy was 98.9%.

concluded that Imprint cytology is simple, rapid, inexpensive and accurate method for intraoperative diagnosis of breast lesions and can be used as adjunct to frozen section.[15]

Sumiyoshi et al described total of 420 patients underwent breast cancer surgery. Subjects comprised 160 patients who underwent breast-conserving surgery and touch smear cytology. Results of the touch smear cytology were compared to those of the histological tissue analysis. Touch smear cytology displayed 70% sensitivity (14/20), 97.1% specificity (136/140) and a diagnostic accuracy of 93.8% (150/160).[28]

Ranjahan et al showed IC for benign and locally infiltrative tumors gave 100% accuracy and that for malignant tumor it was 34/35 (97%) accuracy.[12]

Frozen section

The frozen section technique is one of the reliable and accepted method in intraoperative consultation for more than 100 years.[29] It is used to identify the nature of the lesion, to evaluate the involvement of surgical margins in malignant tumors and to determine the adequacy of diagnosable material.[29] The main indication of frozen section is to determine the tissue sampled is malignant or benign.[30] The overall accuracy of frozen section reported in different studies vary from 91.5 to 97.4%.[31]

Table 5 :shows sensitivity, specificity and accuracy of other studies

Authors	No of cases	Sensitivity	Specificity	Accuracy
Abbasi [31] (2012)	200	92.3%	96%	90.3%
Bolkainy[20](2008)	128	100%	100%	100%
Haeri[29] (2002)	125	92.4%	100%	95.4%

The earliest use of frozen section technique is attributed to Dr.Welch of Johns Hopkins Hospital , in 1891 on benign breast tumor removed by Dr.Halstead.[32] Cullen described a rapid method of making permanent specimens from FS in 1895.[32] Wilson of Mayo clinic , developed FS staining method (methylene blue) in 1905.

[32]Hazard and Stevenson in 1949 introduced a technique which was compared to modern procedures using the cryostat. In this technique the fresh specimen was fixed with alcohol . The fixed block is then frozened between pieces of dry ice and cut at 10-15 um with a microtome knife.[32,33]

Dockerty in 1953 modified the Hazard and Stevenson technique in which the tissue was subjected to froze, unfixed, directly subjected to a Spencer microtome and cut at 10- 15 um section [34]In 1957 Teloh HA again modified the technique by directly placing the unfixed specimen into the freezing stage of the microtome and subjected the tissue to intermittent release of carbon dioxide gas causing rapid freezing, then cutting the tissue at 25um.[35,36]

FS helps to inform the surgeon about the HPE type, grade, adequacy of excision in malignancy with high concordance with paraffin report.[36,37]

Study done by David et al showed that 90% of FS block turn around times were within 20 minutes. This study included 700 laboratories world wide which measured from the time the pathologists received the FS specimens to the time that pathologists returned FS diagnoses to surgeons. [49]

Haeri et al in 2002 performed frozen section on 125 specimens of breast lesions and evaluated the accuracy of frozen section which showed sensitivity, specificity and accuracy for

cytological method were 87.5%, 95%, 90.5% and for the frozen section 92.4%, 100% and 95.4% respectively.[29]

Dener et al, examined 190 tumors in 186 patients with early invasive breast cancer treated by BCS. Breast tumor was excised with 2 cm macroscopic margin, and microscopicmargins examined by intraoperative frozen section. 160 cases had negative margins (no re-excision), but 30 patients (16%) underwent re-excision because of close/positive margins. Negative margins were obtained in 24 patients but six patients underwent mastectomy due to persistent involved margins. Local recurrence was 2.1% and systemic recurrence was 2.6% with mean 62 months follow-up. Tumor type, tumor size, extranodal extension and extensive intraductal component were significant predictors of re-excision by multivariate analysis.[26]

Bolkainy et al, The study was conducted on 130 breast mass lesions The specimens were subjected to FS and TSP.The accuracy of FS was found to be higher than TSP (100%). Thus, a sensitivity of 100% and a specificity of 100%. Whereas, TSP had a sensitivity of 92.99%, a specificity of 93.33% and an overall accuracy of 92.5%. TSP had also a negative predictive value of 80% and the positive predictive value was 97.65%.[20]

Dener et al ; described 190 tumors were detected in 186 patients with early invasive breast cancer treated by breast conservative surgery. Breast tumor was excised with 2 cm macroscopic margin, and microscopic margins examined by intraoperative frozen section**.** 160 cases had negative margins (no re-excision), but 30 patients (16%) underwent re-excision because of close/positive margins. Negative margins were obtained in 24 patients but six patients underwent mastectomy due to persistent involved margins. Local recurrence was 2.1% and systemic recurrence was 2.6% with mean 62 months follow-up. They concluded intraoperative frozen section is an effective procedure in reducing the need of second operation.[26]

Karve et al in 2005 showed 237 breast lumps comprised of 157 malignant lesions and 80 benign lesions. The false negativity rate was 0.42%, false positive rate 0%,deferral rate 0.84% and accuracy rate was 99.57% .False negative rate for axillary nodes was 20% and accuracy was 80% . Concluded that frozen section despite the ragging popularity of FNAC in the following setting (1) Difficult cytology (2)Evaluation of lumpectomy margins(3)intraoperative nodal status.[38]

Yun 2007 examined 13,243 breast lesions diagnosed with intra-operative frozen sections. **117** cases (0.9%) were falsely diagnosed, with one false positive case and 116 false negative cases. The diagnosis of 47 cases (0.4%) was delayed. Concluded that Intra-operative frozen section can accurately identify breast lesions in many instances, leading to few errors on account of more diagnostic experience and understanding of diagnostic limitations.[39]

Ceserni et al frozen section was obtained for 2110 cases . the study showed 22 cases of false negative and 1 case of false positive. [40]Also explained the errors were due to [40]

1. Misinterpretation
2. Poor quality of frozen section
3. Sampling error during sectioning
4. Ignorance of macroscopic finding
5. Lesions difficult to interpret (DCIS)
6. Sections not deep enough.

MATERIALS AND METHODS

This study was done in the department of pathology,SDUMC,Kolar in coordination with department of surgery. Over a period of 2year study total 82 patients presented with palpable lump in the breast were considered for the study . All clinically palpable breast lesions which are surgically resected were examined by both imprint cytology and frozen section and compared with histopathological sections which was considered as gold standard.

Imprint slide preparation:

Clear glass slide was touched gently on the cut surface of the specimen at several places. Three slides were fixed immediately in ethanol for methylene blue, pap and H&E stain. Another slide is air dried for giemsa stain. Methylene blue stained slide examined immediately to assess cellularity and for the possible diagnosis.

Frozen section was done using Leica CM 1100 cryostat.

Procedure:

Specimen is sent for frozen section in normal saline. Tissue is measured then it is sectioned at an interval of 1cms, tissue is examined for obvious features.

The tissue was frozened as quick as possible in order to avoid ice crystal formation resulting in artifact and poor morphological preservation.

Cryostat was is kept ready at -20° C to -30° C before hand.

Tissue from representative area is taken and full form kept on the slide with proper orientation, OCT was added till it covers the tissue and freezed.

OCT frozen section embedding medium was placed on a cryostat object disk.

The frozen specimen was positioned in the center of the object disk and the disk was placed on the cryobar in the cryostat to begin the quick freeze process.

As the OCT freezes it turns from a clear gel to white solid substance.

Before the disk is frozen solid OCT was added to cover the top of the specimen and quickly a heat extractor was placed on top of the specimen

Object will be placed on disk in the microtome by object disk holder and the screw will be tightened or clamped.

The ratchet was engaged on the micrometer gear, trimmed until tissue appears and the first two or three sections were discarded.

Cutting was done slowly and continued until a full tissue section were obtained.

The slides were lowered onto the blade, keeping the slide parallel to the section. As the tissue comes into contact with the slide the OCT and tissue will melt causing the tissue to adhere to the slide.

The slides were placed in fixative of 95% ETOH for 30 sec and sections were stained with rapid H&E, pap stain.

On imprint cytology cellular details, pattern of arrangement of cells, nuclear morphology and other specific nuclear details with respect to particular tumor was analysed .

Frozen section was analysed based on tissue architecture, cellular details, mitotic activity, necrosis and lymphovascular invasion.

Both morphological features of imprint and frozen section were compared with histopathological feature.

Rest of the tissue is fixed in 10% formalin and processed in Leica Histokinete. Processed tissue is embedded in paraffin wax and blocks are made.

The stains used in the study

1. Papanicolaou method[50]

   Procedure: fix the smears in 95%alcohol for 15 – 30minutes

   Hydrate in 70% alcohol and 50% alcohol for 2 minutes

   Rinse in water ,1minute

   Stain in Harris's haematoxylin, 5 mins

   Rinse in water 2min

   Differentiate in 0.5% aqueous hydrochloric acid 10 seconds

   Rinse, bluing done in water 2 min

   Dehydrate in 50%,70%,90% alcohol for 2 min each

   Stain in OG 6, 2 mins

   Rinse in 95%alcohol for 2min each

   Stain in EA50 ,3mins

   Rinse in 95%alcohol,1min

2. Rapid haematoxylin and eosin stain[50]

   Fixation in alcohol for 20 secs

Rinse in water

Stain inHarris's haematoxylin for 1.5min to 2 min

Rinse in water and dip in 1%acid alcohol and again rinse in water

Stain in eosin for 10sec to 15 sec

Rinse in tap water

Dehydrate clear and mount in DPX

3. Standard haematoxlin and eosin for paraffin section[50]

   Dewax sections, hydrate through graded alcohols to water

   Remove fixation pigments if necessary

   Stain in harris haematoxlin for 10-20 min

   Wash in running tap water until sections blue (for 5min or less)

   Differentiate in1%acid alcoholfor 5-10seconds

   Wash well in tap water untilsections for again blue(for min or less)

   Stain wih 1%eosin for 1min

   Wash in running tap water for 1-5min

   Dehydrate through alcohol,clear and mount

4. Methylene blue stain(3-4mins of timing)[50]

   Slides placed in 90% alcohol for fixations a

   Keep in a slide rack flood with 1% methylene blue and leave for ½-1min

   Rinse rapidly in water transfer to pad of filter paper and blot firmly

   Flood with xylene and mount in DPX

RESULTS

Over a period of 2 year study 82 patients resected surgical specimen were selected for further study. Each slide of imprint cytology, frozen section and histopathology were coded with the help of guide and observations were recorded on coded slides. Two pathologists observed the findings.

- Age distribution:
- In our study benign tumors were commonly seen between less than 30yrs of age group(18-30 yrs) and patients with more than more 40 yrs frequently presented with malignant tumors.

  Mean age of subjects was 37.55 ± 14.24 years.

Table 6: Age distribution of subjects

		Frequency	Percent
Age grouping	**<30 yrs**	30	36.6
	31 to 40 yrs	21	25.6
	41 to 50 yrs	18	22.0
	> 50 yrs	13	15.8
	Total	82	100.0

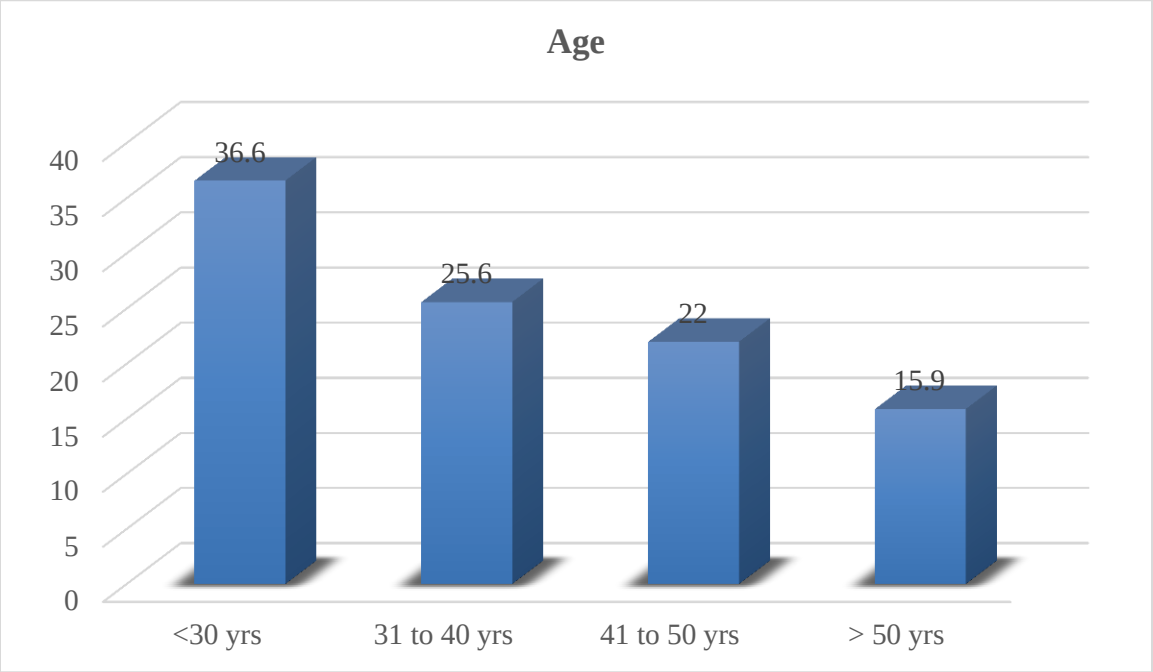


Figure 1: Age distribution of subjects

- In our study 97.6% female presented with breast lesions compared to 2.4% male.

1. Sex distribution : Females were frequently affected compared to males

Table 7: Sex distribution of subjects

		Frequency	Percent
Sex	**Female**	80	97.6
	Male	2	2.4
	Total	82	100.0

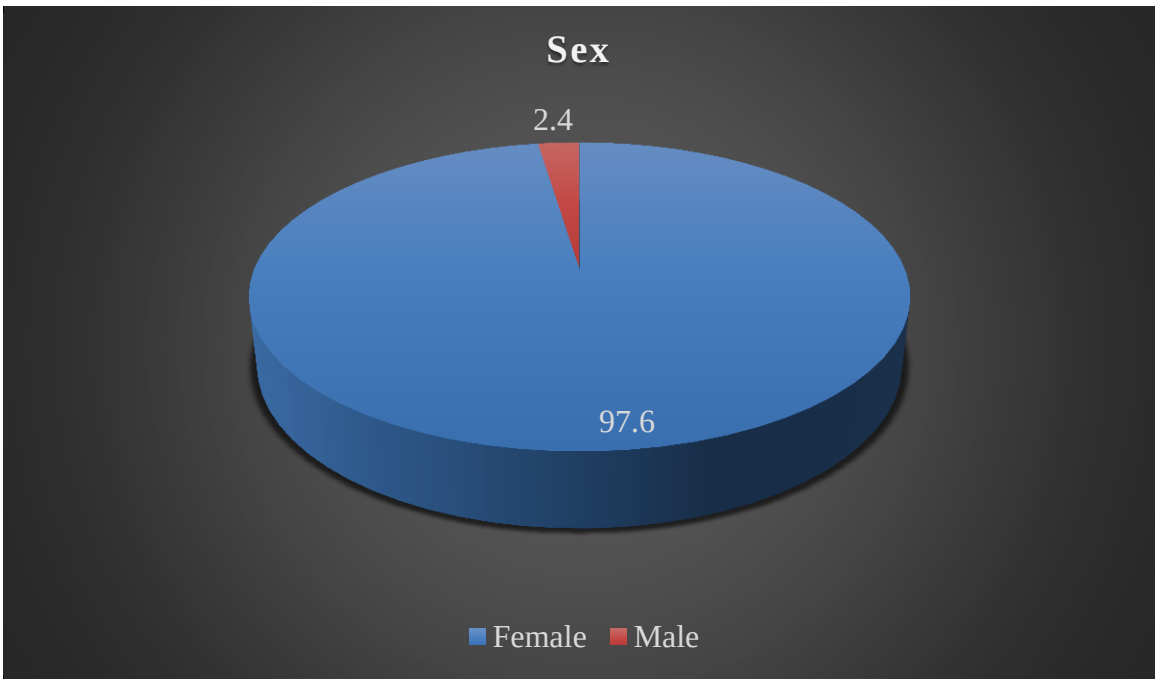


Figure 2: Sex distribution of subjects

In our study breast lumps were frequently seen on left side compared to right. As represented in table 8

2. Site distribution:

Table 8: Site of Breast Lesion

		Frequency	Percent
Site	**Left**	45	54.9
	Right	37	45.1
	Total	82	100.0

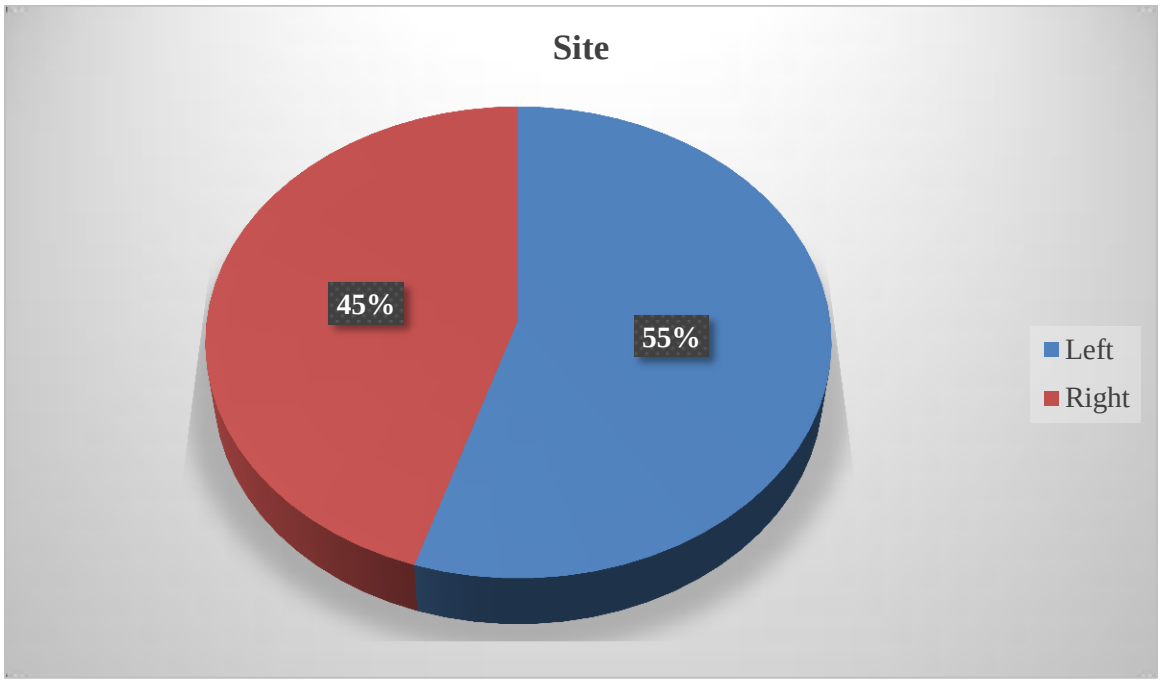


Figure 3: Pie diagram showing site of lesion

Clinically total 45 benign lesion and 37 malignant lesions were seen. Benign lesions (54.88%) were comparatively higher than malignant lesions (45.12%). Predominantly fibroadenoma was commonly seen followed by carcinoma breast.

Table 9: Distribution of subjects according to clinical diagnosis

Clinical diagnosis	**Frequency**	**Percentage**
Carcinoma breast	37	45.12%
Fibroadenoma	37	45.12%
Fibrocystic disease	4	4.90%
Breast abscess	2	2.43%
Mastitis	2	2.43%
Total	82	100.00

- Gross findings : Tumors were divided in two groups based on following findings.

	Benign lesions	Malignant lesions
Size	Small	Large
Consistency	Firm	Firm to hard
Appearance	Well circumscribed	Poorly circumscribed
Cut section	Grey white appearance	Gritty to cut, with grey white appearence
Surrounging areas	May be cystic change	Hemorrhagic,necrosis,cystic

- Smears of imprint categorized into 2 groups

Benign smears	Malignant smears
Thin	Thick
Uniform shaped cells	Pleomorphic cells
In clusters	Sheets and clusters
Hypocellular	hypercellular

- Total 82 cases comprising 47 benign lesions and 35 malignant lesions on HPE

Benign lesions	47
Malignant lesions	35
Total	82

- Total 82 cases were examined by both imprint cytology and frozen section and compared with gold standard HPE.
- Out of 82 cases 4 cases were inadequate i.e; both imprint cytology and frozen section of 4 cases were inadequate so these cases were excluded for statistical analysis.
- **Comparison of imprint cytology with gold standard HPE**: showed out of 78 cases 43 were benign lesions which were correctly diagnosed by imprint cytology and out of 35 malignant lesions 29 were correctly diagnosed and 6 false negative were seen. There was no false positive results in our study which was statistically significant.

- In our study imprint cytology compared with gold standard HPE showed sensitivity, specificity, PPV, NPV and accuracy were 100%,82.86%,87.76%,100% , 92.31%. represented in table 10

Table 10 : Diagnostic ability of Imprint cytology in breast lesions with respect to gold standard HPE.

		HPE		**Total**	**χ 2 , df, p value**
		Benign	**Malignant**		
Imprint cytology	**Benign**	43	6	49	56.715, 1, 0.0001**
	Malignant	0	29	29	
Total		43	35	78	

** statistically significant.

Parameter	**Estimate**	**Lower - Upper 95% CIs**
Sensitivity	100%	(92.44, 100)
Specificity	82.86%	(67.32, 91.9)
Positive Predictive Value	87.76%	(77.42, 94.71)
Negative Predictive Value	100%	(88.3, 100)
Diagnostic Accuracy	92.31%	(84.94, 96.6)
Cohen's kappa (Unweighted)	0.842	(0.6332 - 1.061)

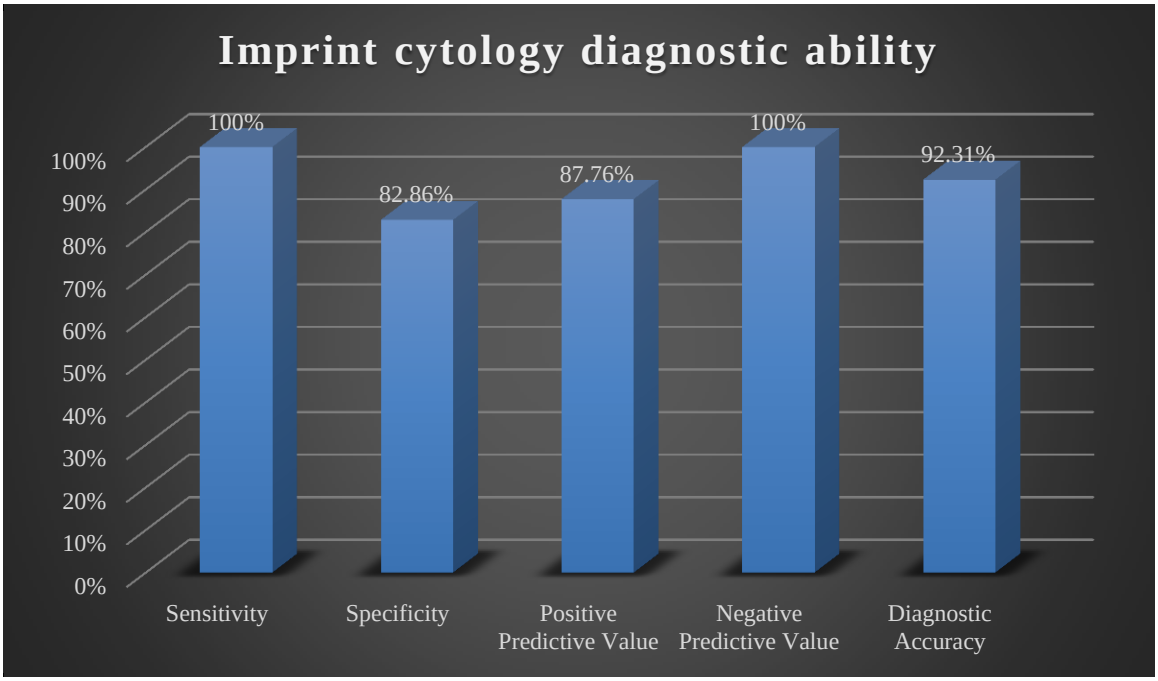


Figure 4: Bar diagram showing diagnostic ability of Imprint cytology

Comparion of frozen section with gold standard HPE:

Out of 78 cases frozen section diagnosed correctly 43 benign lesions and out of 35 malignant lesions 33 were correctly diagnosed and 2 false negative (FN) results and no false positive results were seen. Frozen section showed sensitivity, specificity, PPV, NPV, accuracy shows 100%,94.29%,95.56%,100% and 97.44% represented in table 11.

Table 11: Diagnostic ability of frozen section in breast lesions with respect to gold s tandard HPE.

		HPE		**Total**	**χ 2 , df, p value**
		Benign	**Malignant**		
Frozen section	**Benign**	43	2	45	70.274, 1, 0.00001**
	Malignant	0	33	33	
Total		43	35	78	

** statistically significant

Parameter	**Estimate**	**Lower - Upper 95% CIs**
Sensitivity	100%	(92.44, 100)
Specificity	94.29%	(81.39, 98.42)
Positive Predictive Value	95.56%	(86.29, 98.87)
Negative Predictive Value	100%	(89.57, 100)
Diagnostic Accuracy	97.44%	(91.54, 99.33)
Cohen's kappa (Unweighted)	0.9479	(0.7336 - 1.166)

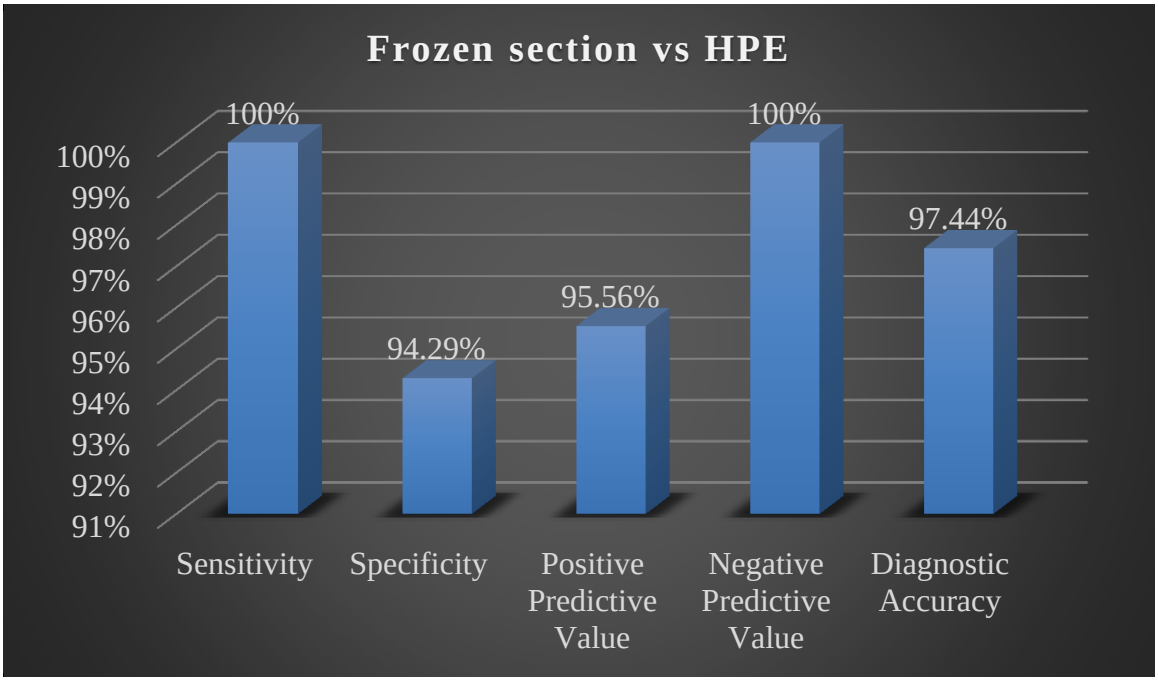


Figure 5: Bar diagram showing diagnostic ability of frozen section in Breast lesions

- Comparison of both imprint cytology and frozen section showed out of 78 cases 45 benign lesions were correctly diagnosed. Out of 33 malignant lesions 29 were correctly diagnosed. 4 false negative were seen with no false positive results. Sensitivity, specificity, PPV,NPV and diagnostic accuracy were 91.84%,100%,100%87.88% and 94.87% as represented in table 12

Table 12: Association between Imprint cytology and frozen section

		Frozen section		**Total**	**χ 2 , df, p value**
		Benign	**Malignant**		
Imprint cytology	**Benign**	45	0	45	62.950, 1, 0.0001**
	Malignant	4	29	33	
Total		49	29	78	

** Significant

Parameter	**Estimate**	**Lower - Upper 95% CIs**
Sensitivity	91.84%	(92.73, 100)
Specificity	100%	(72.67, 95.18)
Positive Predictive Value	100%	(82.14, 97.03)
Negative Predictive Value	87.88%	(88.3, 100)
Diagnostic Accuracy	94.87%	(88.12, 98.09)
Cohen's kappa (Unweighted)	0.8932	(0.6812 - 1.112)

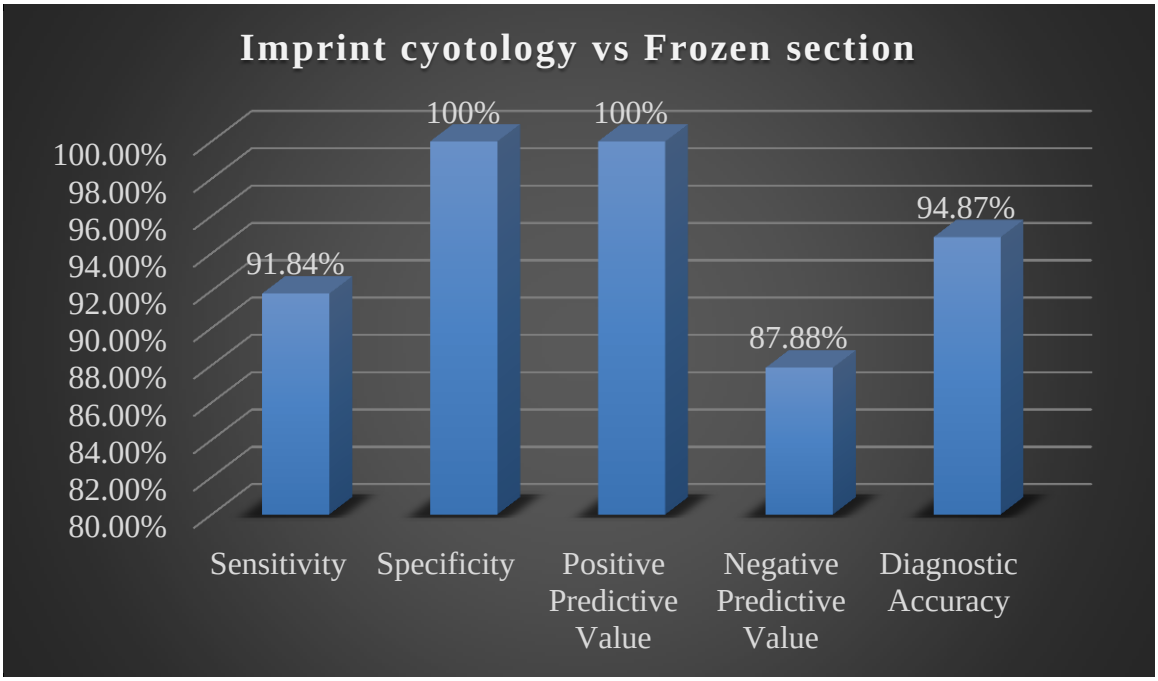


Figure 6: Bar diagram showing diagnostic ability of Imprint cytology in comparison with frozen section

Association of both imprint cytology and frozen section with HPE showed 43 benign lesions which were correctly diagnosed but out of 35 malignant lesions 2 were false negative and 4 cases showed both benign and malignant component. There was significant association between HPE and Imprint with Frozen section. As represented in table 13

Table 13: Comparison of HPE findings with Imprint and Frozen section combined

		HPE		Total
		Benign	**Malignant**	
Both imprint and frozen	**Benign**	43	2	45
	Benign + Malignant	0	4	4
	Malignant	0	29	29
Total		43	35	78

χ 2 = 70.274, df = 2, p <0.0001**

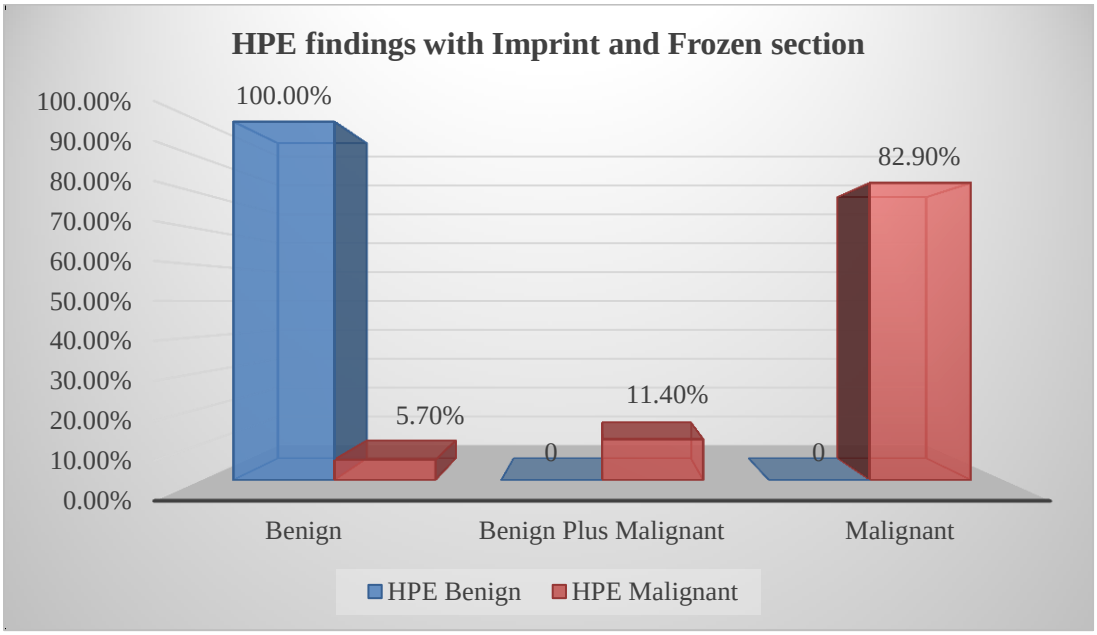
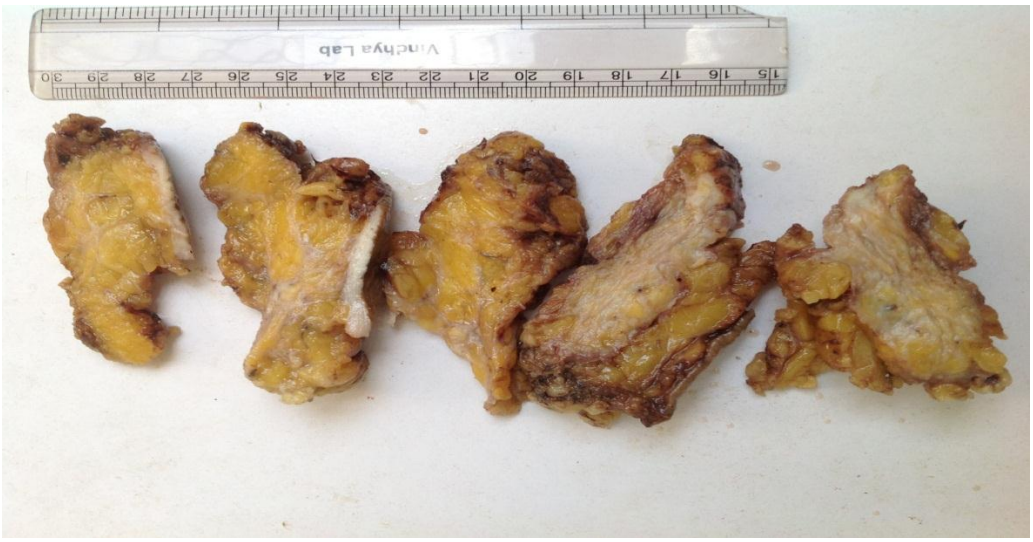
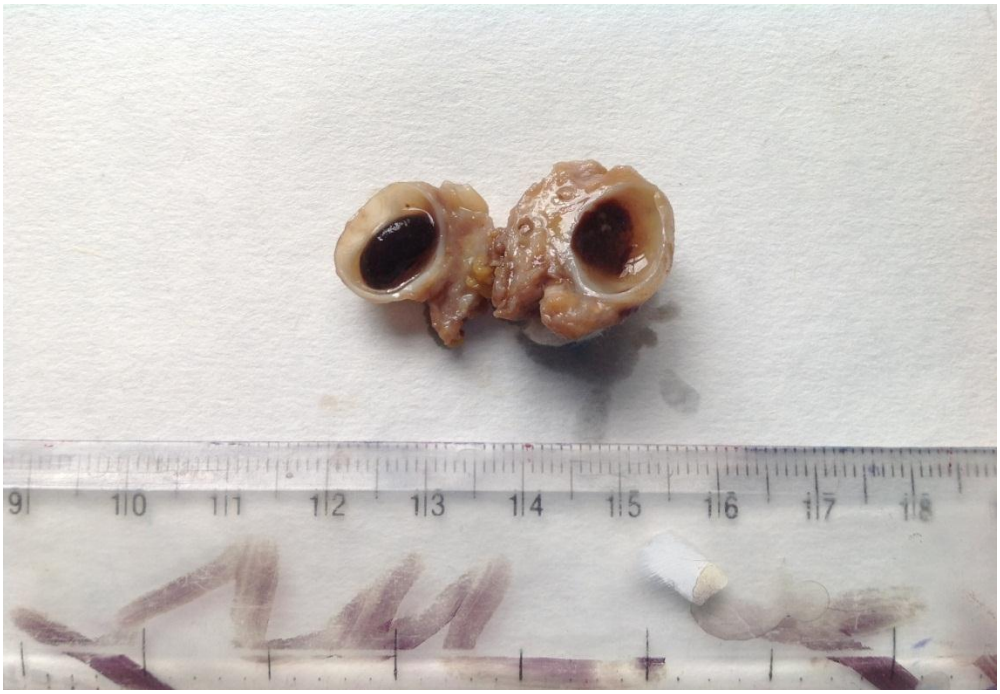


Figure 7: Bar diagram showing HPE findings with imprint and frozen section



Gross specimen of carcinoma breast



Gross specimen of fibrocystic disease.

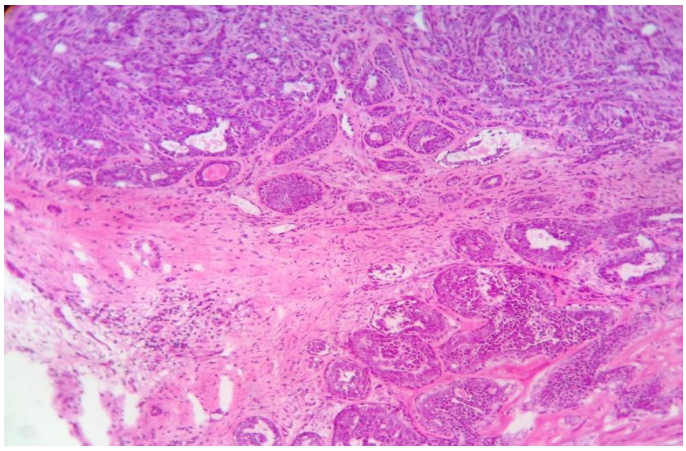


Fig 23: Infiltrating ductal carcinoma (HPE)

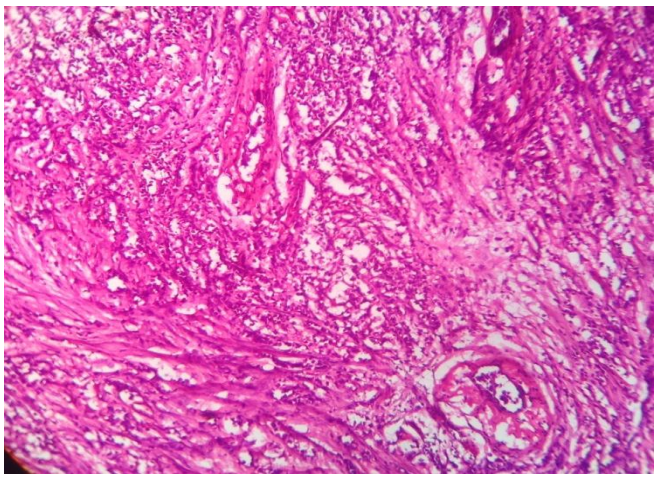


FIG24: Frozen section : ductal carcinoma

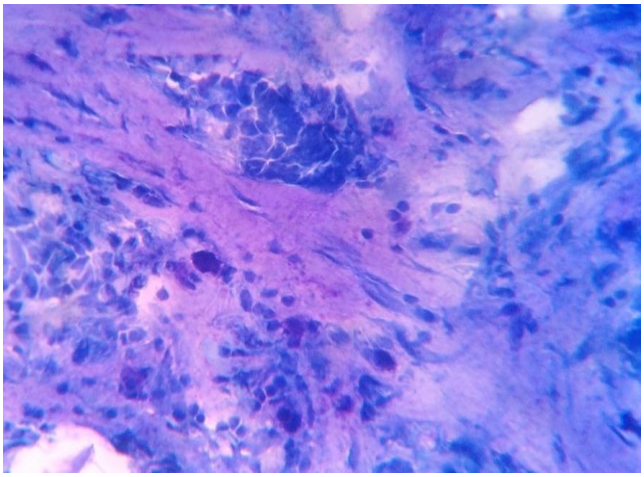


Fig 25: Frozen section of ductal carcinoma(methylene blue stain)

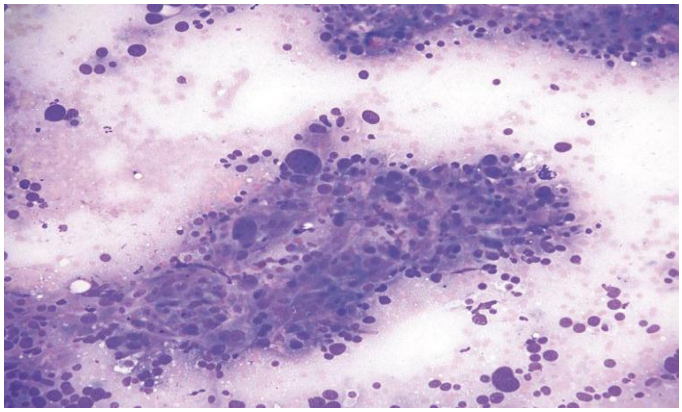


Fig 26:Imprint of ductal carcinoma

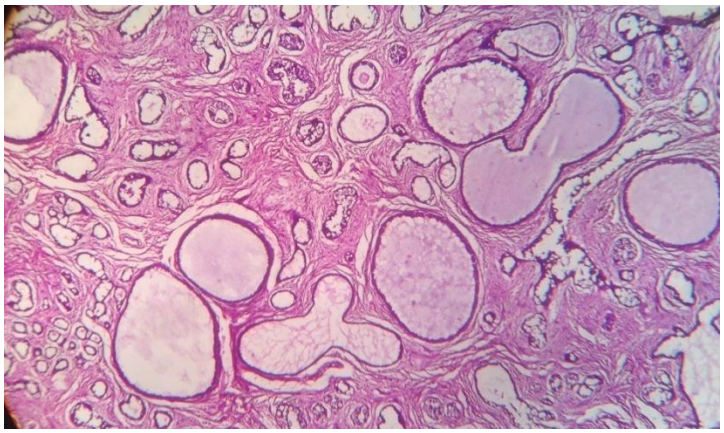


FIG 27: HPE of fibrocystic disease

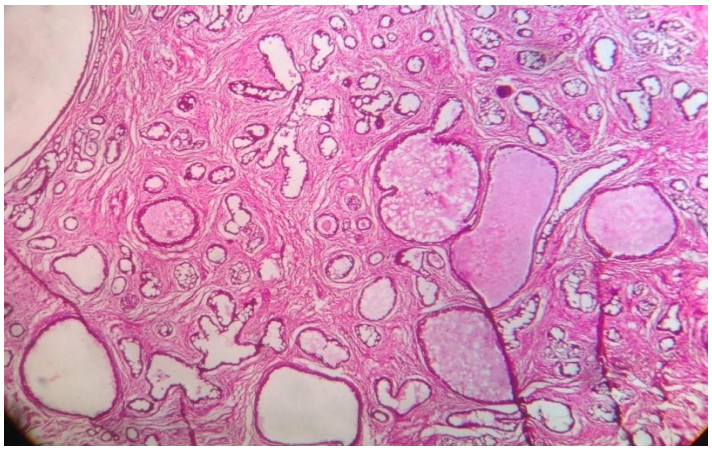


Fig28 : Frozen section of Fibrocystic disease

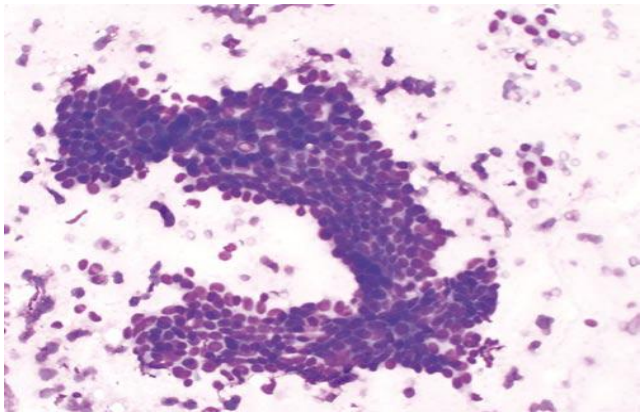


Fig29: Imprint of fibroadenoma

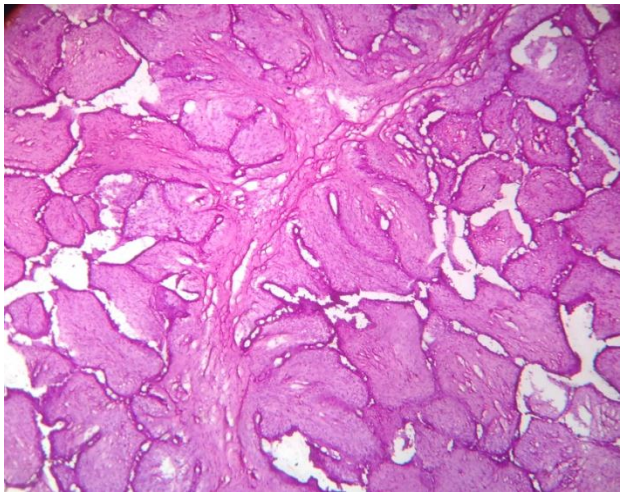


Fig30: Frozen section of fibroadenoma

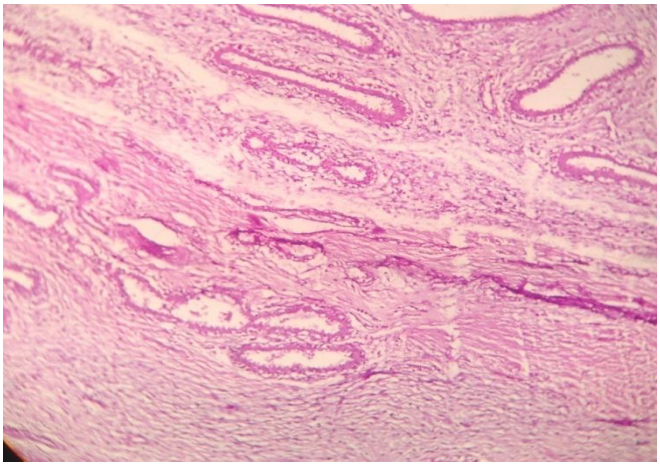


FIG 31: HPE of fibroadenoma

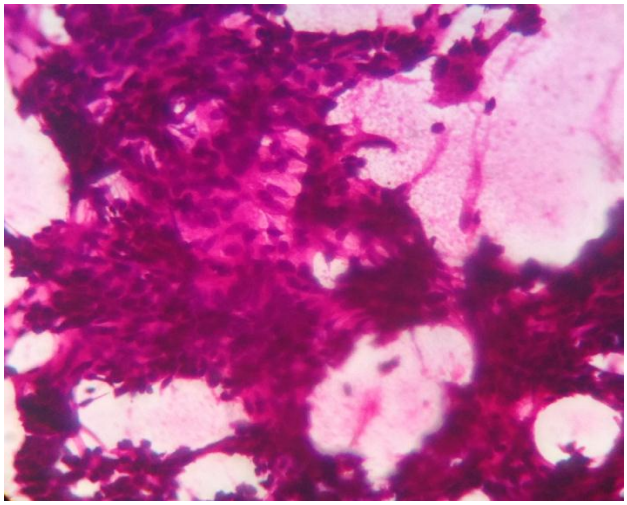


Fig32: Imprint of papillary neoplasm

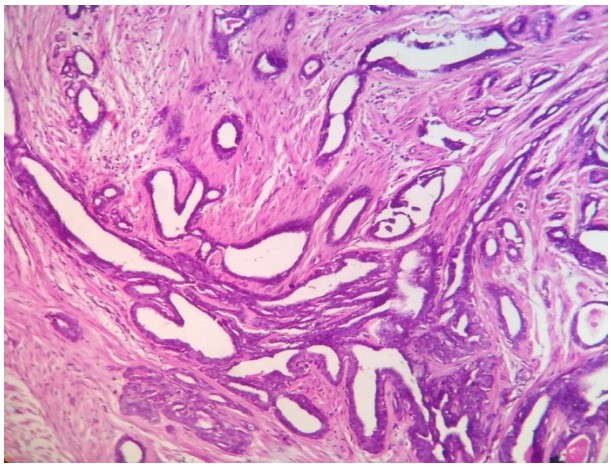


Fig33: Frozen section of intraductal papilloma

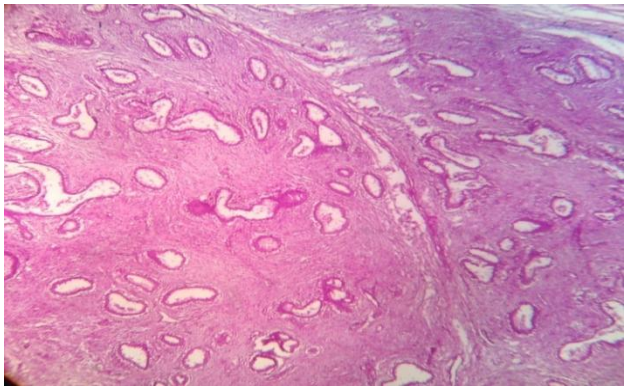


Fig 34 HPE of intraductal papilloma

DISCUSSION:

Breast carcinoma is the second most common malignant tumor among Indian women.[1] Intraoperative procedure like imprint cytology(IC) and frozen section(FS) have an important role despite the ragging popularity of aspiration cytology in cases of difficult cytology, evaluation of lumpectomy margins, intraoperative nodal status.[41]

In our study benign tumors were commonly seen between less than 30yrs of age group(18-30 yrs) and patients with more than more 40 yrs frequently presented with malignant tumors.

Our study observations are similar to the study of Khudier et al(2009) which showed most of the benign tumors were seen in second decade and most of the malignant tumors were seen in fourth decade of life.[15]

Breast cancer are observed rarely below the age of 40 but the proportion of tumors classified as such in young breast cancer cases are also similar.[1] it may depend on these risk factors including geographical, cultural ,lifestyle, reproductive variables.[1]

Comparison of age with other studies:

Authors	Age range	Mean age
Khudier 2009[15]	15-70	36- 14
Ramraje2012[2]	12-70	35-14
Present study	16-70	37-14

In our study 97.6% female presented with breast lesions compared to male 2.4% which was similar to the study done by Ramraje et al[2] 2012. In their study 96.6% of females presented with breast lesions compared to males 2.4%.[2] Study done by Harnish et al also represents same showing (60%) femles are more commonly affected compared with (40%)males.[14]

Comparison of sex distribution with other studies:

Authors	Female		Male	
	No of cases	Percentage	No of cases	Percentage
Ramraje 2012[2]	90	96.6%	90	3.3%
Harnish 2010[14]	119	60%	119	40%
Present study	82	97.6%	82	2.4%

In our study clinically 45 patients presented with benign lesions and 37 patients presented with malignant lesion. In a study done by Ramraje et al[2] showed out of 90 cases 45 cases clinically presented with benign lesion and rest with malignant lesions.[2]

Distribution of subjects according to clinical diagnosis & comparison with other studies

Authors	Benign tumors	Malignant tumors
Ramraje 2012[2]	45 (50%)	45(50%)
Present study	45 (54.87%)	37(45.12%)

In the Present study total 82 HPE showed 47 benign lesions and 35 malignant lesions which is similar to Khudier et al 2009 in this study total 110 cases benign lesions were more 81 compared to malignant lesions 29 cases.[15]

Also present study showed fibroadenoma and IDC being more common .Similar to our study Solanki et 1977 al showed FA, IDC.[21]

Comparison with HPE diagnosis with other studies:

Authors	Benign lesions	Malignant lesions	Total
Bolkainy 2008[22]	35	95	130
Ramraje 2012[2]	45	45	90
Present study	47	35	82

Gross features of our study showed benign lesions small, firm, well circumscribed with cut section showing grey white with whorled appearance with cystic change and malignant lesion were large, firm to hard, irregular shape, gritty to cut. Cut section shows hemorrhagic, cystic, necrosis .Similar to the study of Ramraj 2012.[2]

In the present study imprint smears were categorized into two,i.e benign and malignant lesions which showed Benign lesion - thin, uniform, hypocellular, found in clusters. Malignant lesions were showing thick, hypercellular arranged in sheets, clusters. These observations were similar to the study of Ramraje 2012[2]

In our study total 82 cases were taken for both touch imprint cytology and frozen section. Finally these were compared with gold standard HPE sections. Out of 82 cases 4 cases were inadequate in both imprint cytology and frozen section due to sampling error, so these cases were excluded for statistical analysis. The literature shows limitations of both imprint cytology and frozen section. Imprint cytology shows inadequacy smear rate ranging from 2.95 to 10%.[15] Also limitations of F.S slides were observed suboptimal or inadequate because of necrosis, hemorrhage, calcification, non representative sampling or other technical factors .[31]

Present study was similar to the study done by Khudier et al[15] 2009 showed unsatisfactory imprint smears were seen in 8.2% (9 cases) comprised of fibroadenoma, fibrocystic disease, fat necrosis, gynecomastia. Inadequacy in case of fibroadenoma could be attributed to the excess of fibroadipose tissue , fibrocystic disease showed only inflammatory cells , fat necrosis showed only fat cells, in infiltrating ductal carcinoma(NOS) and phylloids because of excessive fibrosis obscuring the cytological details. Also it could be due to technical or procedural errors which are commonly documented limitations of both the procedures.[2]

The high inadequacy rate described by various authors is attributed to desmoplasia, technical error and inexperience .[15,42] Ceserni et al[40] explained the errors in frozen section were due to

- Misinterpretation
- Poor quality of frozen section
- Sampling error during sectioning
- Ignorance of macroscopic findings
- Lesions difficult to interpret(DCIS,

Comparison of imprint cytology with gold standard HPE: showed out of 78 cases 43 were benign lesions which were correctly diagnosed by imprint cytology and out of 35 malignant lesions 29 were correctly diagnosed and 6 false negative were seen. There was no false positive in our study which was statistically significant.

In our study imprint cytology showed sensitivity, specificity, PPV, NPV and accuracy were 100%,82.86%,87.76%,100% , 92.31%

Comparison of sensitivity, specificity and accuracy of imprint cytology with other studies

Authors	No of cases	No of false positive	No of false negative	Sensitivity	Specificity	Accuracy
Khudier[15] 2009	109	0	1(0.99%)	96.3%	100%	98.9%
Bolkainy[20] 2008[41]	122	1(1.12%)	2(6.06%)	92.2%	93.3%	92.5%
Present study	78	0	6(7.6%)	100%	82.8%	92.5%

Total 6 cases of imprint cytology showed false negative (Paraffin sections of theses cases showed IDC, medullary carcinoma with DCIS, phylloids, DCIS, Microinvasive carcinoma and ILC) these tumors were characterised by low cellularity.

Ramraje et al[2] 2012 described that carcinoma with more fibrous stroma may yield less cells which can be mistaken for benign lesions in imprint cytology. [2]

Comparion of frozen section with gold standard HPE: Out of 78 cases frozen section diagnosed correctly 43 benign lesions and out of 35 malignant lesions 33 were correctly diagnosed and 2 false negative (FN) results and no false positive results were seen. Frozen section showed sensitivity, specificity, PPV, NPV, accuracy shows 100%,94.29%,95.56%,100% aan 97.44%.

Comparison of sensitivity, specificity and accuracy of frozen section with other studies

Authors	No of cases	Sensitivity	Specificity	PPV	NPV	Accuracy
Abbasi[31] 2012	200	92.3%	80	96	66	90.3
Bolkainy[20] 2009	128	100	100	100	100	100
Present study	78	100	94.2%	95.9%	100%	97.56%

The 2 false negative results HPE showed DCIS with desmoplastic stroma and malignant phylloids which was due to sampling error.

.A study of large retrospective analysis of frozen section diagnoses documented that diagnostic errors related to intraoperative consultations can be divided into the following 4 groups: those resulting from interpretation (57%), microscopic sampling (24%), gross sampling (9.5%), and lack of communication between the pathologist and surgeon (9.5%).[37]

Literature shows false negative are often associated with diagnostic discrepancies ranging from 0.4 – 2.56% .

Ceserni et al[48] 2110 cases of frozen section showed 22 cases of FN and 1 FP. Explained the errors were due to

- Misinterpretation
- Poor quality of frozen section
- Sampling error during sectioning
- Ignorance of macroscopic findings
- Lesions difficult to interpret
- Sections not deep enough

Also described the limitations of FRZ diagnosis only small pieces of tissue is selected for forzen section. Intraoperatively cant represent overall tumor profile of tumor especially phylloids and adenofibroma, atypical hyperplasia and DCIS and microinvasive carcinoma. [48]

Comparison of both imprint cytology and frozen section alone showed out of 78 cases 45 benign lesions were correctly diagnosed. Out of 33 malignant lesions 29 were correctly diagnosed. 4 false negative were seen with no false positive results. Sensitivity, specificity, PPV,NPV and diagnostic accuracy were 91.84%,100%,100%87.88% and 94.87%. In those 4 cases(HPE diagnosis showed DCIS with medullary carcinoma, microinvasive carcinoma,ILC and IDC) . As earlier qoated limitations of both imprint cytology and frozen section[37,40,42]

Comparison of imprint cytology and frozen section with gold standard HPE showed following results in the present study

Comparison	No of cases	Sensitivity	Specificity	PPV	NPV	Accuracy
Imprint cytology	78	100%	82.8%	88.6%	100%	92.5%
Frozen section	78	100%	94.9%	95.9%	100%	97.5%

Present study is similar to the study of Bolkainy et al[20] 2009 FS is superior to TSP in intraoperative diagnosis of breast mass lesions. [41]Accuracy of frozen section (100%) was higher compared to imprint cytology(92.6%).

.4 cases of imprint cytology which were false negative, on doing frozen section 3 tumors turned out to be malignant. By this we can tell that frozen section is preferable over imprint for intraoperative consultation however cytology is better than frozen section.

Another one case which was false negative by frozen section may be due to sampling error as quoated in literature (Ciserani et al)[40] This highlights the importance of frozen section over imprint cytology. Only 4 cases showed both benign and malignant component in imprint cytology and frozen section when compared with gold standard HPE, so a reasonable statistical analysis could not be done.

SUMMARY:

Total 82 cases were considered for the study.

Both imprint cytology and frozen section of breast lesions were compared with gold standard HPE

Mean age of the patient is 37

Breast lesions were observed more frequently in females (97.4%) compared to males (2.4%)

Breast lesions were more common on left side (54.9%) compared to right side(45.1%)

Clinically benign lesion(54.88%) were more compared to malignant lesions(45.12%)

HPE diagnosis showed benign lesions being higher than malignant lesion and more common were fibroadenoma and infiltrating ductal carcinoma.

Grossly lesions were grouped into 2 i.e benign and malignant

Out of 82 cases 4 cases were inadequate as both imprint cytology and frozen section both were inadequate so these cases were excluded from statistical analysis

Imprint cytology diagnosed all 43 benign lesions correctly and out 35 malignant lesions 29 were diagnosed correctly with 6 FN results and no FP results were seen

Imprint cytology showed sensitivity, specificity and accuracy of 100%,82.86% and 92.31%

Frozen section diagnosed all 43 benign lesions correctly and out of 35 malignant lesions 33 were correctly diagnosed with 2 FN results and no FP results were seen

Frozen section showed sensitivity, specificity and accuracy of 100%,94.29% and 97%

Correlation of both imprint and frozen section alone showed 45 benign lesions and out of 33 malignant lesions 29 were correctly diagnosed with4 FN results and no FP results were seen.

Imprint cytology and frozen section combined efficacy showed sensitivity, specificity and accuracy of 91.84%, 100% and 94.87%

Both imprint cytology and frozen section compared with HPE showed 4 both benign and malignant component which showed significant association was found.

CONCLUSION

FS is superior to imprint cytology when compared with gold standard HPE in intraoperative diagnosis of breast mass lesions.

When the FS equipment is lacking, imprint cytology could be a reliable alternative with limited technical, financial provided that an experienced cytopathologist is available

The predictive value analysis indicated that a positive diagnosis by imprint cytology is more reliable than a negative one.

Imprint cytology can be used as an adjuvant to FS in the intraoperative consultations.

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PROFORMA

- Name:
- Age:
- Sex:
- Religion:
- Occupation:
- Chief complaints:
- History of presenting illness:
- History of trauma:
- History of radiation exposure:
- Dietory history:
- Personal history:
- Marital history –

  Married or unmarried:

  Consanguineous marriage:

  Method of contraception used: Duration:

- Past history:
- Family history:

- Drug history:
- Obstetric history:

  Age of menarche: Age of menopause:

  No. of pregnancies: Age of pregnancy:

  GPLA: Menstral history:
- Lactational history- Breast fed or not:

  Duration:

  Any burning sensation during lactation:
- Physical examination –

  Pallor: clubbing: oedem: jaundice: lymphadenopathy:
- Per vaginal and per rectal examination:
- Systemic examination:

  CNS:

  RS:

  CVS:

  P/A:
- Local examination:

  Engorged veins/ skin

  Number

  Site

  Margins

  Nodules

  Unilateral or bilateral

  Size

  Consistency

Surface

Tenderness

Adherence to underlying structures

Nipple – position, size,retraction,shape,discharge,surface

Areola – crack, discharge

Ulcer-

Lymphnodes – number,site,size,consistency, mobile

- Investigation –
- FNAC
- Imprint cytology
- Frozen section
- Gross
- Histopathology
- Conclusion