A STUDY ON ESTROGEN AND PROGESTERONE RECEPTOR STATUS IN FINE NEEDLE ASPIRATES OF BREAST CARCINOMA: COMPARISON OF THE IMMUNOHISTOCHEMICAL EXPRESSION OF ER PR IN CELL BLOCK AND BIOPSY

Dissertation submitted on partial fulfillment
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INSTITUTE OF PATHOLOGY
MADRAS MEDICAL COLLEGE,
CHENNAI – 600 003.

MADRAS MEDICAL COLLEGE
THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY
CHENNAI - TAMILNADU

MAY 2018

CERTIFICATE

This is to certify that this dissertation entitled **"A STUDY ON ESTROGEN AND PROGESTERONE RECEPTOR STATUS IN FNAC BREAST CARCINOMA: COMPARISON OF IMMUNOCYTOCHEMISTRY AND IMMUNOHISTOCHEMISTRY"** is the original work of **Dr. S.KAVITHA,** in partial fulfilment of the requirement for **M.D., (Branch III**) in Pathology examination of the Tamilnadu Dr.M.G.R. Medical University to be held in May 2018.

PROF. DR.R.PADMAVATHI, M.D.,
Professor of Pathology,
Institute of Pathology,
Madras Medical College,
Chennai- 600003.

PROF. DR.BHARATHI VIDHYA JAYANTHI, M.D.,
Director and Professor of Pathology,
Institute of Pathology,
Madras Medical College,
Chennai- 600003.

PROF.DR.R. NARAYANA BABU, M.D. D.C.H.,
DEAN,
Madras Medical College and Rajiv Gandhi
Government General Hospital,
Chennai - 600003.

DECLARATION

I, **Dr. S.KAVITHA,** solemnly declare that the dissertation titled **"A STUDY ON ESTROGEN AND PROGESTERONE RECEPTOR STATUS IN FNAC BREAST CARCINOMA: COMPARISON OF IMMUNOCYTOCHEMISTRY AND IMMUNOHISTOCHEMISTRY"** is the bonafide work done by me at the Institute of pathology, Madras Medical College under the expert guidance and supervision of **Prof. Dr.R. Padmavathi M.D.,** Professor of Pathology, Institute of pathology, Madras Medical College. The dissertation is submitted to the Tamilnadu Dr. M.G.R Medical University towards partial fulfilment of requirement for the award of M.D., Degree (Branch III) in Pathology.

Place: Chennai Signature of the candidate

Date: **Dr. S.KAVITHA**

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INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI 600 003

EC Reg.No.ECR/270/Inst./TN/2013
Telephone No.044 25305301
Fax: 011 25363970

CERTIFICATE OF APPROVAL

To
Dr. Kavitha S
II nd year, Post Graduate in M.D. Pathology
Madras Medical College, Chennai

Dear ,

The Institutional Ethics Committee has considered your request and approved your study titled **"A STUDY ON ESTROGEN AND PROGESTERONE RECEPTOR STATUS IN FINE NEEDLE ASPIRATES OF BREAST CARCINOMA: COMPARISON OF THE IMMUNOHISTOCHEMICAL EXPRESSION OF ER PR IN CELL BLOCK AND BIOPSY " NO.27092016 .**

The following members of Ethics Committee were present in the meeting hold on **06.09.2016** conducted at Madras Medical College, Chennai 3

1. Prof. C. Rajendran, MD.	Chairperson
2. Prof. Dr. M.K. Muralidharan, M.S, M.Ch., MMC ,Ch-3	Deputy Chairperson
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7. Tmt.J.Rajalakshmi, Junior Administrative Officer,MMC,Ch	Layperson
8. Thiru.S.Govindasamy., B.A.B.L., High Court, Chennai-1	Lawyer
9. Tmt.ArnoldSaulina, MA., MSW.,	Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

27/9/16

Member Secretary – Ethics Committee

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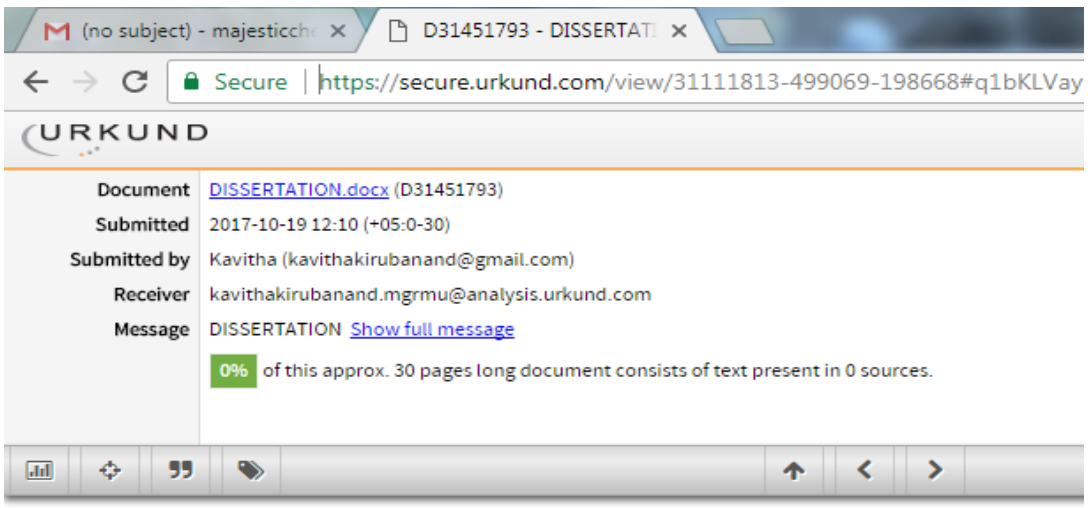


ABBREVIATIONS

IHC	-	Immunohistochemistry
ICC	-	Immunocytochemistry
ER	-	Estrogen Receptor
PR	-	Progesterone Receptor
FNAC	-	Fine needle aspiration cytology
TDLU	-	Terminal duct lobular unit
ADH	-	Atypical ductal hyperplasia
HRT	-	Hormone replacement therapy
Her 2 neu	-	Human epidermal growth factor
DCIS	-	Ductal carcinoma in situ
FISH	-	Flouerescent insitu hybridization
PMT	-	Primary medical therapy
BRCA1	-	Breast Carcinoma 1 Gene
BRCA2	-	Breast Carcinoma 2 Gene
EGFR	-	Epidermal growth factor receptor
IDC NOS	-	Invasive ductal carcinoma not otherwise specified
ICMR	-	Indian Council of Medical Research
WHO	-	World Health Organisation
PPV	-	Positive predictive value

CONTENTS

INTRODUCTION

INTRODUCTION

Breast cancer is the most common malignant disease and accounts for 21% of all cancers in the worldwide.(1) It is reported as the leading cause of cancer deaths in females with more than 1 million cases globally annually.(2) As per ICMR 1.5 lakh (over 10% of all cancers) new cases of breast cancer were diagnosed during 2016 and it is the number one among all cancer incidence overall. Statistics from the Madras Metropolitan tumour Registry shows the incidence of breast cancer as 35.8 per one lakh women.

The incidence of breast cancer is on the rapid rise and becoming the most common cancer among females in India and incidence of cervical cancer is next only to carcinoma breast.

The crude incidence rate of breast cancer in India is 85 per one lakh women per year[3] .

The important role of the histopathologist is to evaluate the prognostic factors to assess the outcome of the patient in handling and reporting the invasive breast carcinomas.

The important prognostic factors of breast cancer include tumor size, histological grade and axillary lymph node status, lymphatic and vascular invasion, hormone receptor status and surface epithelial growth factor expression. The other markers include Human epidermal growth factor (Her 2 neu), DNA ploidy, Cathepsin D and angiogenesis.(4)

However, none of these show independent significance, to be considered as a single prognostic factor. Grading of breast carcinoma has a prognostic value, as better survival is seen in patients with grade II than grade III tumors.(5) In breast carcinoma the histological grading is well established whereas the cytological grading is not practiced widely.

The main purpose of grading of breast carcinoma by fine needle aspiration cytology (FNAC) is to plan preoperative management as high grade tumors respond better to chemotherapy and low grade tumors are treated with tamoxifen.(6)

The morbidity associated with under treatment of breast tumors of high grade and overtreatment of low grade tumors can be avoided.(7)

The most widely accepted protocol followed for the diagnosis of breast lumps is "Triple assessment" which includes clinical, radiological, and cytopathological assessment.[8] Detection of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor (Her2/neu) receptors in breast carcinoma is necessary for pre- and post-operative chemoendocrine therapy and for predicting prognosis.[9,10] Conventionally, trucut biopsy and immunohistochemistry (IHC) are carried out on tissue sections to determine the hormone receptor status of the tumor. However, fine-needle aspiration cytology (FNAC) for breast lumps is a simple initial outpatient procedure which is easy, accurate, reliable, repeatable and give rapid diagnostic information equivalent to that of frozen sections . Other indications

of FNAC are staging of multiple tumors or suspicious zones and apparition of a new suspicious lesion during neoadjuvant chemotherapy.

Hence, it would save time and cost if these markers are performed on cytological material at the time of diagnosis using immunocytochemistry (ICC) without doing trucut biopsy.

The prognostic and therapeutic implications of hormone receptors –ER and PR in breast carcinoma have been studied extensively. ER is expressed in upto 75% of primary breast carcinoma, PR coexpressed in about 50% and no expression of ER or PR in 20% of breast carcinoma.[12]. The Hormone receptors determination primarily acts as a predictive factor for the response to therapeutic and adjuvant hormonal therapy[13]

The presence of ER has a favourable response to tamoxifen therapy and improved overall survival.

AIMS & OBJECTIVES

AIMS AND OBJECTIVES

1. To grade breast carcinoma and to determine Estrogen (ER) and progesterone (PR) expression in fine needle aspiration cytology (FNAC) samples.
2. To compare the results with histological grading and immunohistochemistry for ER and PR on surgical specimens.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

HISTORICAL ASPECTS

Breast cancer may be one of the known oldest forms of cancer in humans. The oldest description in Egypt for breast cancer dates back to around 1600BC.The lesions of the breast were treated by cauterization ,described by Edwin Smith Papyrus. The writing says," There is no treatment for breast cancer". For centuries, same conclusion was described by physicians in similar cases .In the 17th century doctors achieved greater understanding of the circulatory system and they could establish a link between breast cancer and the lymph nodes in the axilla. William stewart Halstead started doing mastectomies in 1882. The Halstead radical mastectomy included removing of both breasts, associated lymph nodes and the underlying chest muscles. This led to long term pain and disability, but it was necessary in order to prevent the cancer from recurrence. Radical mastectomies remained as the standard treatment till 1970, a new understanding of metastasis made to perceive cancer as a localized one as well as systemic illness, and more sparing procedures were developed which proved to be equally effective.

ANATOMY OF BREAST[12]

Breast is a specialized modified skin appendage which demonstrates morphologic alteration throughout the reproductive life cycle. Breast is suspended from anterior chest by ligaments of Cooper attached to skin and fascia of major and minor pectoral muscles and covered by skin and

subcutaneous tissue anteriorly. It extends from 2nd rib to 6th rib vertically, horizontally from the lateral border of sternum to the mid axillary line. Nipple areolar complex is a cone shaped protuberance located slightly medial and inferior on breast, at the level of 4th intercostal space.

BLOOD SUPPLY

Mammary gland is highly vascular and it is supplied by the branches of internal thoracic artery (branch of subclavian artery), superior thoracic, lateral thoracic, thoracoacromial branches of the axillary artery and lateral branches of the posterior intercostals arteries.

NERVE SUPPLY

The breast is supplied by the anterior and lateral cutaneous branches of the 4^{th} and 6^{th} intercostal nerves.(12)

LYMPHATIC DRAINAGE

Lymphatic drainage of the breast has a great importance because carcinoma of breast spreads along the lymphatics to the regional lymphnodes.(13)

The breast drains 75% of the lymph into axillary nodes, 20% into internal mammary nodes and 5% into the posterior intercostal nodes.(13)

PHYSIOLOGY OF BREAST[13]

Oestrogen cyclically stimulates the growth of the breast in females and the complete breast development occurs during pregnancy in which the placenta produces large amount of oestrogen that induces the growth, division, elongation of tubular duct system, maturation of nipple and fat deposit. Besides oestrogen, insulin, growth hormone, thyroxine and glucocorticoids of suprarenal gland also influence the milk secretion.

Estrogen and progesterone has a specific inhibitory effect on the milk secretion. Prolactin, produced by pituitary gland, have a important role in the initation and maintainence of lactation in puerperium. The prolactin level decreases after delivery to normal level and the prolactin secretion is enhanced by breast stimulation such as the act of nursing. The myoepithelial cells of mammary alveoli undergoes contraction by the action of oxytocin, which make them to expel milk from the secretory tissue to the nipple.

CYTOLOGY OF NORMAL BREAST[14]

Ductal cells are highly cohesive, in two dimensional flat sheets of small epithelial cells. The cells are uniform with scant and delicate eosinophilic cytoplasm ,nuclei round to oval with smooth regular nuclear membrane. The chromatin is fine, evenly distributed with inconspicuous and single nucleoli.

Myoepithelial cells are seen as naked, bipolar, oval to elongated stripped of their cytoplasm. The chromatin is dark but bland without nucleoli and the nuclear outlines are fine and regular.

HISTOLOGY OF NORMAL BREAST [15]

The breast contains 15-25 lactiferous ducts which start at the nipple, then branch into smaller ducts and end in the terminal duct lobular unit(TDLU) and all the ducts and ductules are lined by inner layer of cuboidal or columnar epithelial cells and an outer layer of myoepithelial cells. The connective tissue stroma with in the lobule (intralobular stroma) is composed of fibroblasts, occasional lymphocytes, histiocytes in a background of collagen and mucin. The interlobular stroma is hypocellular and it contains fibroadipose tissue.

INCIDENCE OF BREAST CARCINOMA

In INDIA

According to National Cancer Registry Programme ICMR (2009-2011), the most common malignancy in India is breast carcinoma which accounts for 25-30% of all cancers in women and is the second most common cancer next to cervical cancer [16].

The crude incidence rate of breast cancer is 85 per one lakh women per year. The death per incident ratio in India is highest with 50%, compared to 30% in China and 18% in United States.

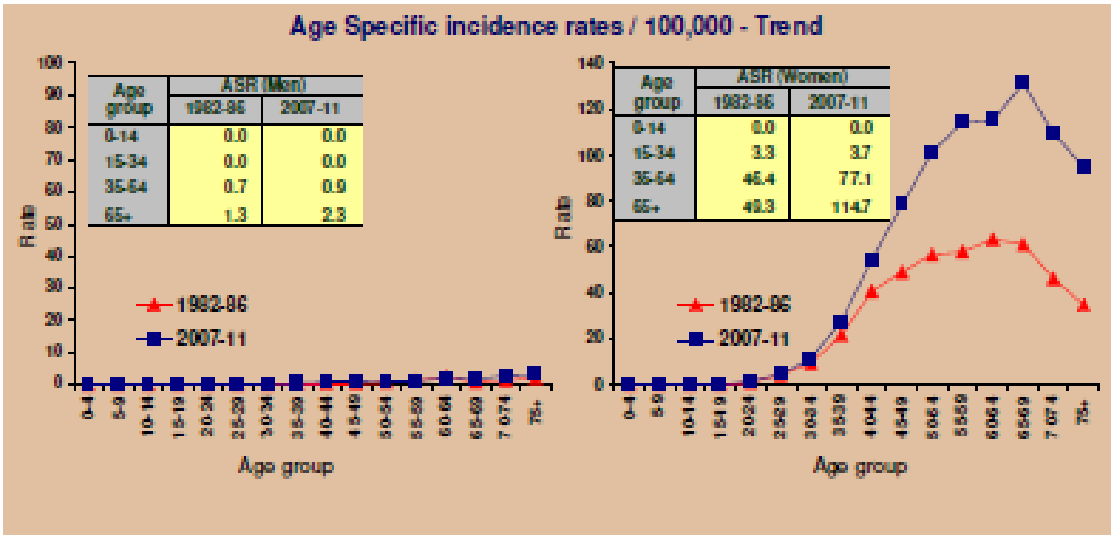
Breast cancer is more common in 50-60 years of age group constituting 69% of breast cancer. India is rapidly moving towards industrialization which results in drastic change in life style and this may be probable reason for increased incidence of breast cancer in India.

The annual age-adjusted rate is 30 to 33/ 1 lakh in urban and 8.6 / 1, 00,000 in rural women.

ACCORDING TO MADRAS METROPOLITIAN TUMOR REGISTRY (MMTR)

Breast cancer was ranked second in 1982-86, and became the first since 2002 among women. The histological diagnosis of breast cancer rose from 68% to 86% in 2007-11 ,diagnosis by imaging modalities from 1% to 4% and diagnosis by clinical evaluation only decreased from 25% to 8% in the corresponding period [17](Figure 1)

FIGURE 1: AGE SPECIFIC INCIDENCE RATE OF BREAST CANCER BETWEEN 1992-96 AND 2007 TO 2011 IN CHENNAI (*COURTESY MMTR*)



DEPARTMENT STATISTICS

In the institute of pathology, Madras medical college ,the total pathological specimens received in the year 2016 was 11362 .Among them the total number of breast specimens were 379 ,out of which 238 were carcinoma specimens.

RISK FACTORS

1.**Family history:** Women who have first degree relative with breast cancer have increased risk of 2-3 times to that of general population.[18]

2.**Menstrual and reproductive history**: Increased risk is correlated with early menarche ,first child birth at late age, nulliparous and late menopause.[19,20] The risk of breast carcinoma is increased in postmenopausal women with increased levels of androgen in plasma.[21]

3.**Atypical ductal hyperplasia**:.[22] Risk for breast cancer is higher for younger women and those with multiple foci of ADH and the relative risk of carcinoma is 4 – 5 times.

4.**Exogenenous estrogens**: Recent study have showed a strong evidence for increased risk of breast carcinoma in women using hormone replacement therapy(HRT) than in using ostrogens alone.[23]. The hormone estrogen was declared a known human carcinogen in December 2002 by the National Toxicology Program.

***5* .Contraceptive agents.** Many epidemiologic studies have shown that no increased risk, or at most a very low risk among young long-term users.[24]

***6* .Ionizing radiation.** Increased risk of breast carcinoma with exposure to ionizing radiation, particularly if this exposure occurred during the time of breast development. Example, those received irradiation to the mediastinum for Hodgkin lymphoma at early age.[25]

***7.*Breast augmentation.** Breast carcinomas are also detected in women who have underwent augmentation mammoplasty.[26]

***8.*Others**. Ataxia–telangiectasia syndrome and Cowden syndrome patients have an increased risk of breast carcinoma.[27,28]

Genetic predisposition

Around 5 to 10% of all breast cancers are familial.[29] There are two high-penetrance susceptibility genes, when they are affected by germline mutations, it is associated with an increased life-time risk of breast cancer as well as few other cancers like ovarian carcinoma . They are *BRCA1*, located on chromosome 17q21, and *BRCA2*, located on 13q12.3 chromosome .[30]. *BRCA1* mutations has increased percentage of breast carcinomas with medullary carcinoma features and they are triple negative.[31,32] *BRCA2*-mutations cancers does not have a specific morphological feature and are positive for ER, PR(hormone receptors).[33]

SPORADIC BREAST CANCER

The risk factors are hormone exposure, sex, age at menarche and age at menopause, use of exogenous estrogens.

CLINICAL EXAMINATION

Triple assessment test is the screening method done for breast diseases which includes clinical examination, imaging and tissue sampling.

PALPATION

This is considered as one of the best mode and remains as the extremely useful for diagnosis of breast carcinoma.

RADIOLOGICAL IMAGING

1. Mammogram:

- The widespread use of mammography brought a dramatic change in the diagnosis of breast carcinoma. which is used to detect small non palpable carcinoma which are asymptomatic.
- The primary signs of carcinomas detected in mammogram include density and calcification.

USG

- To distinguish between solid and cystic lesions and to delineate the borders more accurately in case of solid masses.

MRI

- ❖ It is helpful for screening the high risk women and those with dense breast, evaluating cases of breast implants with rupture.

FNAC VALUE IN CLINICAL PRACTICE [34]

Advantages

- ❖ Cost effectiveness
- ❖ Minimal discomfort
- ❖ Rapid results and potential bedside diagnosis
- ❖ Allows moving the needle in different directions,which permits better and more extensive sampling of the lesion.
- ❖ Can be done as outpatient procedure

Accuracy

- ❖ Highly operator dependent
- ❖ Sensitivity for malignancy 65-98%
- ❖ Specificity 34-100%
- ❖ False positive results 0-2%
- ❖ False-negative results occur because of inaccurate sampling, interpretation or both.

USES OF FNAC IN BREAST

All palpable lesions

- Radiologically detected nonpalpable cystic lesions
- Therapuetic procedure when surgical biopsy is not possible
- Tumor sampling for prognostic/predictive factors and response to therapy

TECHNIQUES

- FNAC on non palpable lesions are usually done under ultrasound or mammogram guidance.
- The average number of passes recommended for adequate sampling of most palpable masses is 2 to 4, more passes may be necessary in selected cases.
- Direct smearing is the preferred method for preparation of slides.
- Air dried Romanowsky type stains (Modified giemsa stain) and alcohol fixed papanicolaou (PAP) stains are optimal for diagnosis
- The cell block is prepared mainly when special stains and prognostic/predictive factor studies are anticipated. A separate pass can be dedicated for cell block preparation.

SPECIMEN ADEQUACY

- ❖ There is no specific requirements for a minimum number of ductal cells.
- ❖ A specimen is considered adequate when it represents the lesion for which the biopsy is performed.

DIAGNOSTIC TERMINOLOGY

- ➢ Category system of reporting
- ➢ **Benign**: No evidence of malignancy
- ➢ **Atypical /Indeterminate**:Applied to adequate samples that represent entities difficult to diagnose by cytology ,For example
    - Atypical ductal hyperplasia versus low grade ductal carcinoma in situ(DCIS)
    - Papillary lesions:Intraductal papilloma versus papillary carcinoma
    - Fibroepithelial lesions:Fibroadenoma versus benign phyllodes tumour
    - Mucinous lesions:Mucocele like lesions versus mucinous carcinoma

- **Suspicious:** The findings are highly suggestive of malignancy ,but other factors likepreservation or the amount of cells fall short for the diagnosis.
- **Malignant:** The cellular findings are diagnostic of malignancy
- **Unsatisfactory/non diagnostic**: due to scant cellularity, artifacts, obscuring blood etc.

CLASSIFICATION OF BREAST CANCER

In situ Carcinoma refers to proliferation of tumor cells which are limited within ducts and lobules by the basement membrane. Invasive carcinoma ("infiltrating" carcinoma) refers to those tumors that have breached the basement membrane and invaded into the stromal tissue .

INVASIVE CARCINOMAS

INFILTRATING DUCTAL CARCINOMA NOS[35]

Cytology:

Hypercellular smears, frequently isolated cells and poorly cohesive groups, variable degree of pleomorphism, eccentric enlarged nuclei with hyperchromatic ,fine or coarse granular chromatin, prominent nucleoli in a dirty background.

Microscopically, it is composed of solid sheets, tubules, nests, single cells in varying proportions depending on the degree of differentiation. Grading

of the tumors is by Nottingham Modification of Richardson System. (ANNEXURE I)

INFILTRATING LOBULAR CARCINOMA[36,37,38]

Cytology: Low to moderate cellularity,Presence of noncohesive single cells or as small balls of slightly enlarged nuclei,uniform cells with mild atypia and presence of small nucleoli.A single cell distribution and Indian file pattern is characteristic

Microscopically there is poorly cohesive tumor cells that infiltrates the stroma in single file arrangement or in in sheets or as loosely arranged clusters. There is characteristic loss of E-cadherin This carcinoma shows positivity for HMW keratin and lack of p53.[39] p20 catenin has been recently added marker and the lobular carcinoma shows a characteristic cytoplasmic staining. .[40]

MEDULLARY CARCINOMA

Cytology: :Very cellular smear with predominance of single cells, naked atypical nuclei or syncytial sheets of atypical cells showing scant to abundant cytoplasm with indistinct cell borders, bizarre nuclei with macronucleoli ,irregular nuclear in membrane, coarse chromatin .Numerous mitosis, lymphocytes and plasma cells in the background.

Microscopically, more than 75% of the tumor is composed of solid sheets of neoplastic cells, pleomorphic vesicular nucleus with prominent nucleoli admixed with lymphoplasmacytic infiltrate.

MUCINOUS CARCINOMA

Cytology: Highly cellular, loosely cohesive clusters and dissociated cells with intact cytoplasm and nuclei with mild atypia, no oval bare nuclei Also seen thin walled capillaries, micropapillary pattern of angulated clusters or abortive papillae and ball-like clusters with abundant extracellular mucin May show spindle cells at edge of nests and in background, which represent either tumor cells compressed by mucin or fibroblasts and variable psammoma bodies

Microscopically, show the clusters of tumor cells floating in pools of mucin. The tumor cell clusters shows acinar or micropapillary architecture or may be solid.[55] When more than 90% of tumor content is mucin, it is known as Pure mucinous carcinoma,. They shows strong positivity for MUC2. The neoplastic cells show ER, PR positive and HER2neu negative.

APOCRINE CARCINOMA:

Cytology: Cellular smears with loosely cohesive clusters of atypical apocrine cells with abundant finely granular eosinophilic cytoplasm ,round to irregular highly pleomorphic nucleus usually eccentrically placed with prominent nucleoli.

Microscopically, two types of apocrine cells are seen. Type A cells with abundant granular eosinophilic cytoplasm and Type B cells showing clear foamy cytoplasm. There is also glandular differentiation with characteristic apocrine snouts. These tumors exhibits positivity for C-KIT and negative for ER, PR and BCl2.

METAPLASTIC CARCINOMA

Cytology: Very cellular smears ,the mesenchymal cells are elongated, atypical and pleomorphic in a myxoid background.

Microscopically, it is composed of heterogeneous components like Squamous, spindle, mesenchymal elements like chondroid and osseous material in different proportions.

TUBULAR CARCINOMA

Cytology: Smears are variable cellular, with presence of angular glandular or tubular structures with sharp borders .Oval cells perpendicularly arranged along the edges of the cellular clusters, regular enlarged nuclei with occasional grooves with singly dispersed epithelial cells with minimal atypia.

Microscopically shows irregular and angulated glands are arranged haphazardly in a desmoplastic stroma.

PROGNOSTIC FACTORS

AGE OF THE PATIENT

Better prognosis is seen below fifty years of age. Prognosis declines after fifty years of age

SIZE

In minimal carcinoma size is one of the two criteria, which includes all insitu carcinomas regardless of size and the invasive carcinomas of <1cm in size.

SITE

Tumors located in the upper inner and lower inner quadrants show greater risk of (50%) relapse and death than the laterally located tumors.

CYTOARCHITECTURAL TYPE

There is no significant prognostic difference between ordinary infiltrating ductal and lobular carcinoma .[66] Morphological variants like Mucinous, Papillary ,Medullary, Tubular , secretory , Cribriform and Adenoid cystic carcinoma have good prognosis(41).

Variants like Metaplastic, Squamous cell carcinoma, Inflammatory, Neuroendocrine and Signet ring cell carcinoma are aggressive tumors having poor prognosis. (42)

PRESENCE OR ABSENCE OF INVASIVENESS

The amount of insitu component correlates with incidence of multicentricity and indirectly with probability of occult invasion. [43].

Insitu ductal carcinoma of the comedocarcinoma type also associated with metastases in the absence of a detectable invasion.(44)

TUMOR NECROSIS

Tumor necrosis correlates with increased nodal metastasis and reduced survival rates . [45]

TYPE OF MARGINS

Tumors with infiltrating margins have a worse prognosis compared with the tumors with pushing margins.[46,47]

MICROSCOPIC GRADE(ANNEXURE I)

Grading is based on Nottingham Modification of Scarff Bloom Richardson system . Ellis et al observed that there is an excellent correlation between the Nottingham grading system and patient's survival rate and metastasis.

SKIN INVASION

Breast carcinomas with skin infiltration are associated with reduced survival rate.[48]

NIPPLE INVASION

Carcinomas involving the nipple areolar complex is associated with higher incidence of axillary node metastasis.[49]

BLOOD VESSEL EMBOLI

Vascular emboli have high association with size of the tumor , tumor type, histological grade, lymph node status and distant metastasis. Tumors with vascular invasion is associated with poor prognosis.

LYMPHATIC TUMOUR EMBOLI

There is also increased risk of tumor recurrence if lymphovascular invasion is seen.[50,51]

LYMPH NODE STATUS

Metastatic deposits in the axillary nodes is considered as a poor prognostic factor.

METASTASIS

Locally advanced disease with distant metastasis have bad prognosis.

BRCA-1 STATUS

In BRCA 1 mutation carriers developing carcinomas are associated with overall poor survival rate, if they have not received adjuvant chemotherapy.[52]

STAGING (TNM) (ANNEXURE II)

PROLIFERATION RATE

The proliferation rate is measured with mitotis. Poor prognosis is observed in tumors with high proliferation rate but they respond to the chemotherapy better. It can be also be measured using S-Phase fraction (SPF) and with thymidine labeling index.

OTHER PROGNOSTIC FACTORS

Many factors like lymphocytic infiltration, [54] Tumor necrosis association with pregnancy and lactation, [53] and vimentin expression[55] have variable prognostic implications in breast carcinoma.

HORMONE RECEPTORS

In Breast carcinoma, the tumour cells generally express ER, PR and Human Epidermal Growth Factor Receptor (HER2neu) for breast cancer formation and tumor progression.

Immunohistochemistry was discovered 30 years back which was used for the classification of breast carcinomas. Nuclear hormone receptors detected by IHC correlated with good prognosis and also predicts the response to hormonal therapy.[55, 56]

ER positive neoplastic cells depends on estrogen for their growth and so the use of anti-estrogenic agents (eg.Tamoxifen) inhibits cell proliferation.[57, 58]

Both ER and PR receptors are co-independent variables. Estrogen receptor, a better predictor for the response to hormone therapy than the Progesterone. [59] HER2neu positive carcinomas have worse prognosis inspite of having good response to Transtuzumab, a monoclonal antibody. [60] which can be measured by IHC or FISH and a better correlation observed between these methods.[61, 62]

Fisher et al found that there is significant association between ER expression and older age group with high nuclear grade, marked tumor elastosis and absence of necrosis . [63]

Harvey et al in 1999 suggested the cut off values for ER/PR score in the treatment of advanced stage diseases.

0 score Endocrine therapy will not work definitely .

2-3 score 20 percent possibility of response to therapy.

4-6 score 50 percent possibility of response to therapy.

7-8 score 75 percent possibility of response to therapy.

IMMUNOHISTOCHEMISTRY

In 1941 Dr .Albert Coons first described immunohistochemistry.] The most commonly used technique is the Peroxidase- antiperoxidase immune complex technique, developed by Sternberger in the year 1970. The newer, biotin-avidin immunoenzymatic technique was developed by Heitzman and Richards in 1974.[64,65]

APPLICATIONS OF IHC IN BREAST PATHOLOGY

1) The myoepithelial markers are used to assess the stromal invasion.

2) To differentiate between various types of breast cancers. Eg.

E -cadherin to differentiate between ductal and lobular carcinoma.

3) To differentiate between the precursor lesions and the malignant lesions.Eg. Usual Ductal Hyperplasia and Ductal carcinoma insitu can be distinguished using HMWCK.

4) The site of origin of metastatic carcinomas can be found.

5) To detect the sentinel node metastasis.

6) Estrogen and Progesterone receptor status and HER2neu overexpression which can be assessed using specific antibodies to receptor proteins.

7) Evaluation of Metaplastic carcinoma from that of mesenchymal lesions.

Bozetti et al in 1994[66] have done ICC evaluation of ER, PR hormone receptors and Ki 67, expression in 100 patients with primary breast carcinoma. They found the significant association between Ki 67 values and progesterone receptors (p=0.003) and also the estrogen (p=0.02) and progesterone(p=0.04) negativity with high Ki 67 growth fraction (P=0.005) associated with clinical evidence of axillary involvement. This study suggests that FNAC represents a effective practice for a simultaneous evaluation of multiple biologic indicators and useful as preoperative procedure in patients for neoadjuvant chemotherapy and /or endocrine therapy.

A. Makris et al 1997[67] have done a study to evaluate the ability to detect molecular markers of prognosis and response to treatment in fine needle aspirates (FNA) from patients with primary breast carcinomas. They included 147 patients with operable primary breast carcinomas planned for primary medical therapy (PMT) versus adjuvant chemoendocrine therapy ..The percentage positive values obtained are : 74% for ER and 70% for PR, The concordance for ER and PR, FNA when compared to ICC of matching histological sections was 91.5%, 75.5%, and 75% respectively. These results indicate that molecular markers can be adequately tested on cytological

preparations from primary breast tumours. These markers can be used to determine prognosis and predict response to PMT.

Marianne Briffod et al 2000 [68]assessed the reliability of prognostic biologic markers by means of ICC on cell blocks obtained from diagnostic fine-needle cytopunctures of breast carcinomas and their lymph node metastases. ICC estrogen receptors (ER), progesterone receptors (PR), were performed in 55 cases of primary breast carcinoma on cell blocks (cytoblock technique) and on their corresponding tissue samples (46 mastectomy specimens and 9 Trucut biopsies) and in 38 cases on cell blocks from fine-needle cytopunctures of both the primary breast tumors and their concurrent lymph node metastases. A good correlation was observed between immunostaining assessment on cell blocks and on the corresponding tumor tissues with ER (96%) and PR (82%) They concluded that the cell blocks prepared from fine-needle cytopuncture specimens of breast carcinomas and their node metastases are useful when planning neoadjuvant treatment.

Savitri krishnnamurthy et al 2003 [69] re used Pap-stained smears to assess the estrogen receptor (ER) status of breast carcinoma. The objective of this study was to compare ICC evaluation of ER status on FNA smears by three methods: 1) ER-ICC performed on slides fixed in formaldehyde–methanol–acetone; 2) destained Pap slides and 3) Pap-stained slides without destaining. Two representative Pap smears of breast carcinoma were selected from 48 cases of breast carcinoma in which ER was previously evaluated by ER-ICC.

One of these Pap smears was used as such and the other was destained prior to immunostaining. The number of cells with positive nuclear staining was expressed as a percentage and the intensity of staining was semiquantitatively scored on a scale of 1+ to 3+. Thirty cases (63%) showed varying degrees of positive staining while 18 cases (38%) were entirely negative by all three methods. Significant discrepancies in the number of cells with positive staining and in the intensity of staining between the three methods occurred in 40% and 23% of the cases and was mainly due to a reduction in the number of cells with positive staining and the intensity of staining using Pap slides in comparison to ER-ICC. Weighted kappa agreement of the percentage of cells with positive staining using Pap-stained slides and destained Pap-slides in comparison to ER-ICC was 0.75 and 0.64, respectively, and that for the intensity of staining was 0.75 and 0.66, respectively. Therefore, ICC evaluation of ER using Pap-stained smears as such or destained Pap smears compared favorably with ER-ICC.

Zoppi et al 2002[70] have done the study in 101 primary breast carcinoma with FNAC smears and corresponding tissue sections and results were ER sensitivity 96.1% specificity 86.9% and concordance 94.1%.In case of PR sensitivity 65.7%,specificity 83.3%,and concordance 71.2%

Guillerma Cano et al 2003 [71] have done ICC assessment of estrogen receptor (ER) and progesterone receptor (PR) status on alcohol-fixed smears obtained by fine-needle aspiration (FNA) from 40 breast cancer patients using anti ER and anti PR without any antigen retrieval. A series of 40 aspirates were

analyzed and the results of ER and PR status were compared with the respective formalin-fixed tissue using the same procedure and with antigen retrieval on paraffin sections. ER showed positive in twenty-four out of the 40 cases in two methods and 16 were negative. In one case the material was insufficient to interpret the reaction in the cytological specimen and only one case, with focal positivity reaction on paraffin sections, was negative in the cytological specimen. The intensity of nuclei staining in cytological smears of breast cancer cells was stronger compared to histochemical methods. Out of 40 cases examined, PR showed positive in 8 cases and 22 were negative in both methods and he concluded that the application of the ER and PR receptors on alcohol-fixed smears / paraffin sections, provide several advantages, such as high sensitivity and specificity of the reaction, stronger immunostaining, shorter procedures times, and avoidance of antigenic retrieval methods le as

Malaviya et al 2006[72] have done a study to correlate of ICC and IHC Determination of ER and PR Receptors in 101 cases of Breast Cancer and obtained ER cytohistologic correlation 94%,sensitivity 96%,specificity 86.9%. In PR cytohistologic correlation 71.2% sensitivity 65.7% and specificity 83.8%. The results concluded that ICC correlates with IHC for ER

Ahmad shabaik et al 2010[73] compared ICC ER and PR testing performed on 42 formalin-fixed, paraffin-embedded cell blocks from 27 fine needle aspirations (FNA). ER testing showed 85.7% sensitivity, 100% specificity, 100% positive predictive value (PPV), and 85.7% negative predictive value

(NPV). PR testing the results showed 80% sensitivity, 100% specificity, 100% PPV, and 88.8% NPV respectively. He concluded ICC for, ER and PR performed on formalin-fixed, paraffin-embedded cell blocks prepared from fresh FNA correlated with IHC and FISH performed on the corresponding tumor tissue.

H. Hafez et al 2010 [(74)] have done a study to evaluate the reliability of ICC for estrogen and progesterone receptors on previously papanicolaou-stained fine needle aspiration smears of 90 breast carcinoma cases. ICC and IHC on tissue sections were done. Smears were interpreted as positive if 10% of the examined cells showed nuclear staining.

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In estrogen receptor ICC, the cyto-histologic accuracy was 91.1% (82/90) while the discordance rate was 8.9% (8/90). The diagnostic sensitivity, specificity, positive predictive value, and negative predictive value were 93%, 84.2%, 95.7%, and 76.2%respectively.

In progesterone receptor ICC, the cyto-histologic accuracy was 88.9% (80/90) while the discordance rate was 11.1% (10/90). The diagnostic sensitivity, specificity, positive predictive value and negative predictive value were 87.1%, 95%, 98.4%,and 67.9% respectively.

The study concluded that application of estrogen and progesterone receptor ICC on previously Papanicolaou-stained slides provides an overall accuracy of

91.1% for estrogen receptor and 88.9% for progesterone receptor when compared with the IHC.

Radhika K, Prayaga A et al. 2010[75] have done a study to evaluate the degree of correlation between l (ICC) and (IHC) determination of estrogen receptors (ERs) and progesterone receptors (PRs) in breast cancer. The total numbers of cases selected are 100 and cases studied were 76 . The fixatives used in this study were 4% buffered formalin, cold acetone, ether alcohol and destained Papanicolaou smears. Antigen retrieval was performed with 0.9 M Tris-HCl buffer instead of sodium citrate and this was reason for the lower positivity rate.

Of these, 24 (confirmed) expressed hormone receptors and 24 cases were labeled as invalid because of the improper staining. After considering both tests of the 24 positive cases, both ER and PR were positive in five cases, ER was positive in only three, PR in one and both were negative in nine cases. Among individual receptor analysis, the total number of ER-positive cases were 20 (27%) and negative cases were 56 (73%). In this study, cases compared were 24 .

False-negative cases in ICC were nine and in IHC were three. But, false-negative cases were not due to the use of destained smears. In unstained smears, eight (40%) were ER positive and 30 (55%) were ER negative. In destained smears, 12 (60%) were ER positive and 25 cases were (45%) ER negative. More number of ER-positive cases were observed in destained

smears while more number of ER-negative cases were observed in unstained smears.

Total number of PR +ve cases were 10 (27%), destained six (60%) and unstained four (40%). Total number of PR -ve cases were 27 (73%), destained 10 (37%) and unstained 17 (63%).

Sensitivity and specificity were calculated for ER and PR. ER showed 33% sensitivity, 75% specificity, 67% positive predictive value, 43% negative predictive value and 50% concordance. PR showed 25% sensitivity, 33% specificity, 33% positive predictive value, 25% negative predictive value and 29% concordance.

Keykhosro mardanpour et al 2012[76] In this study, markers on cytoblocks and on the corresponding tissue samples were compared to determine the reliability and difficulties of cytoblock assessment on the determination of estrogen receptors (ERs) and progesterone receptors (PRs) in breast cancer. Fine needle aspiration cytology (FNAC) was done on 45 primary breast cancers and paraffin embedded cell blocks were prepared which were Immunostained for ER and PR and IHC was done on corresponding tissue sections.

Assessment of staining:

At least 100 malignant cells were considered suitable for IHC on cytoblocks. Immunoreactivity for ER and PR was graded as negative and positive according to more than 10% of tumor cells in high grade tumor and less than 10% of tumor cells.

In total, 45 cases were assessed by IHC on cytoblock and tissue specimens for the determination of estrogen receptors (ERs) and progesterone receptors (PRs) in breast cancer.. There were three histopathological types of reports between our patients. About 82.2% of patients were diagnosed for invasive ductal carcinoma alone, 2.2% of patients were diagnosed for carcinoma in situ alone, and 15.6% of remaining patients were diagnosed for invasive ductal carcinoma with carcinoma in situ component. All patients expressed ER and PR receptors equally and there is no significant difference observed. of multiple ER expression was positive in 26 cases (57.8%) and negative in 19 cases (42.2%) in IHC on tissue specimen. ER expression in IHC on cytoblock specimen was positive in 17 cases (37.8%) and negative in 28 cases (62.2 %). The sensitivity of IHC stain on cytoblock for expressing ER marker was 65.4%, the specificity was 100%, the positive predictive value was 100% and the negative predictive value was 68%.The concordance rate between IHC stain on cytoblock and tissue specimen was 80% biologic indicators and could be useful as a preoperative procedure in patients who are candidates supported byhe aPR expression in IHC on tissue specimen was positive in 16 cases (35.6%) and negative in 29 cases (64.4 %). PR expression

in IHC on cytoblock specimen was positive in 15 cases (33.3%) and negative in 30 cases (66.7%). The sensitivity of IHC stain on cytoblock for expressing PR marker was 87.5%, the specificity was 96.6%, the positive predictive value was 93% and the negative predictive value was 93 %.

Usha Dalal et al 2015 [77] have done a study to grade breast carcinoma and to determine estrogen receptor (ER) and progesterone receptor (PR) expression on fine-needle aspiration cytology (FNAC) and to compare the results with histological grading and IHC on surgical material. Fifty cases of breast carcinoma diagnosed on FNAC were included.. Immunostaining for ER and PR was done on smears and tissue sections. On both cytological and histological evaluation, 49 cases were infiltrating ductal carcinoma and one case was colloid carcinoma. On comparing cytological and histological grading, 78% were correctly graded on cytology. The sensitivity, specificity, positive predictive value and negative predictive value for ER detection on (ICC) were 55.6%, 95%, 93.8% and 61.3%, respectively. The sensitivity, specificity, positive and negative predictive value for PR detection on ICC were 57.7%, 95.2%, 93.8% and 64.5%, respectively. The correlation for ER and PR between cytology and histology was 72.3% and 74.5%. The study concluded that the grading of ER and PR in breast carcinoma on smears is advocated because of high concordance between cytology and histology. This allows the patient to be treated with hormonal therapy on the basis of FNAC alone.

Kempula geethamala et al 2016 [11] have done the study evaluate the diagnostic reliability of performing ER and PR status on FNAC by ICC and compare the results with IHC on tissue sections. FNAC done on 100 breast carcinomas smears were stained with H and E and material was checked, marked, destained, fixed for 10 min in cold acetone, and then used for ICC. IHC in tissue sections and ICC were performed together .Hundred tumor cells on FNAC and 500 cells on tissue sections were counted for positivity.. ER, PR positivity was denoted by nuclear staining using Allred scoring system which takes into account both intensity of staining.

By ICC, ER+/PR+ 49/100 (49%) .By IHC, ER+/PR+52/100 (52%) Among the individual hormone receptor study by ICC, ER was expressed in 53/100 (53%), PR in 50/100 (50%), and Her2/neu in 22/92 (23.9%).

The ICC results were compared with IHC.In ICC, ER-positive expression was seen in 53/100 (53%) of cases and rest 47/100 (47%) were negative. Among 53/100 (53%) ICC positive cases of ER, all were positive by IHC (true positives). Of 47/100 (47%) ICC negative cases, 45 were negative (true negatives) by IHC and 2 were positive (false negative) by IHC. Compared to IHC, the ICC diagnostic sensitivity, specificity, PPV, and NPV for ER staining were 96.3%, 100%, 100%, and 95.7%, respectively. The overall accuracy and concordance between ICC and IHC were 98%

PR ICC positive cases were 50/100 (50%) and rest 50/100 (50%) were negative. Among fifty ICC positive cases of PR, all fifty were positive by IHC (true positive). In rest 50 ICC negative cases, 47 were negative (true negatives) by IHC and 3 were positive (false negatives) by IHC. The diagnostic sensitivity, specificity, PPV, and NPV for PR immunocytochemical staining were 94.3%, 100%, 100%, and 94%, respectively. The concordance between ICC and IHC was 97%.

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MATERIALS & METHODS

MATERIALS AND METHODS

This study is a prospective study of Primary breast carcinomas conducted in the Institute of Pathology, Madras Medical College and Rajiv Gandhi Government General hospital, Chennai during the period between March 2016 to April 2017.

SOURCE OF DATA

A total of 379 breast biopsy specimens were received in our surgical pathology department during this one year period. Out of which 238 were malignant. Of these 68 cases were diagnosed as malignant in mastectomy specimens.

INCLUSION CRITERIA

- Cases diagnosed as primary carcinoma breast .

EXCLUSION CRITERIA

- Benign tumors,
- Benign and malignant phyllodes,
- Non neoplastic lesions of breast and
- Necrotic and highly desmoplastic tumors with scanty cellularity

METHOD OF DATA COLLECTION

FNAC of 50 breast carcinomas together with corresponding trucut or modified radical mastectomy (MRM) specimens paraffin-embedded tissue sections over a period of 1 year from March 2016 to April 2017 were studied in the Department of Pathology. FNAC was done and the material is fixed

with cold acetone and 3ml of plasma added to it, kept in a refrigerator overnight. Next day centrifuge the sample at 2400 rpm X 5min,supernantant discarded and 5cc of buffered formalin added and again centrifuged. Cell button removed with spatula and place in a tissue paper and submitted for routine processing and paraffin embedded.

Detailed history of the cases regarding age, sex, side of the breast, type of procedure, details of gross characteristics such as tumor size, nodal status details were obtained for those 50 cases from surgical pathology records. Formalin fixed tissue were cut, processed and paraffin embedded.

4μm thick sections of the paraffin tissue blocks were cut and stained with eosin and hematoxylin. Slides were reviewed and graded using the Nottingham modification of the Scarff Bloom Richardson Grading system (Annexure I) and formalin fixed paraffin embedded tissue samples and cell blocks were subjected for IHC for ER and PR expression. Slides were evaluated and scoring was given. The results were recorded with photographs.

INTERPRETATION & SCORING SYSTEM

ER and PR

Hormone receptors like Estrogen and Progesterone receptor, when expressed show a nuclear positivity. The number of cells expressing and their intensity of staining is scored as two values and a composite score based on percentage plus intensity of more than 2 is considered to be positive. (Annexure II).

OBSERVATIONS & RESULTS

OBSERVATION AND RESULTS

In our Institute the total number of pathological specimens received from March 2016 to April 2017 was 11362, out of which the total number of breast specimens received was 379 and 238 of the specimens were malignant. Out of 238 breast malignancies, the total number of breast carcinomas enrolled in this study period was 50 cases.

The age wise distribution of these 50 cases is given below

Table -1:**Age wise distribution of breast cancer**

Age Group in years	NO OF CASES(n)	Percent(%)
30-40 YEARS	11	22.0
41-50 YEARS	7	14.0
51-60 YEARS	19	38.0
61-70 YEARS	9	18.0
ABOVE 70 YEARS	4	8.0
Total	50	100.0

The highest incidence of breast cancers was found in the age group of 51-60 years. The median age of the patient in this study was 49. The youngest age of presentation of breast cancer was 30 years in this study.

Figure 2 : Age wise distribution of breast cancer

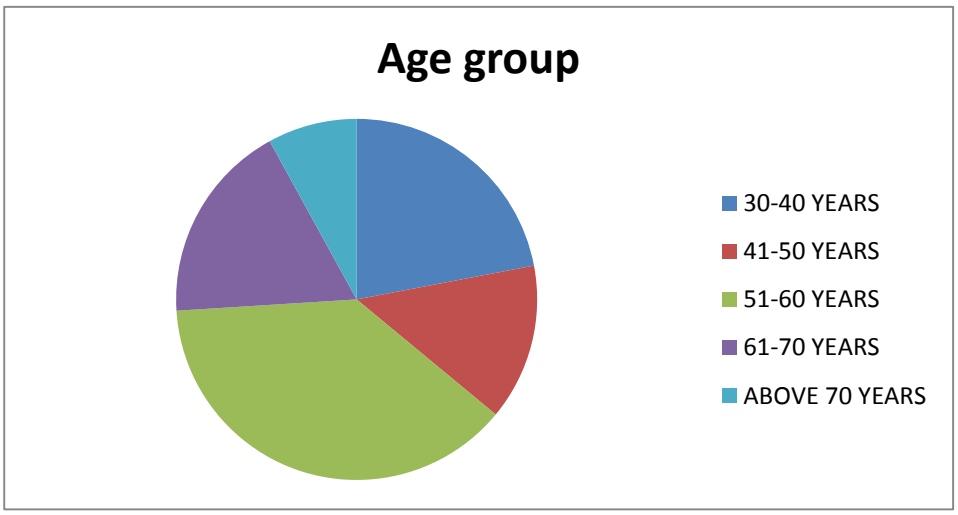


Table-2: **Side of the breast involved**

SIDE	NO OF CASES(n)	Percent
Lt breast	19	38.0%
Rt breast	31	62.0%
Total	50	100.0%

31 cases of primary breast carcinomas were reported in right breast and 19 cases were reported in left breast which are stastically insignificant.

Figure: 3 **Side of the breast involved**

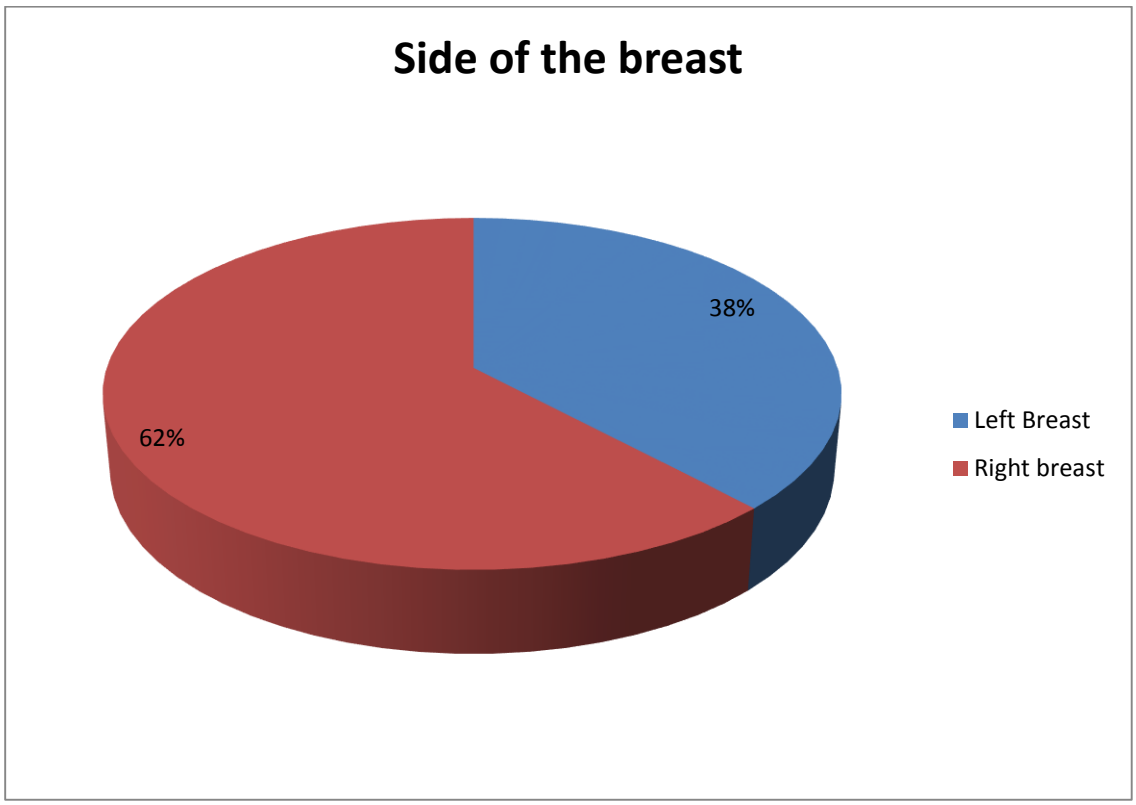


Table-3: **Tumour Location among the breast cancers**

Tumour location	NO OF CASES(n)	Percent(%)
Central	6	12.0
LIQ	1	2.0
LOQ	3	6.0
UIQ	10	20.0
UOQ	30	60.0
Total	50	100.0

30 cases of breast carcinoma were located in upper outer quadrant.

Figure: 4 **Tumour Location among the breast cancers**

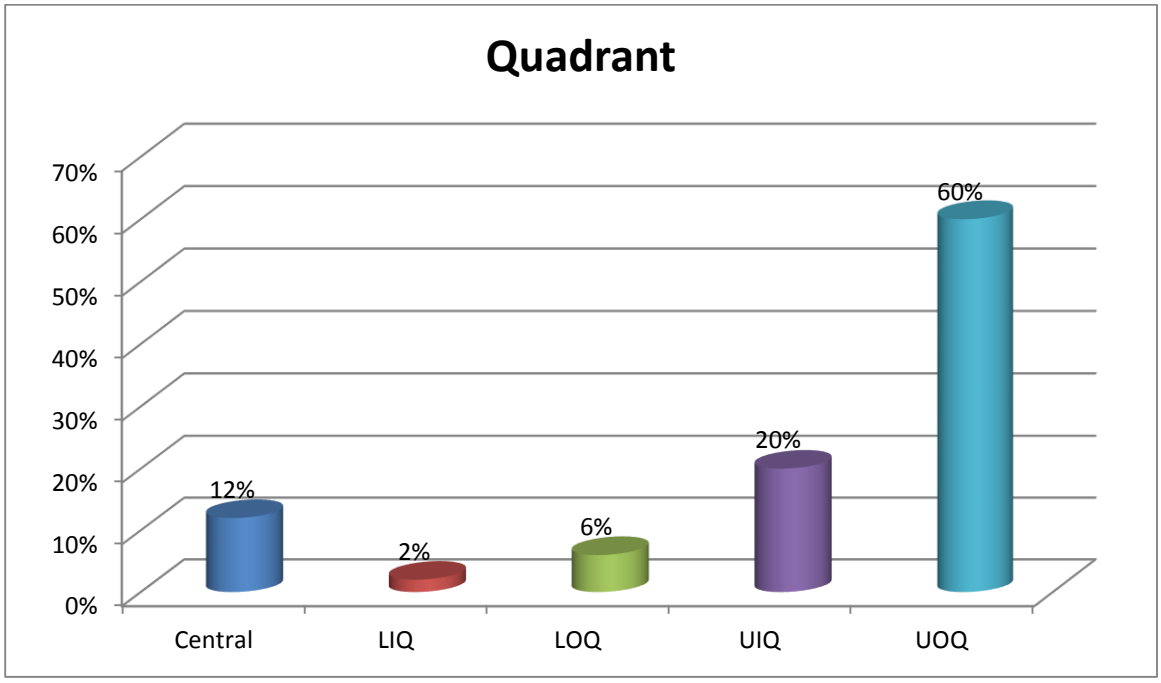


Table-4: Distribution of Breast Cancers with Tumour Size

T SIZE	NO OF CASES(n)	Percent(%)
<2cm	11	22.0
2-5 cm	8	16.0
>5cm	31	62.0
Total	50	100.0

11 cases (22%) had tumor less than 2 cm,8 cases (16%) were of 2to 5 cm in size and 31 cases (62 %) were more than 5 cm in size.

Figure 5: **Distribution of Breast Cancers with Tumour Size**

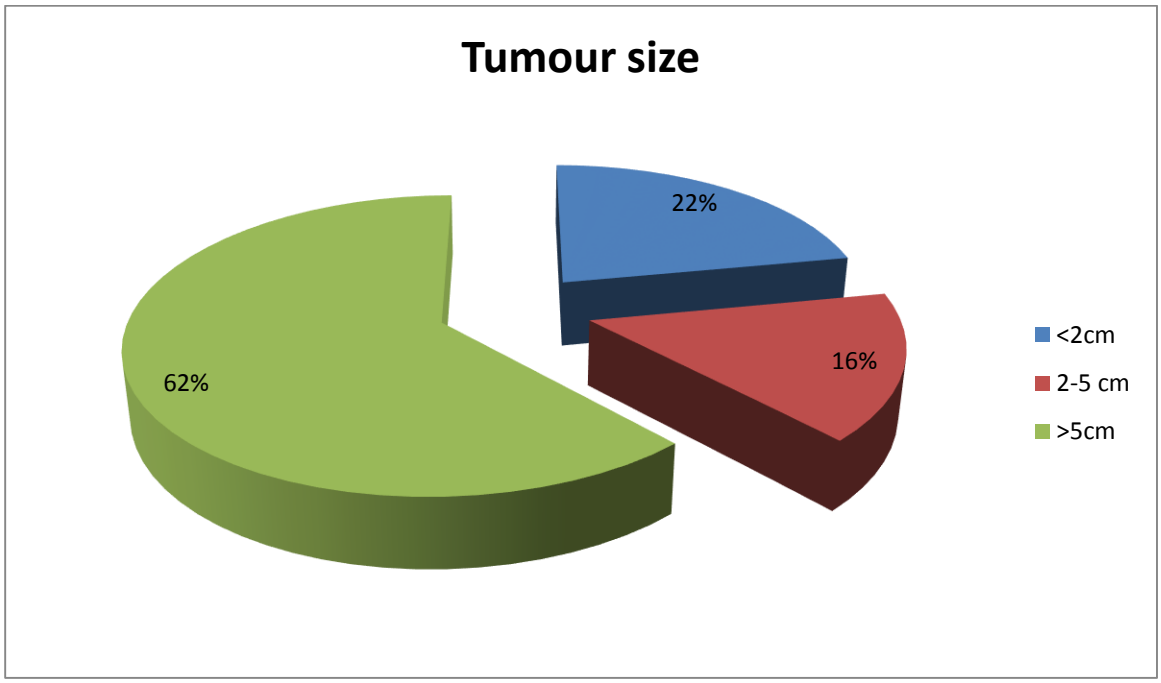


Table-5:**Distribution of breast cancers in biopsy and resection specimens**

Type of biopsy	NO OF CASES(n)	Percent(%)
Lt MRM	7	14.0
Rt MRM	12	24.0
Trucut Biopsy	28	56.0
Wide local excision	3	6.0
Total	50	100.0

Breast carcinoma reported in 28 cases (56%) of trucut biopsy, 12 cases(24%) right MRM , 7cases(14%) Left MRM and wide local excision 3 cases(6%).

Figure 6: **Distribution of breast cancers in biopsy and resection specimens**

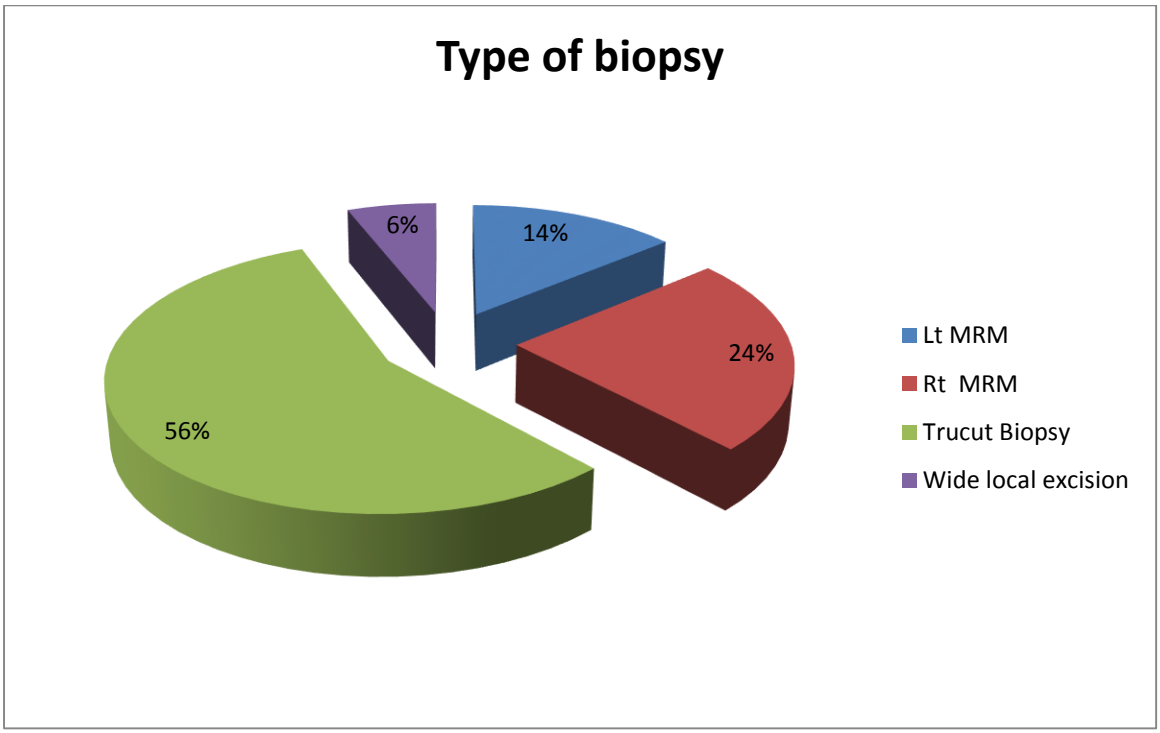


Table-6: Histological type of breast cancer

Histological diagnosis	**NO OF CASES(n)**	**Percent(%)**
IBC-NST	50	100.0

All the cases included in this study are Invasive breast carcinoma-NST.

Figure: 7 **Histological type of breast cancer**

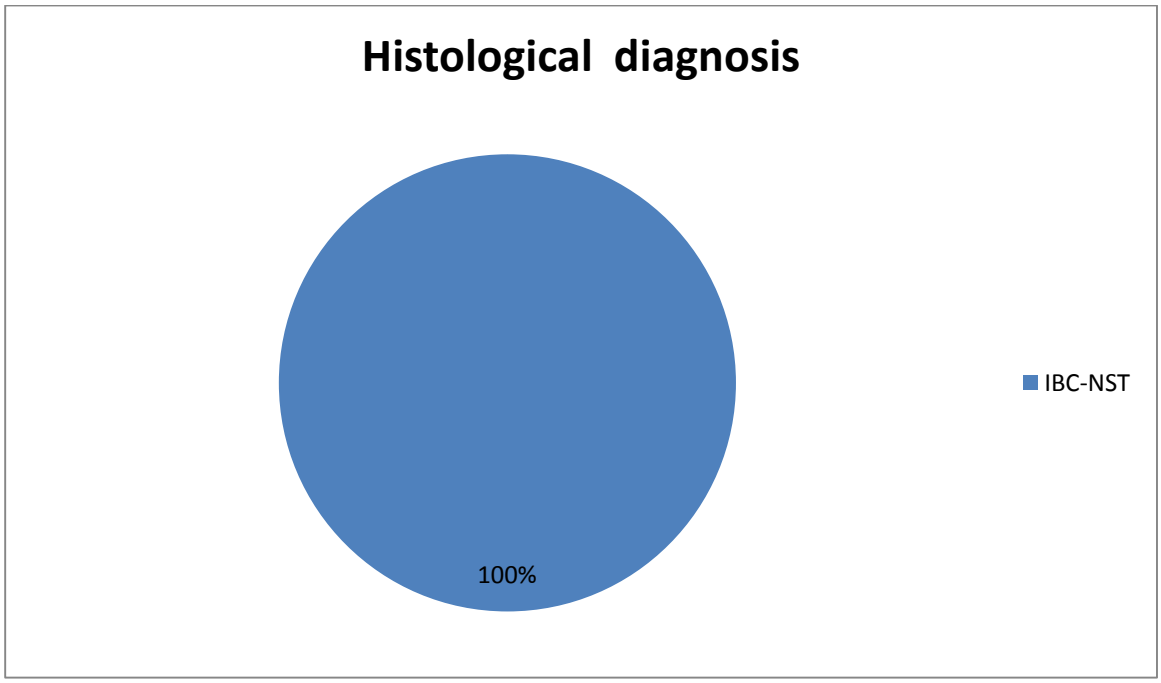


Table-7: Distribution Of breast Cancers with tumour Grade

GRADES	NO OF CASES(n)	Percent(%)
No grade	Nil	Nil
I	0	0
II	45	90.0
III	5	10.0
Total	50	100.0

Tumor grade was done according to modified Scarff-Bloom-Richardson grading system -low grade (grade 2) includes 90%(n=45) and high grade (grade 3) seen in 10%(n=10) only.

Figure: 8 **Distribution Of breast Cancers with tumour Grade**

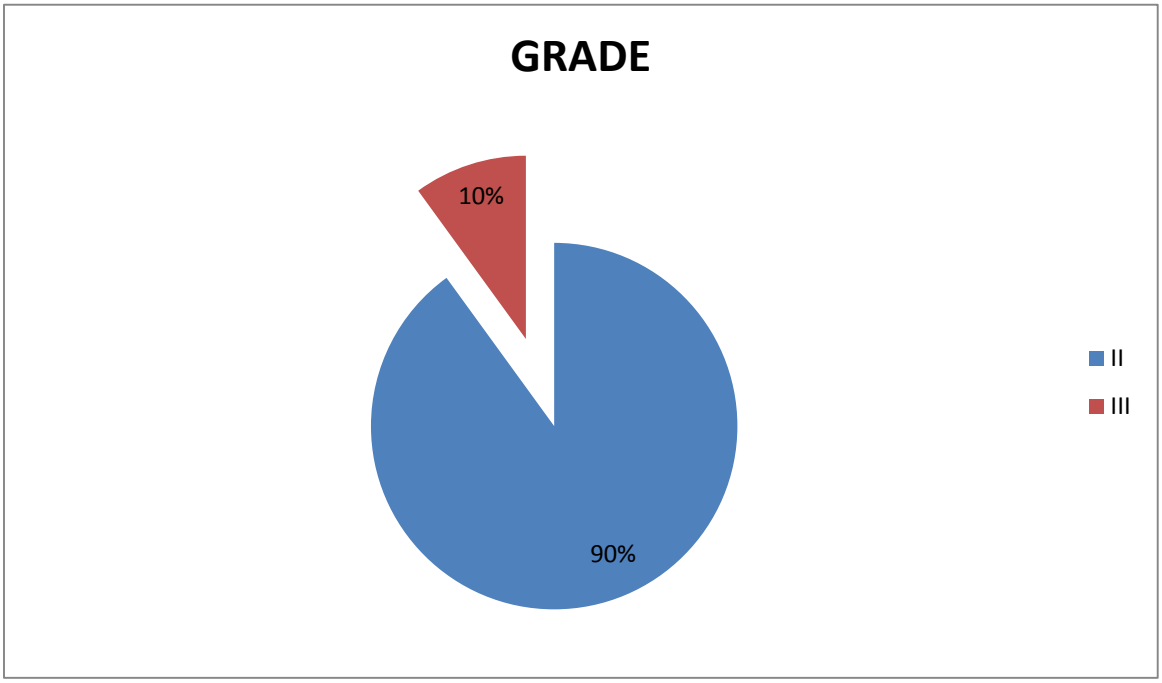


Table-8 Distribution of lymph Node metastasis in breast Cancers in MRM specimens

LYMPH NODE STATUS	NO OF CASES(n)	Percent(%)
NEGATIVE	31	62%
<= 3	13	26%
4-9	3	6%
>= 10	3	6%
Total	50	100

13 cases (26%) had upto 3 nodes with metastatic carcinomatous deposits, 3 cases(6%) had 4 to 9 involved nodes,3 cases(6%) had more than 10 involved nodes, while 31cases (62%) had no nodal involvement.

Figure 9: **Distribution of lymph Node metastasis in breast Cancers in MRM specimens**

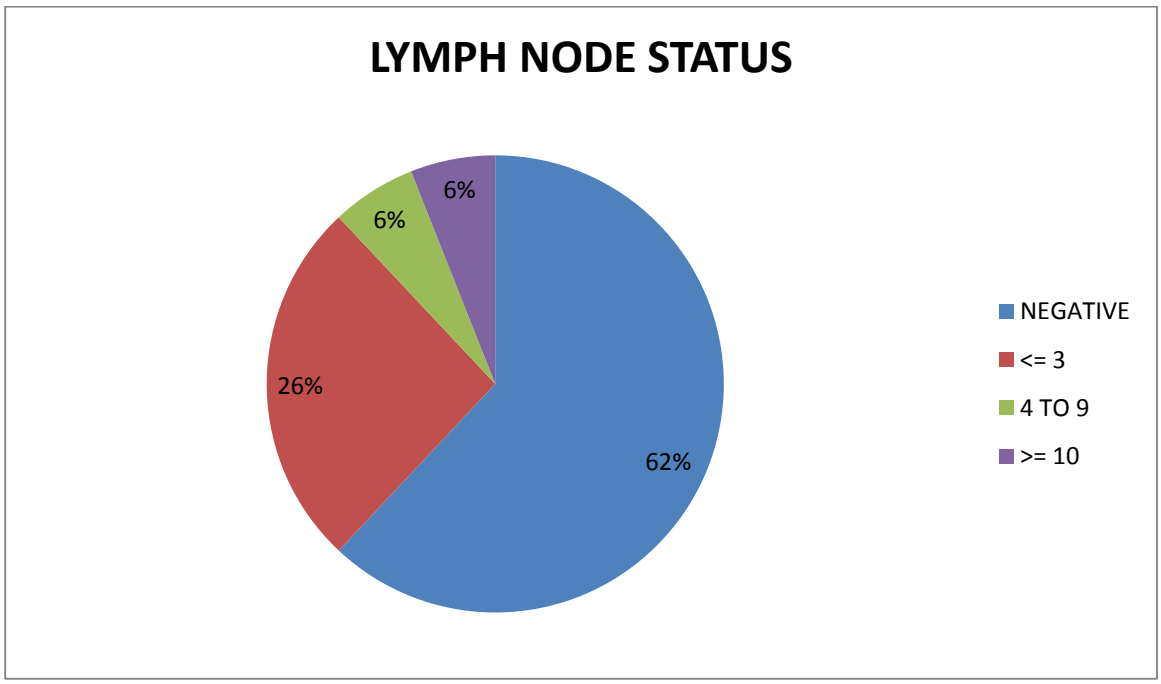


Table-10: **Expression of ER and PR in tissue IHC**

ER	NO OF CASES(n)	Percent(%)	PR	NO OF CASES(n)	Percent(%)
Positive	19	38.0	Positive	11	22.0
Negative	31	62.0	Negative	39	78.0
Total	50	100.0	Total	50	100.0

ER positive in 19 cases (38%),negative in 31 cases(62%) and PR positive in 11 cases(22%),negative in 39 cases(78%).

Figure 10: **Expression of ER and PR in tissue IHC**

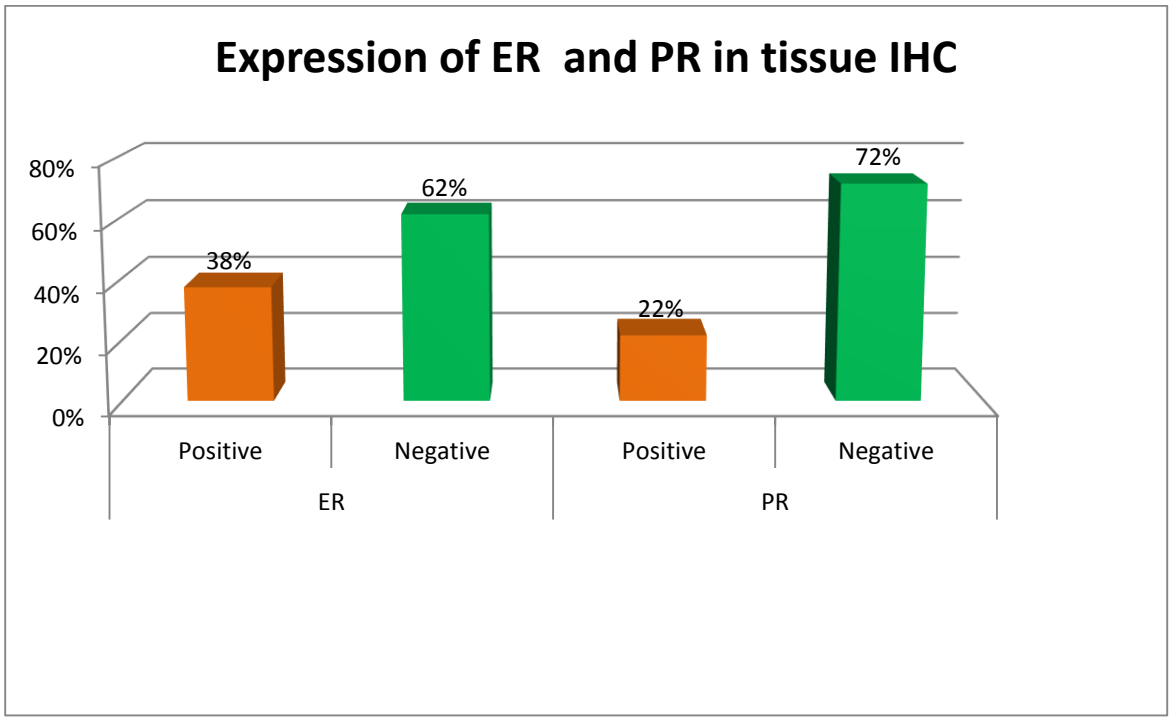


Table-11:Expression of ER and PR in FNAC IHC

ER	NO OF CASES(n)	Percent(%)	PR	NO OF CASES(n)	Percent(%)
Positive	14	28	Positive	7	14
Negative	36	72	Negative	43	86
Total	50	100.0	Total	50	100.0

ER positive in 14 cases (28%),negative in 36 cases(72%) and PR positive in 7 cases(14%),negative in 43 cases(86%).

Figure 11: **Expression of ER and PR in FNAC IHC**

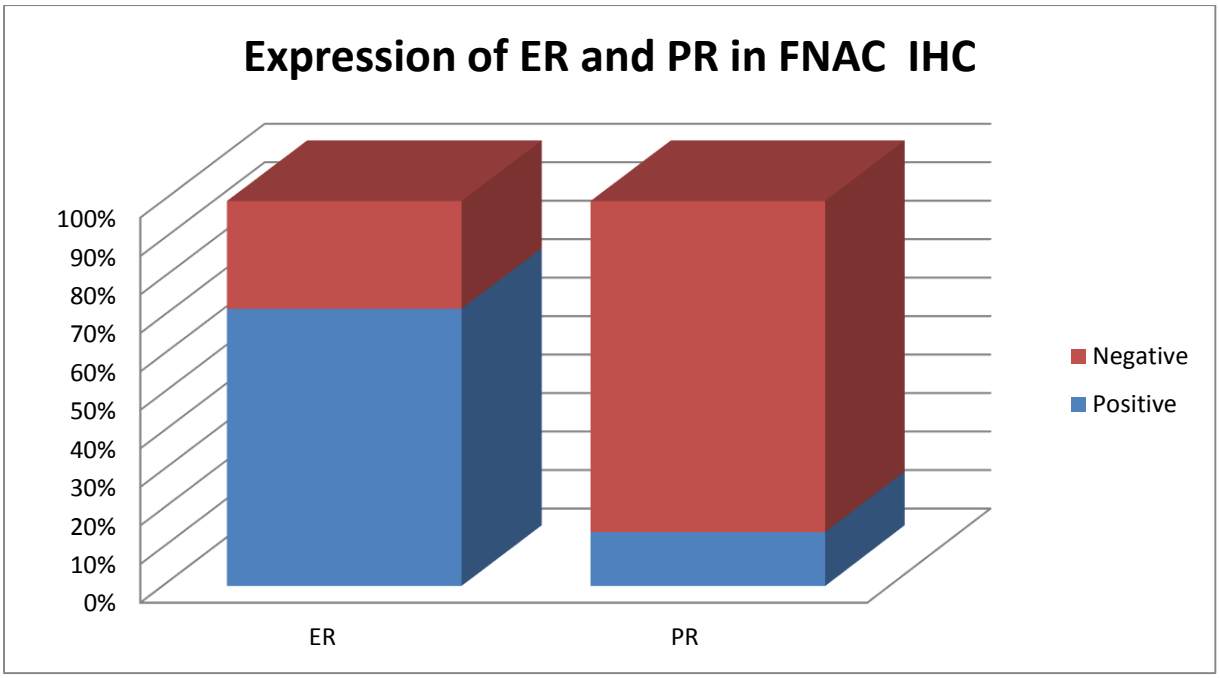


Table12: Expression of ER and PR In MRM specimen IN IHC

ER	NO OF CASES(n)	Percent(%)	PR	NO OF CASES(n)	Percent(%)
Positive	13	68%	Positive	17	89%
Negative	6	32%	Negative	2	11%
Total	19	100%	Total	19	100%

ER positive in 13 cases(68%) negative in 6 cases(32%) and PR positive in 17 cases()

Figure: 12 **Expression of ER and PR In MRM specimen IN IHC**

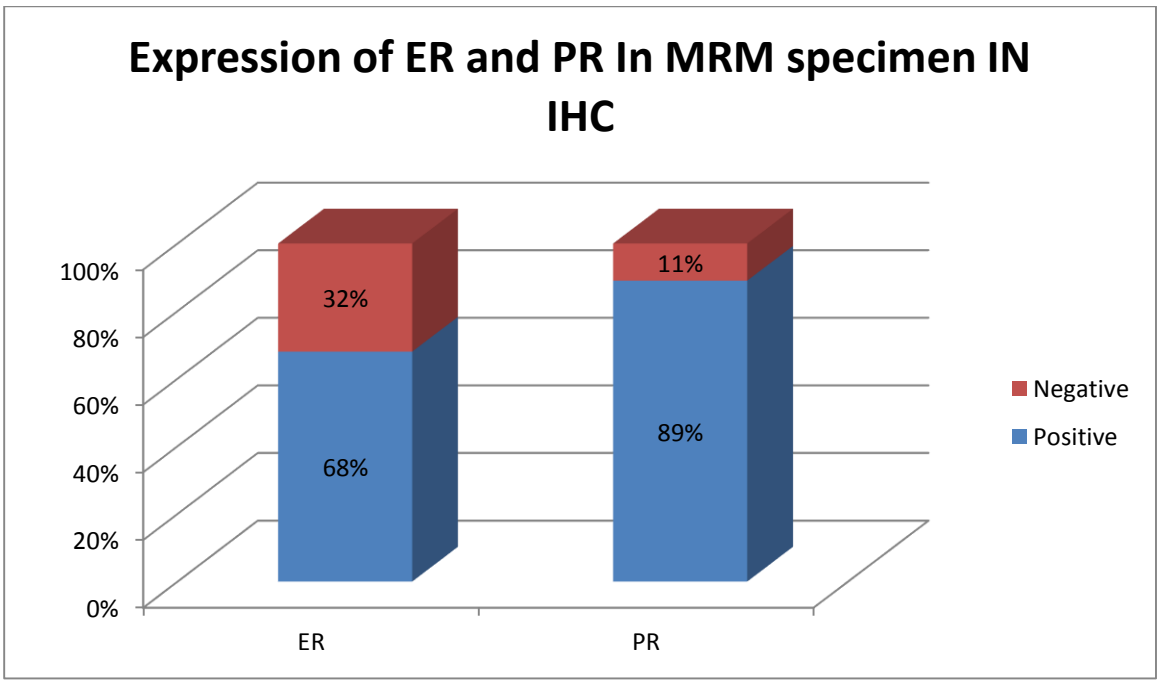


Table 13:**Expression of ER and PR In MRM specimen IN FNAC**

ER	NO OF CASES(n)	Percent(%)	PR	NO OF CASES(n)	Percent(%)
Positive	5	26%	Positive	3	11%
Negative	14	74%	Negative	16	89%
Total	19	100%	Total	19	100%

ER positive in 5 cases(26%),negative in14 cases(74%)and PR positive in 3 cases (11%) and negative in 16 cases(89%).

Figure13: **Expression of ER and PR In MRM specimen IN FNAC**

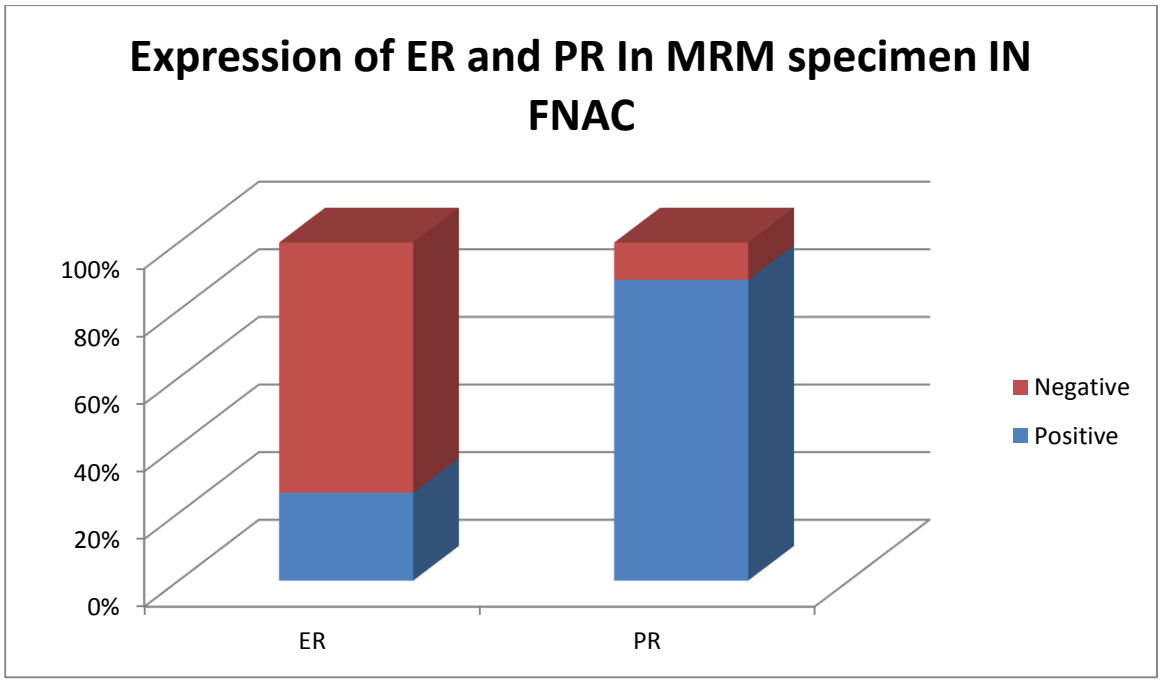


Table 14:Expression of ER and PR In Trucut biopsy IN IHC

ER	NO OF CASES(n)	Percent(%)	PR	NO OF CASES(n)	Percent(%)
Positive	16	54%	Positive	7	32%
Negative	15	54%	Negative	24	68%
Total	31	100%	Total	31	100%

ER positive in 16 cases(54%) ,negative in 15 cases(54%) and PR positive in 7 cases(32%) ,negative in 24 cases(68%)

Figure: 14 **Expression of ER and PR In Trucut biopsy IN IHC**

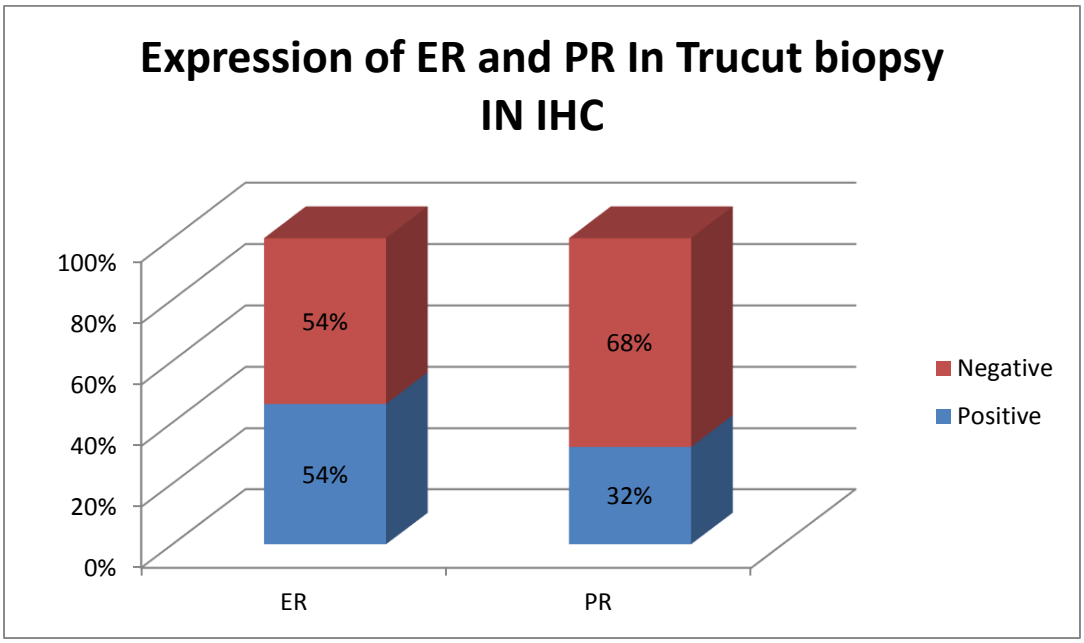


Table 14:Expression of ER and PR In Trucut biopsy IN FNAC

ER	NO OF CASES(n)	Percent(%)	PR	NO OF CASES(n)	Percent(%)
Positive	9	32%	Positive	5	18%
Negative	22	68%	Negative	26	82%
Total	31	100%	Total	31	100%

ER positive in 9 cases(32%) ,negative in 22 cases(68%) and PR positive in 5 cases(18%) ,negative 26 cases(82%).

Figure : 14 **Expression of ER and PR In Trucut biopsy IN FNAC**

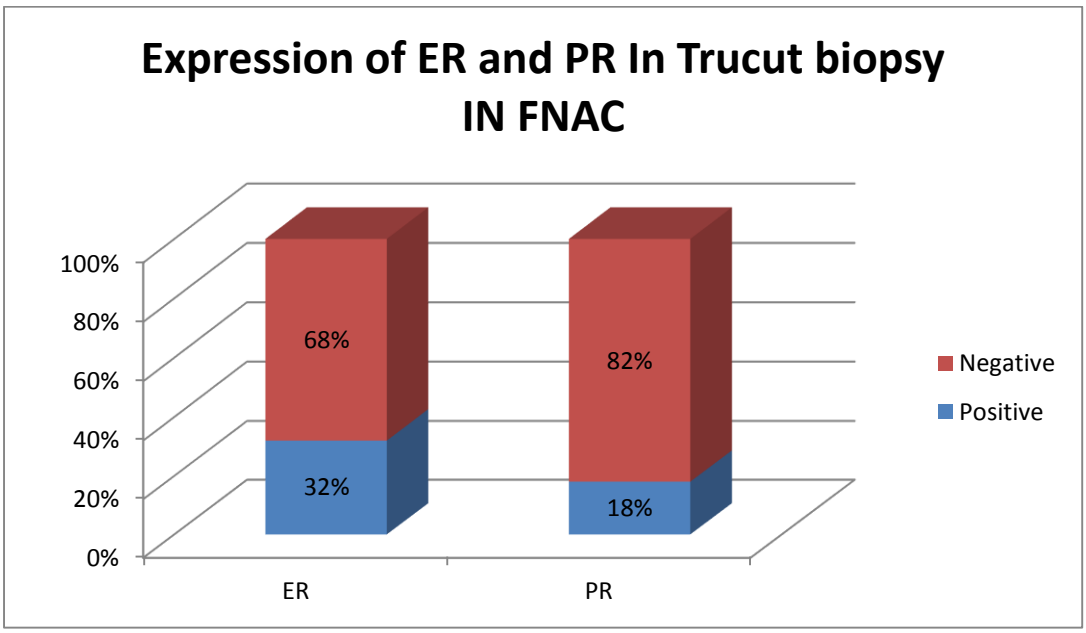


Table 15:Correlation Of ER and PR Expression And Age Of The Patients in TISSUE IHC

AGE GROUP	Tissue IHC HORMONE STATUS ER				PR			
	NEGATIVE		POSITIVE		NEGATIVE		POSITIVE	
	No.of cases	Percent %	No.of cases	Percent%	No.of cases	Percent%	No.of cases	Percent%

CHI SQUARE =6.224 P=0.183

Expression of ER and PR seen more in the age group of 61-70 years.

There is no significant correlation between age ER and PR expression.

Figure: 15 Correlation Of ER and PR Expression And Age Of The Patients in TISSUE IHC

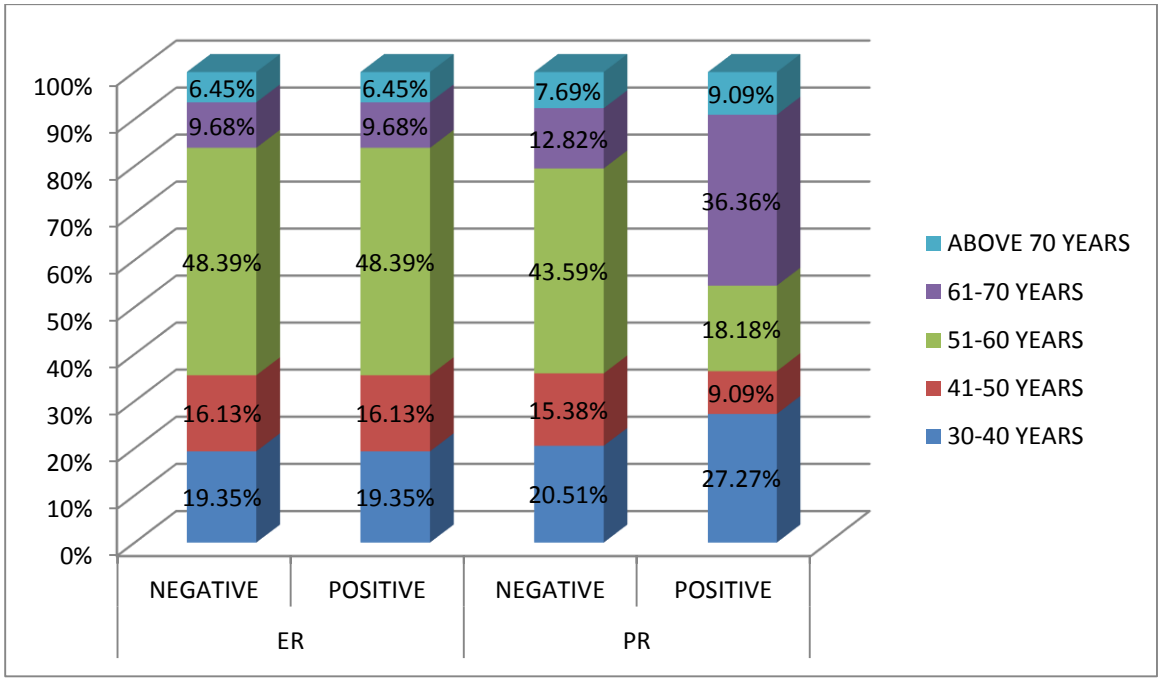
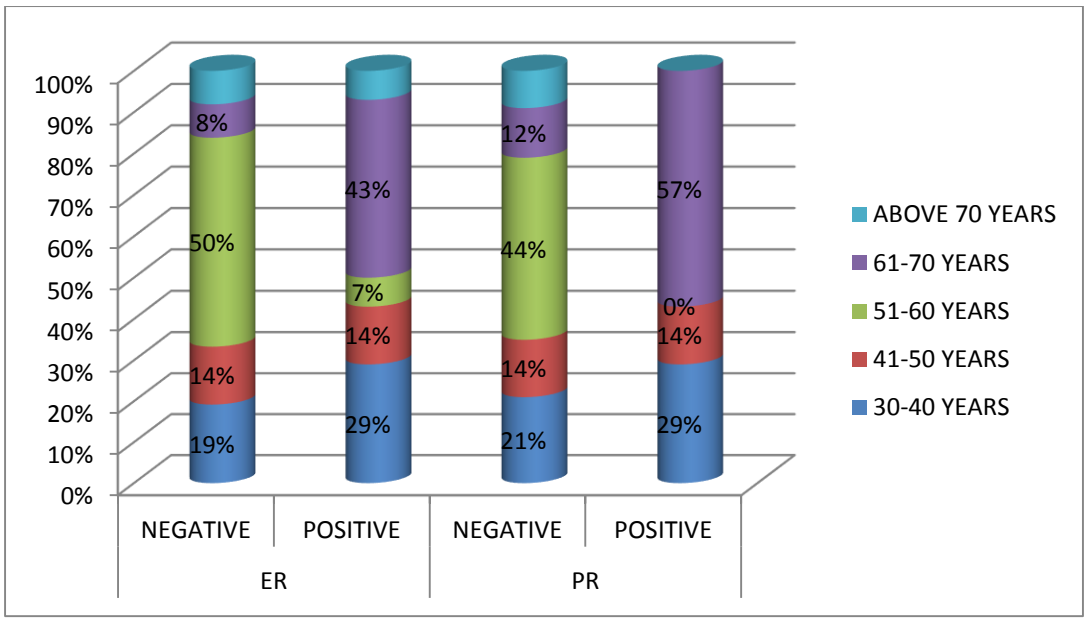


Table 16:Correlation Of ER and PR Expression And Age Of The Patients in FNAC IHC

AGE GROUP		FNAC IHC HORMONE STATUS ER				FNAC PR			
		NEGATIVE		POSITIVE		NEGATIVE		POSITIVE	
		No.of cases	Percent%	No.of cases	Percent%	No.of cases	Percent%	No.of cases	Percent%
	30-40 YEARS	7	19.44 %	4	28.57 %	9	20.93 %	2	28.57 %
	41-50 YEARS	5	13.89 %	2	14.29 %	6	13.95 %	1	14.29 %
	51-60 YEARS	18	50.00 %	1	7.14%	19	44.19 %	0	0.00%
	61-70 YEARS	3	8.33%	6	42.86 %	5	11.63 %	4	57.14 %
	ABOVE 70 YEARS	3	8.33%	1	7.14%	4	9.30%	0	0.00%
	TOTAL	36	100.00 %	14	100.00 %	43	100.00 %	7	100.00 %

Expression of ER and PR was seen more in the age group of 61-70 years. There is no significant correlation between age ER and PR expression.

Figure : 16 **Correlation Of ER and PR Expression And Age Of The Patients in FNAC IHC**



17.Correlation Of ER and PR Expression And Side Of Breast Involvement in tissue IHC

Site of tumour	Tissue IHC HORMONE STATUS ER				PR			
	NEGATIVE		POSITIVE		NEGATIVE		POSITIVE	
	No.of cases	Percent%	No.of cases	Percent%	No.of cases	Percent%	No.of cases	Percent%
Lt breast	9	29.03%	10	52.63%	12	30.77%	7	63.64%
Rt breast	22	70.97%	9	47.37%	27	69.23%	4	36.36%

Chi-square=2.785 P=0.095

		Left breast	Right breast
ER	NEGATIVE	29%	71%
	POSITIVE	52.63%	47.37%
PR	NEGATIVE	30.77%	69.23%
	POSITIVE	63.64%	36.36%

ER positive in Lt breast 10 cases(52.63%),negative in 9cases(29.03%),and
ER positive in Rt breast 9cases(47.37%),negative in 22cases(70.97%).
PR positive in Lt breast 7cases(63.64%),negative in 12 cases(30.77%).
PR positive in Rt breast 4 cases(36.36%)and negative in 27cases(69.23%).

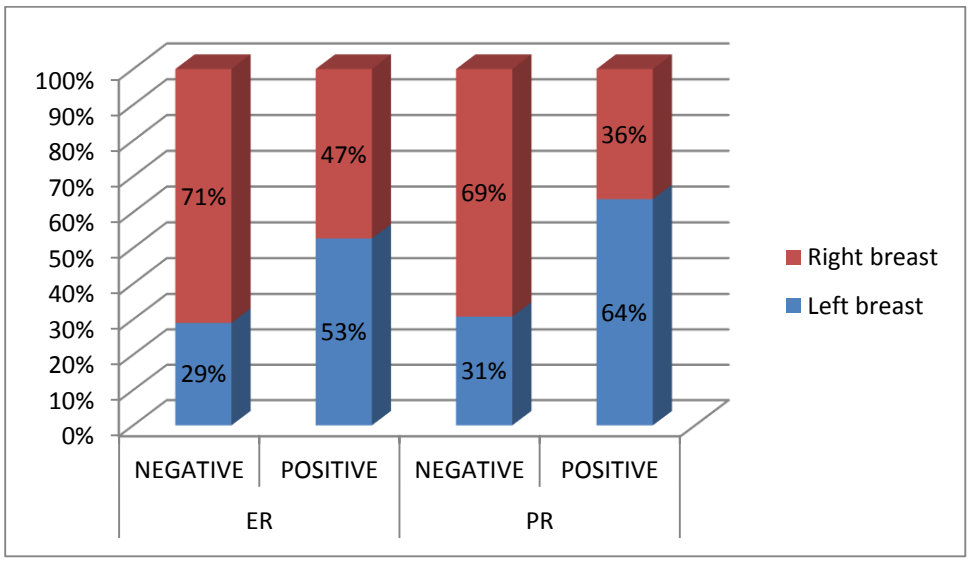


Table18:Correlation Of ER and PR expression And side of breast involvement in FNAC

		FNAC IHC HORMONE STATUS ER				FNAC PR			
		NEGATIVE		POSITIVE		NEGATIVE		POSITIVE	
		No.of cases	Percent %	No.of cases	Percent %	No.of cases	Percent %	No.of cases	Percent %
Site of tumour	Lt breast	13	36.11%	6	42.86%	16	37.21%	3	42.86%
	Rt breast	23	63.89%	8	57.14%	27	62.79%	4	57.14%
	TOTAL	36	100.0%	14	100.0%	43	100.0%	7	100.0%

ER positive in Lt breast 6 cases(42.86%), ER positive in Rt breast 8cases(57.14%),

PR positive in Lt breast 3cases(42.86%), PR positive in Rt breast 4cases(57.14%)

		Left breast	Right breast
ER	NEGATIVE	36%	64%
	POSITIVE	43%	57%
PR	NEGATIVE	37%	63%
	POSITIVE	43%	57%

Figure: 18 **Correlation Of ER and PR expression And side of breast involvement in FNAC**

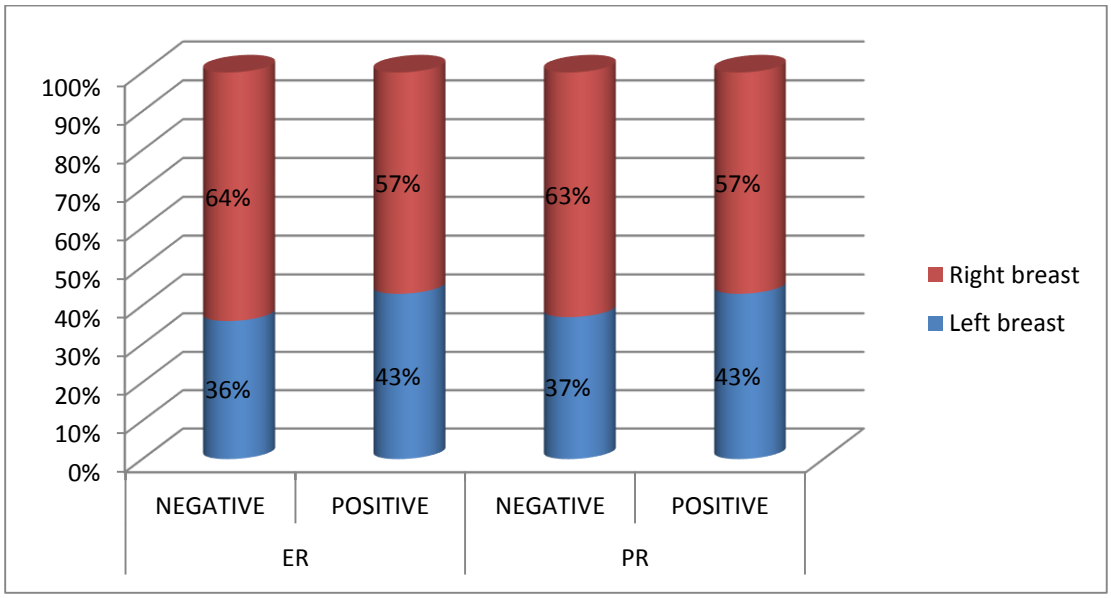


TABLE 19.Tumor Location ER and PR Expression in Tissue FNAC

		FNAC IHC HORMONE STATUS ER				FNAC PR			
		NEGATIVE		POSITIVE		NEGATIVE		POSITIVE	
		No.of cases	Percent%	No.of cases	Percent%	No.of cases	Percent%	No.of cases	Percent%
quadrant	Central	5	13.89%	1	7.14%	5	11.63%	1	14.29%
	LIQ	0	0.00%	1	7.14%	0	0.00%	1	14.29%
	LOQ	2	5.56%	1	7.14%	2	4.65%	1	14.29%
	UIQ	8	22.22%	2	14.29%	10	23.26%	0	0.00%
	UOQ	21	58.33%	9	64.29%	26	60.47%	4	57.14%
		36	100.0%	14	100.0%	43	100.0%	7	100.0%

		Central	LIQ	LOQ	UIQ	UOQ
ER	NEGATIVE	14%	0%	6%	22%	58%
	POSITIVE	7%	7%	7%	14%	64%
PR	NEGATIVE	12%	0%	5%	23%	60%
	POSITIVE	14%	14%	14%	0%	57%

Tumor located in the UOQ showed ER positive 9 out of 30 cases.PR positive 4cases out of 30 cases.

Figure 19: **Tumor Location ER and PR Expression in Tissue FNAC**

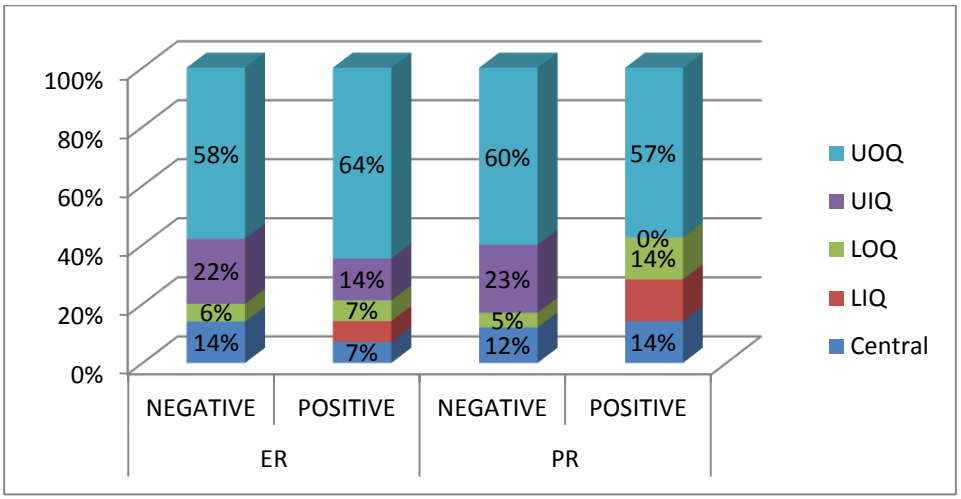


Table20:Lymph Nodal Status And ER PR Expression in tissue and FNAC

Lymph nodal Status	Tissue IHC HORMONE STATUS ER				PR				FNAC IHC HORMONE STATUS ER				FNAC PR			
	NEGATIVE		POSITIVE		NEGATIVE		POSITIVE		NEGATIVE		POSITIVE		NEGATIVE		POSITIVE	
	No. of cases	Percent %	No. of cases	Percent %	No. of cases	Percent %	No. of cases	Percent %	No. of cases	Percent %	No. of cases	Percent %	No. of cases	Percent %	No. of cases	Percent %
NEGATIVE	18	58.06%	13	68.42%	22	56.41%	9	81.82%	22	61.11%	9	64.29%	26	60.47%	5	71.43%
0-3	9	29.03%	4	21.05%	12	30.77%	1	9.09%	10	27.78%	3	21.43%	12	27.91%	1	14.29%
4-9	1	3.23%	2	10.53%	2	5.13%	1	9.09%	1	2.78%	2	14.29%	2	4.65%	1	14.29%

>=10	3	9.68 %	0	0.00 %	3	7.69 %	0	0.00 %	3	8.33 %	0	0.00%	3	6.98%	0	0.00%

Figure 20: **Lymph Nodal Status And ER PR Expression in tissue and FNAC**

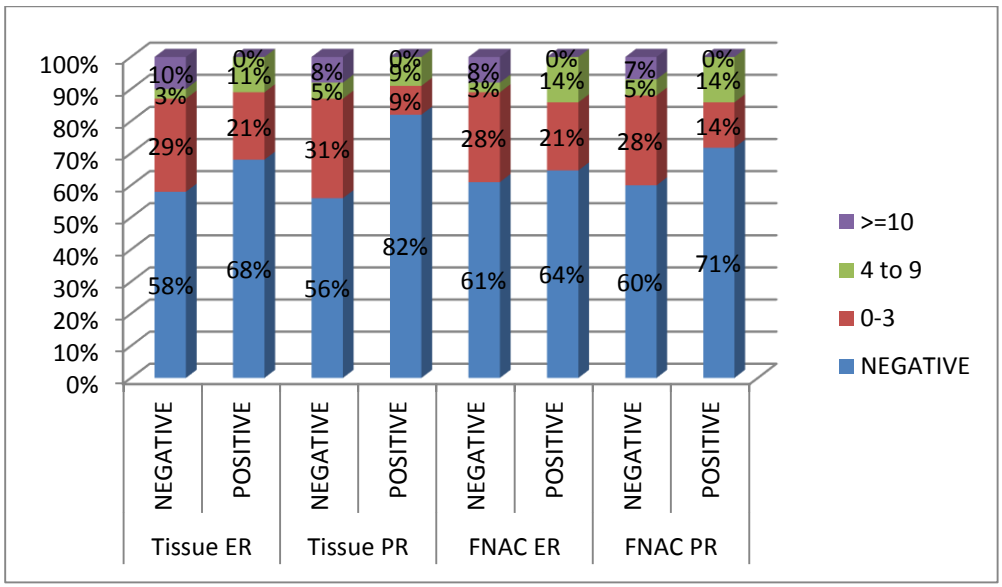


Table 21: Comparison of FNAC ER and Tissue ER Hormone status

			Tissue IHC HORMONE STATUS ER		Total
			POSITIVE	NEGATIVE	
FNAC IHC HORMONE STATUS ER	POSITIVE	No.of cases	14	0	14
		Percent	73.7%	0.0%	28.0%
	NEGATIVE	No.of cases	5	31	36
		Percent	26.3%	100.0%	72.0%
Total		No.of cases	19	31	50
		Percent	100.0%	100.0%	100.0%

Comparison of FNAC ER and Tissue ER Hormone status

SENSTIVITY	73.68%
SPECIFICITY	100.00%
POSTIVE PREDICTIVE VALUE	100.00%
NEGATIVE PREDICTIVE VALUE	86.11%
DISEASE PREVALENCE	38.00%
DIAGNOSTIC ACCURACY	90
FALSE POSITIVITY RATE	0.00%
FALSE NEGATIVITY RATE	26.32%
KAPPA	0.776**

**P<0.001

Table 22: Comparison of FNAC PR and Tissue PR Hormone status

			Tissue IHC HORMONE STATUS PR		Total
			POSITIVE	NEGATIVE	
FNAC PR	POSITIVE	No.of cases	7	0	7
		Percent	63.6%	0.0%	14.0%
	NEGATIVE	No.of cases	4	39	43
		Percent	36.4%	100.0%	86.0%
Total		No.of cases	11	39	50
		Percent	100.0%	100.0%	100.0%

Comparison of FNAC PR and Tissue PR Hormone status

SENSTIVITY	63.64%
SPECIFICITY	100.00%
POSTIVE PREDICTIVE VALUE	100.00%
NEGATIVE PREDICTIVE VALUE	90.70%
DISEASE PREVALENCE	22.00%
DIAGNOSTIC ACCURACY	92
FALSE POSITIVITY RATE	0.00%
FALSE NEGATIVITY RATE	36.36%
KAPPA	0.732**

**P<0.001

COLOR PLATES

INVASIVE DUCTAL CARCINOMA

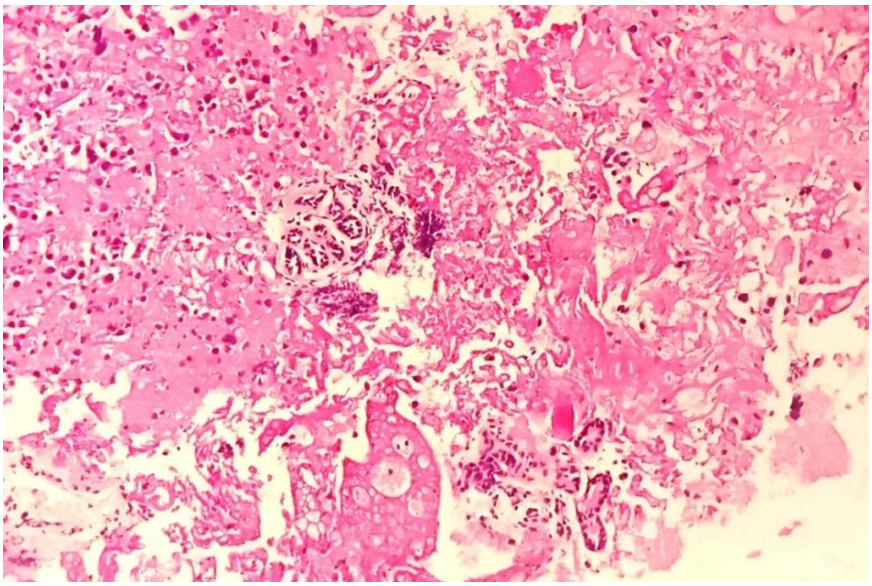


Figure:21 Invasive ductal carcinoma –Tumour cells showing tubule formation. (100X)

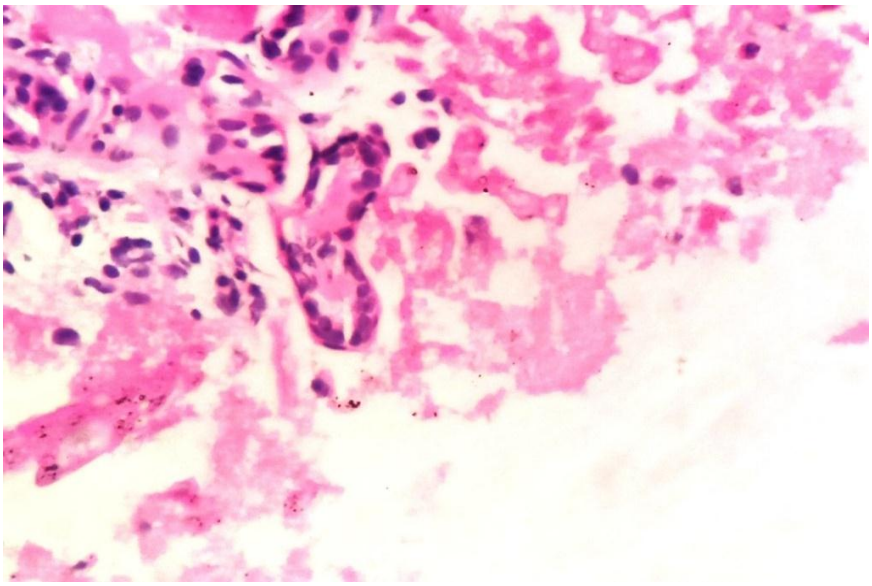


Figure 22: Malignant duct epithelial cells showing mild nuclear pleomorphism (400x)

ER

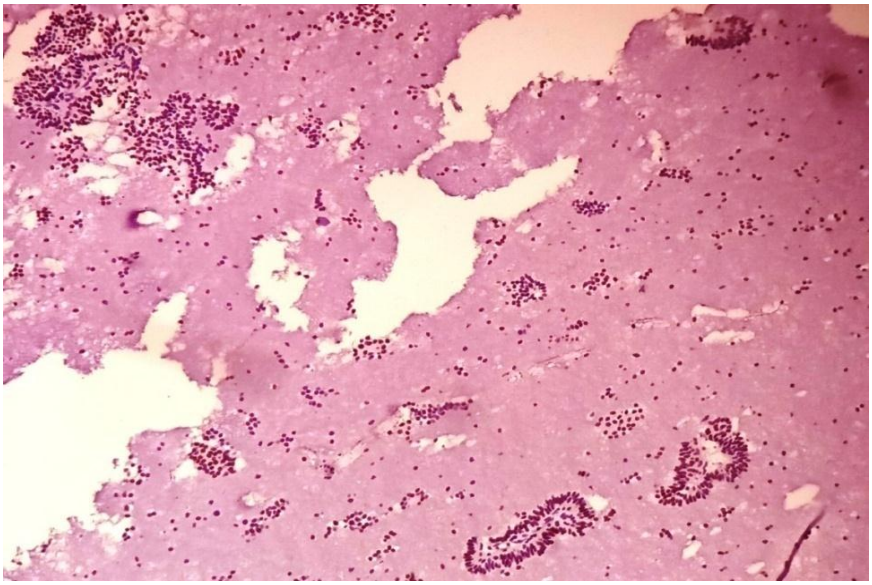


Figure 23:Positive for ER (4+3) 100X

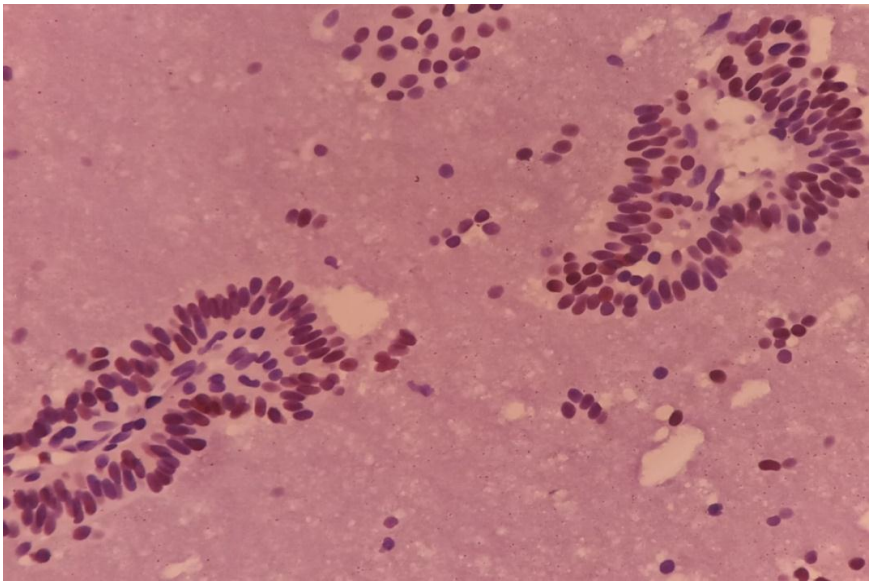


Figure 24:Positive for ER (4+3) 400X

PR

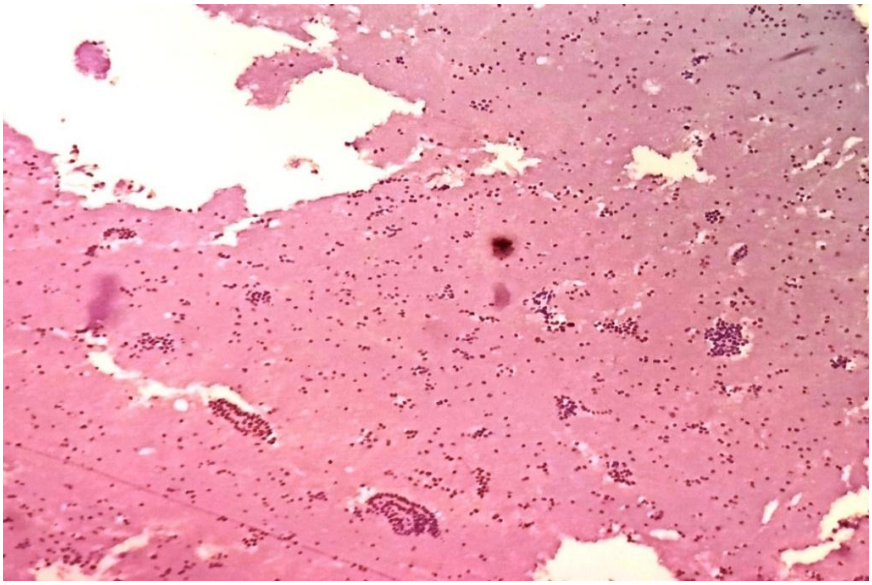


Figure 25:Positive for PR (4+3) 40X

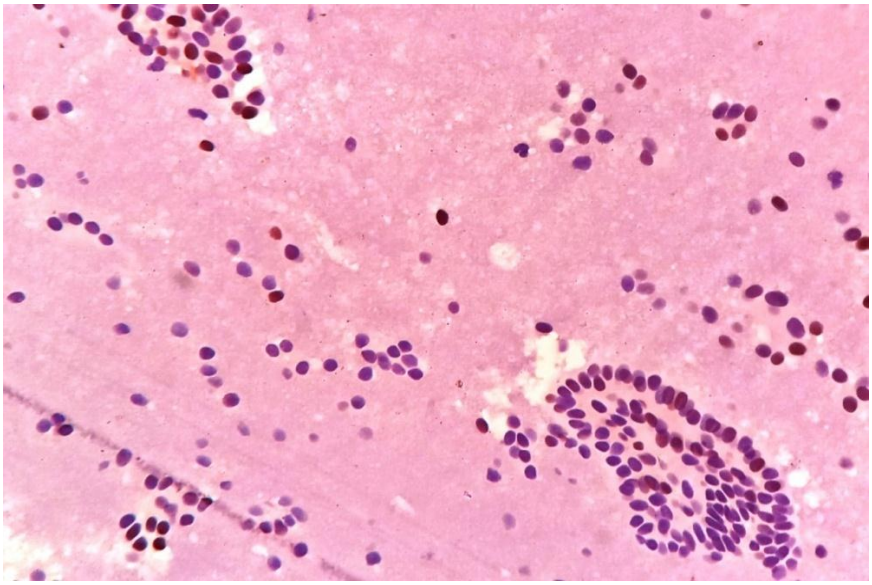


Figure 26:Positive for PR (4+3) 400X

INVASIVE DUCTAL CARCINOMA

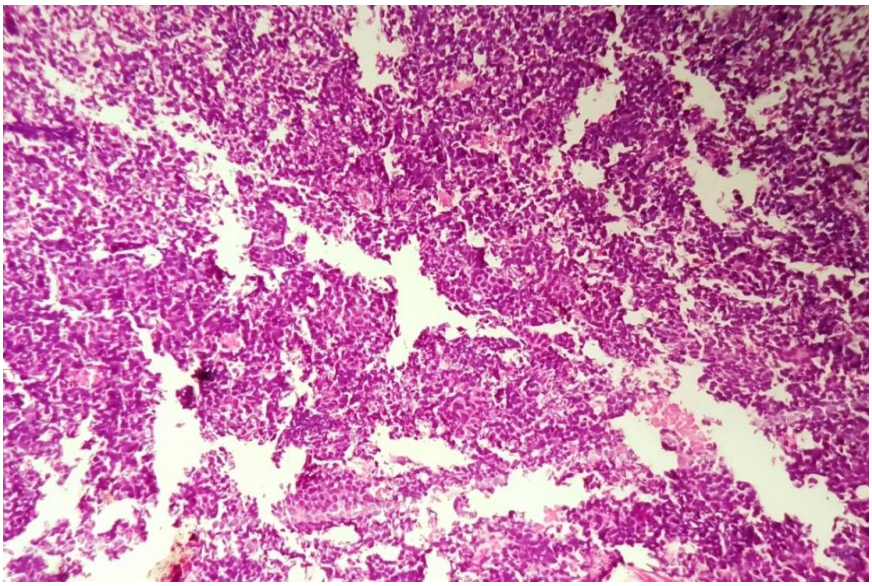


Figure:27 Invasive ductal carcinoma –Tumour cells arranged in sheets (100x)

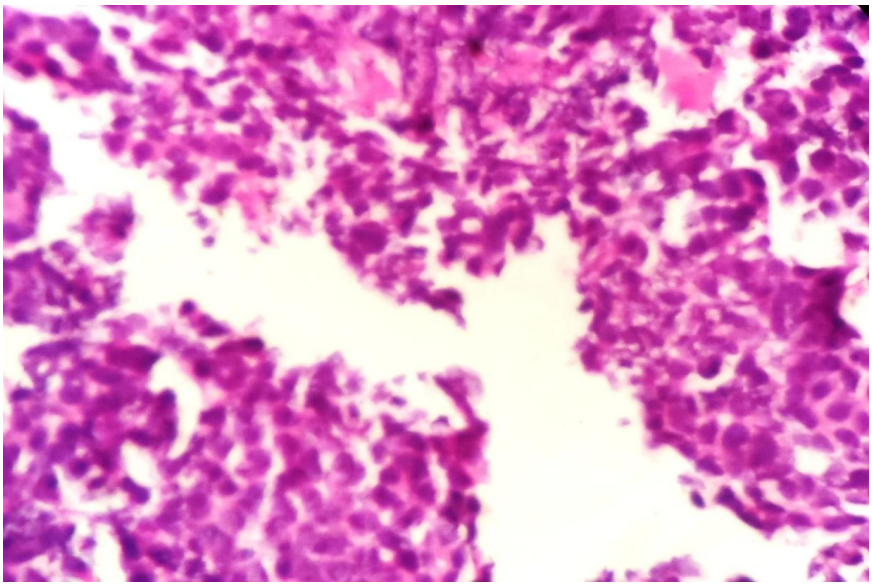


Figure:28 Invasive ductal carcinoma Malignant duct epithelial cells showing moderate pleomorphism(40x)

ER

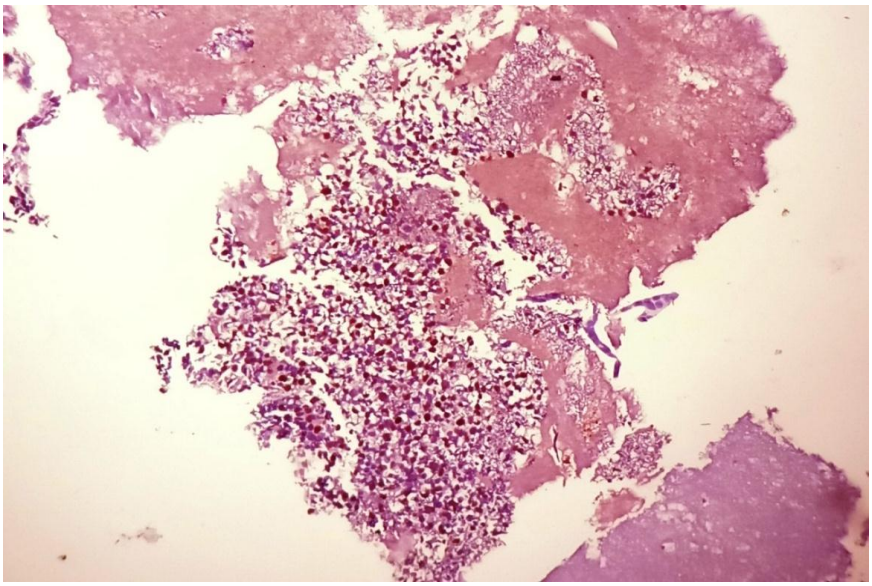


Figure2 9:Positive for ER (3+3) 100X

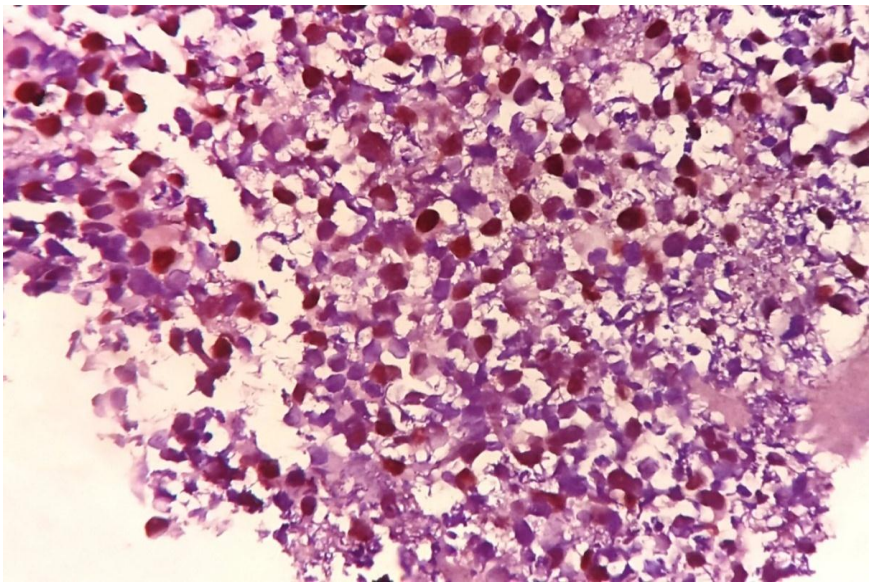


Figure 30: Positive for ER (3+3) 400X

PR

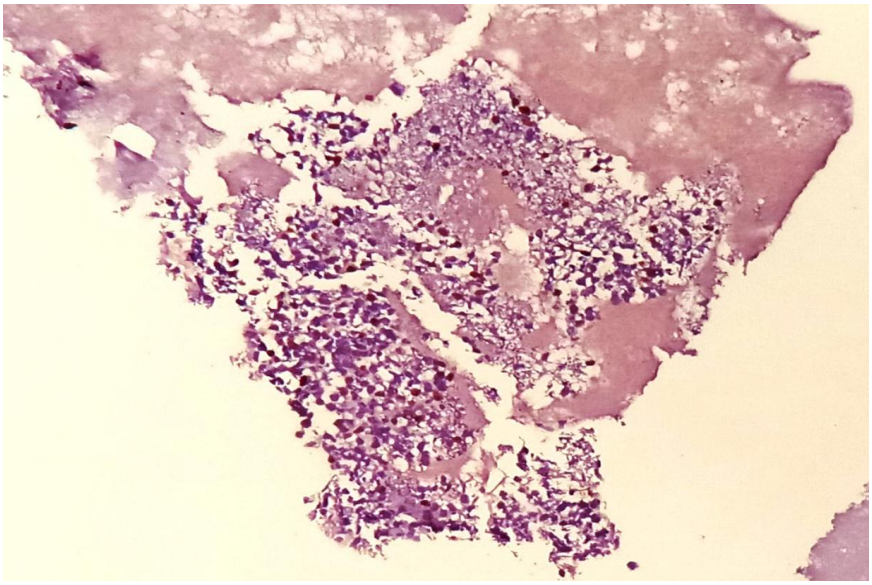


Figure31:Positive for PR (3+3) 100X

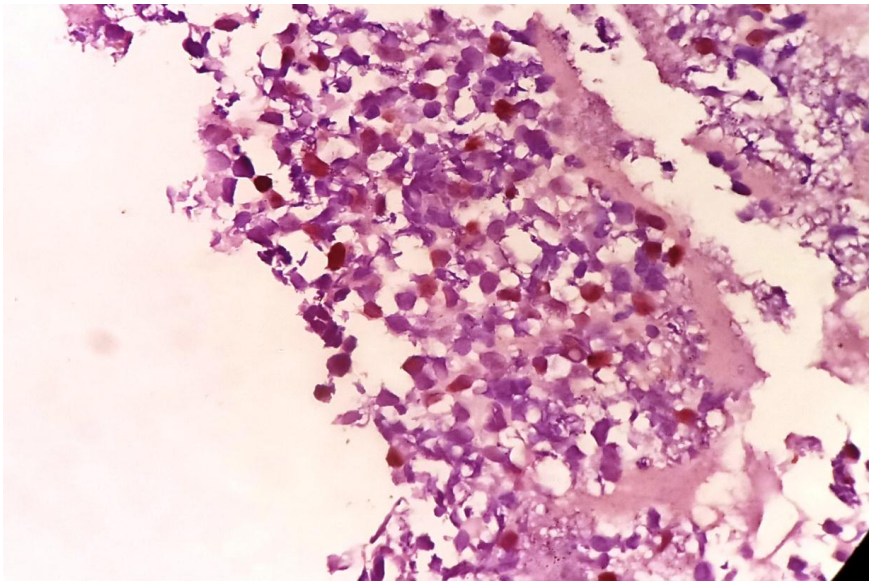


Figure 32: Positive for PR (3+3) 400X

INVASIVE DUCTAL CARCINOMA

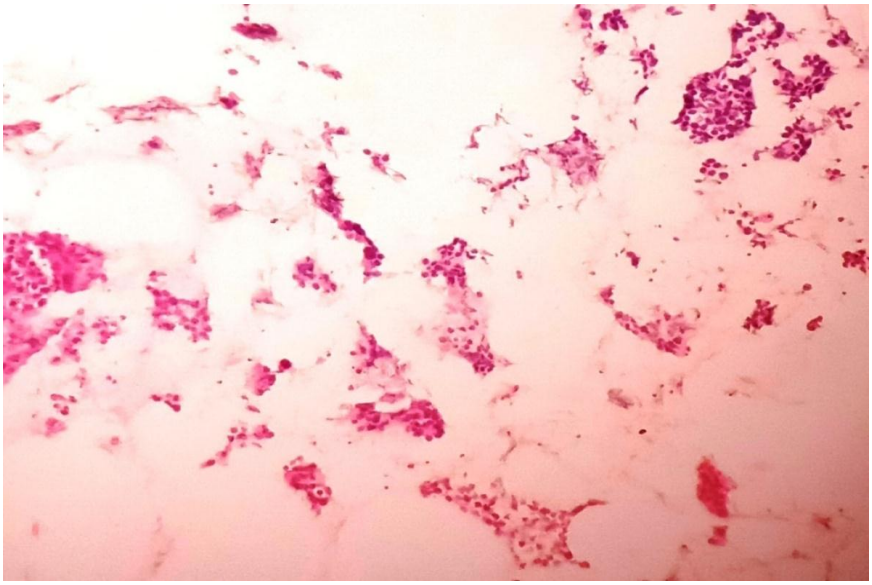


Figure 33: Invasive ductal carcinoma –Tumour cells showing tubule formation (100x)

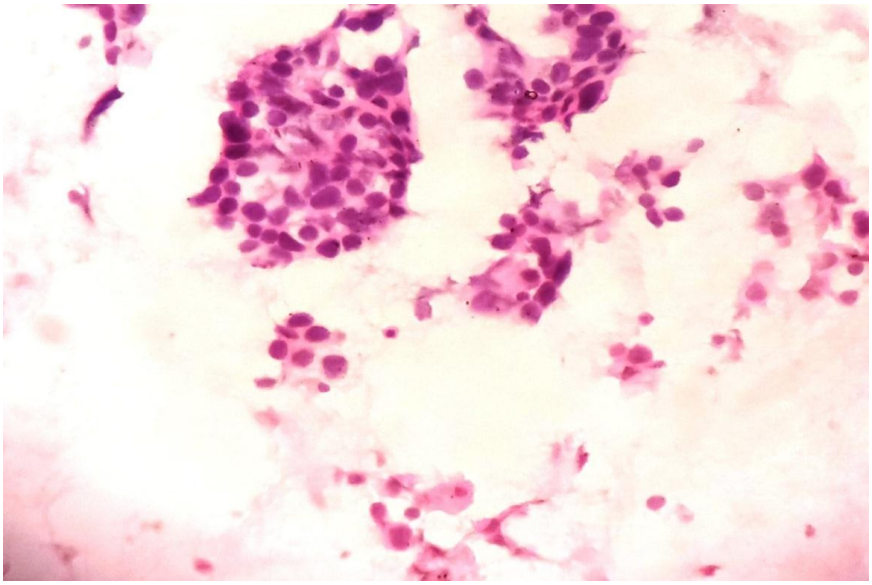


Figure 34:Malignant duct epithelial cells show moderate pleomorphism(400X)

ER

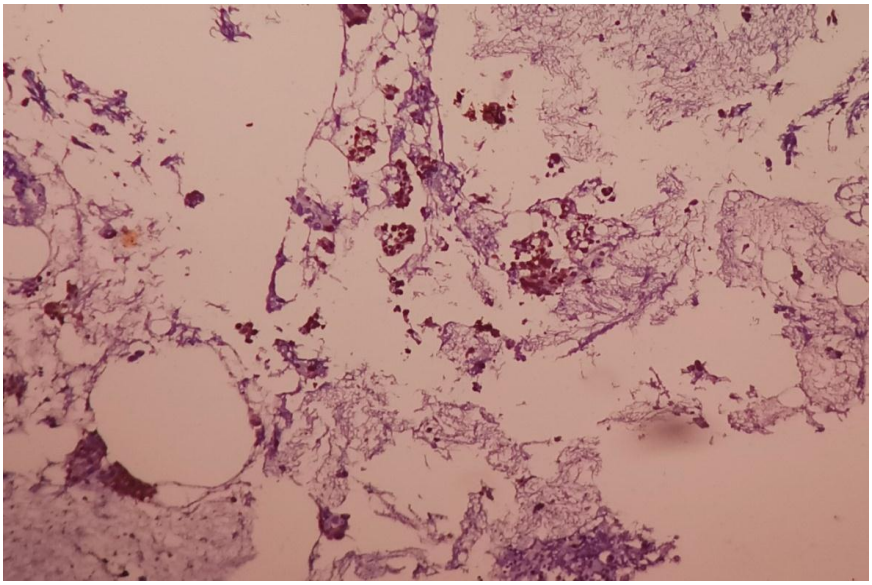


Figure 35 : ER positive (5+5) (100X)

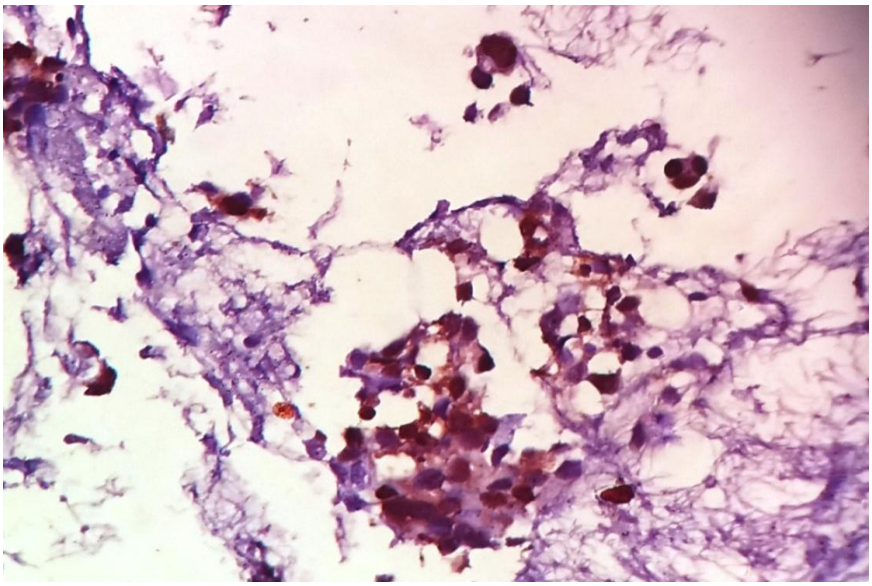


Figure 36:ER positive (5+3) (400X)

PR

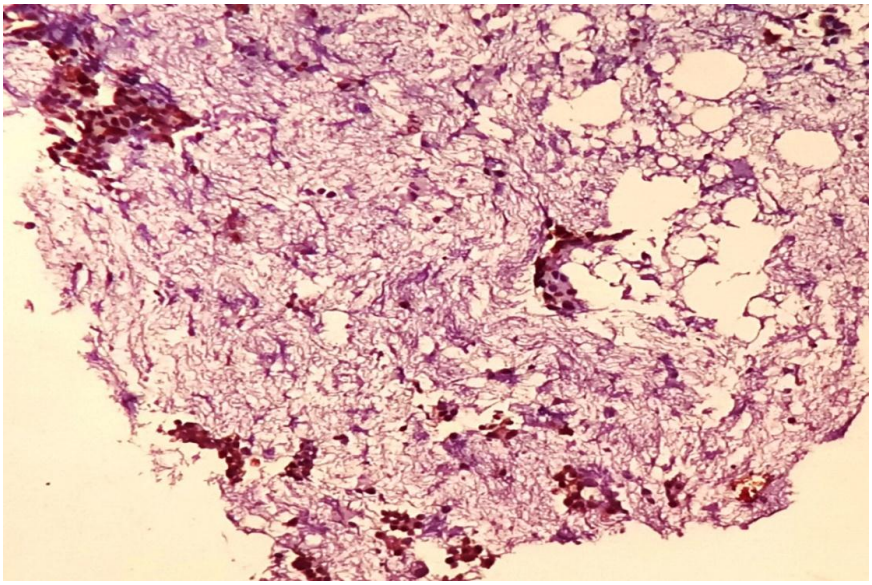


Figure 37:PR positive (5+3) (100X)

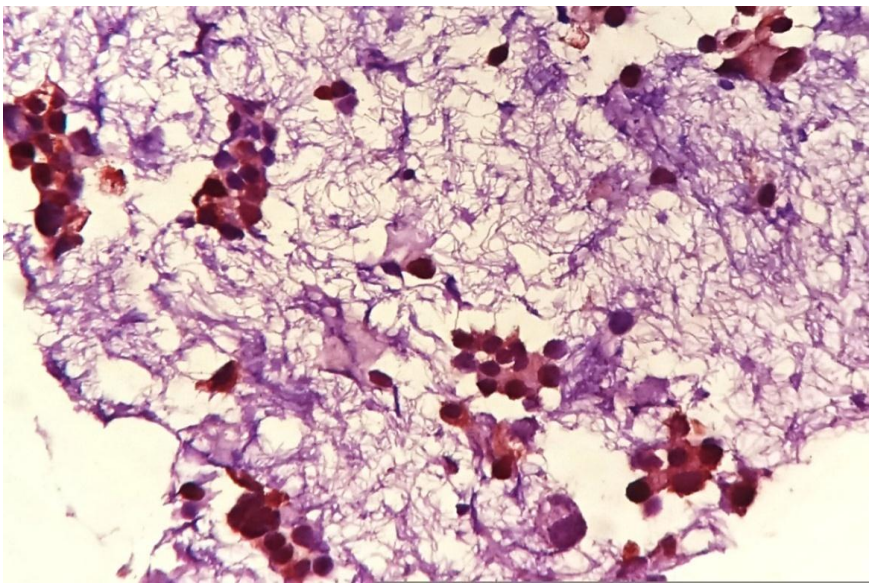


Figure 38:PR positive(5+3) 400X

INVASIVE DUCTAL CARCINOMA

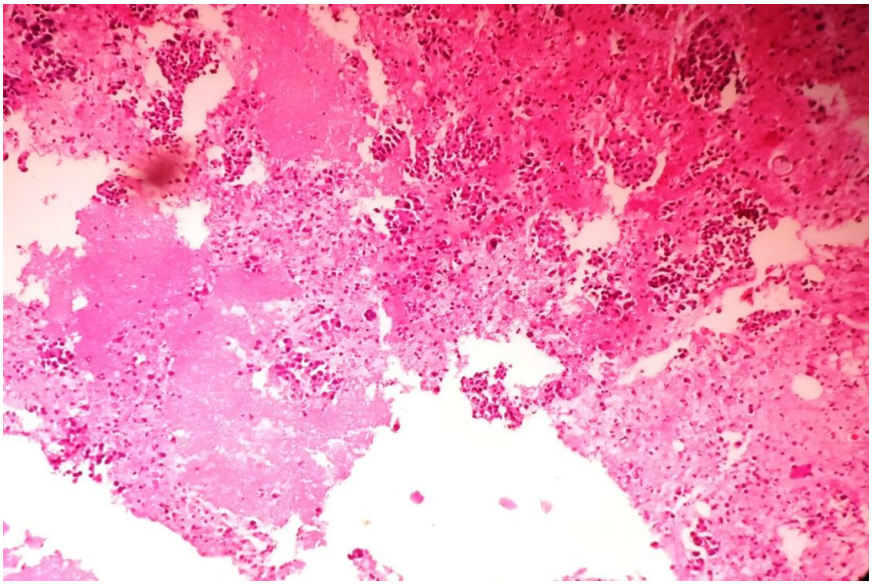


Figure 39: Invasive ductal carcinoma –Tumour cells arranged in sheets (100x)

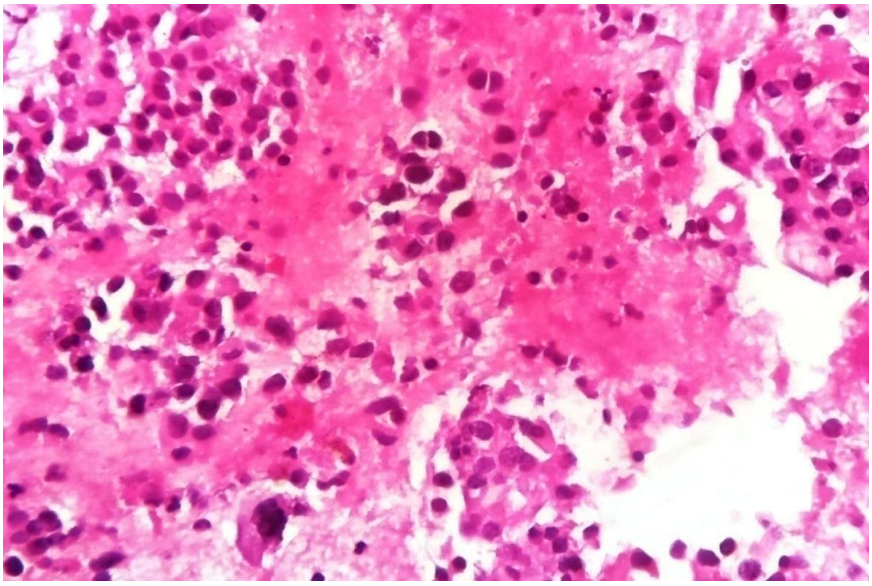


Figure 40:Malignant epithelial cells showing moderate pleomorphism(400X)

ER

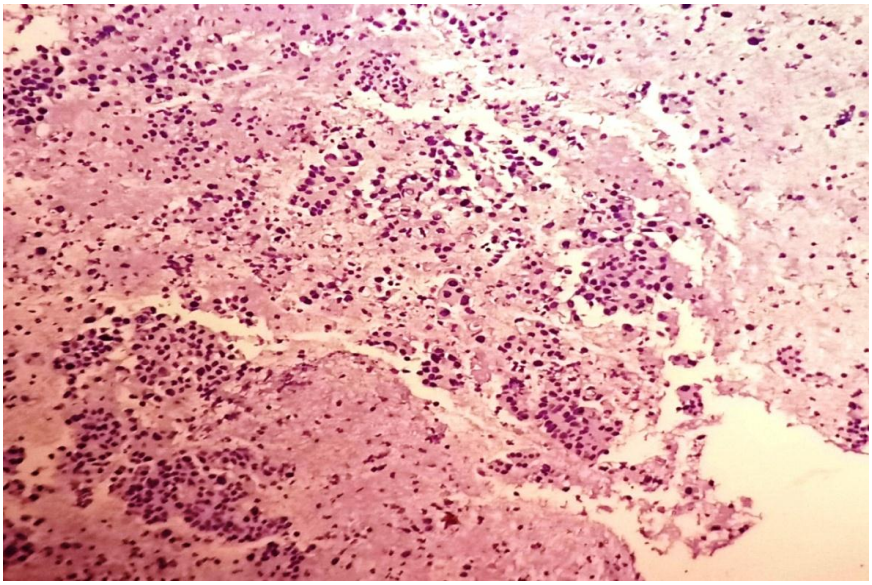


Figure 41:ER-Negative(100X)

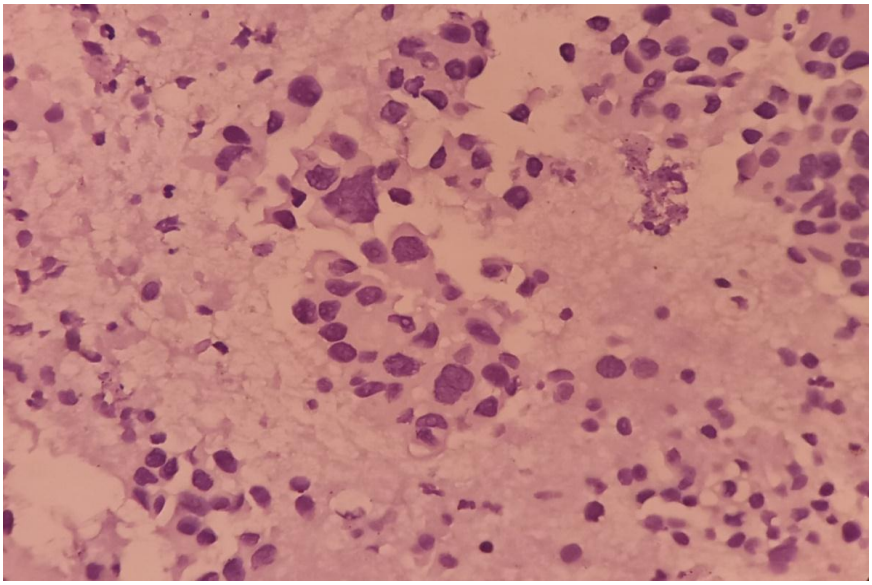


Figure 42:ER-Negative(400X)

PR

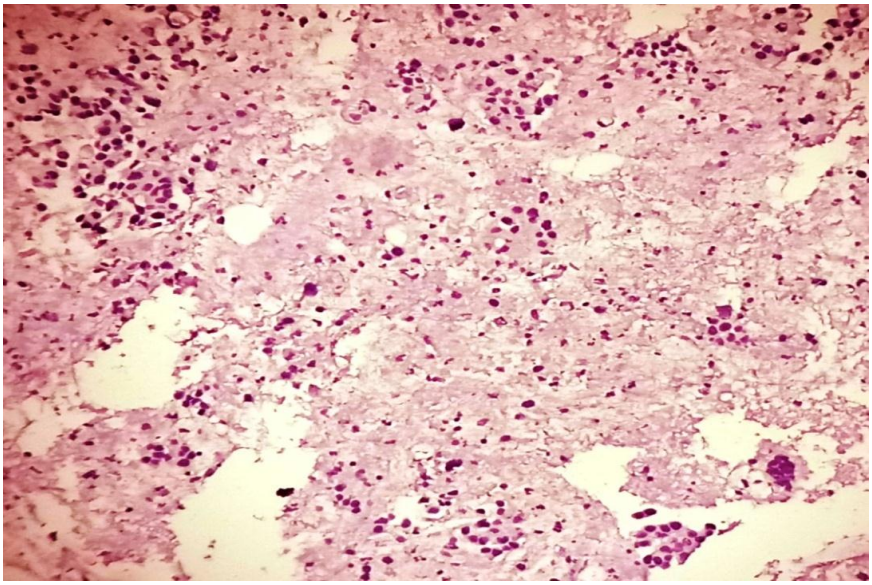


Figure 43:PR-Negative(100X)

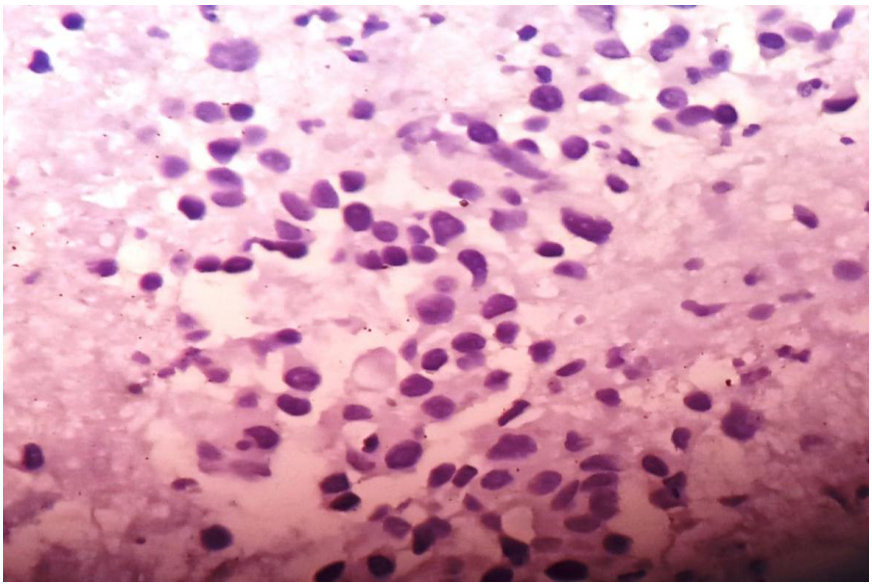


Figure 44:PR-Negative(400X)

INVASIVE DUCTAL CARCINOMA NOS -GRADE II

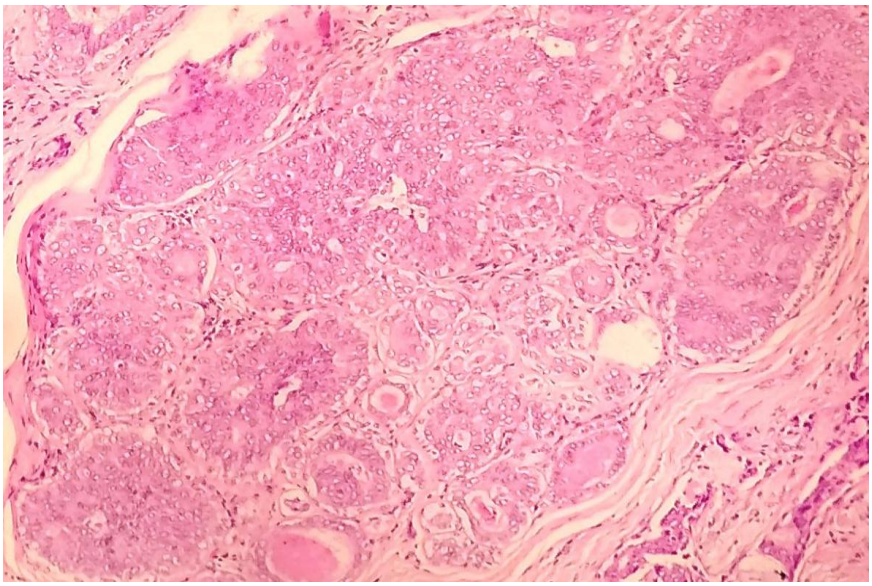


Figure45: **Sheets of malignant epithelial cells, 30 % tubule formation (100 X)**

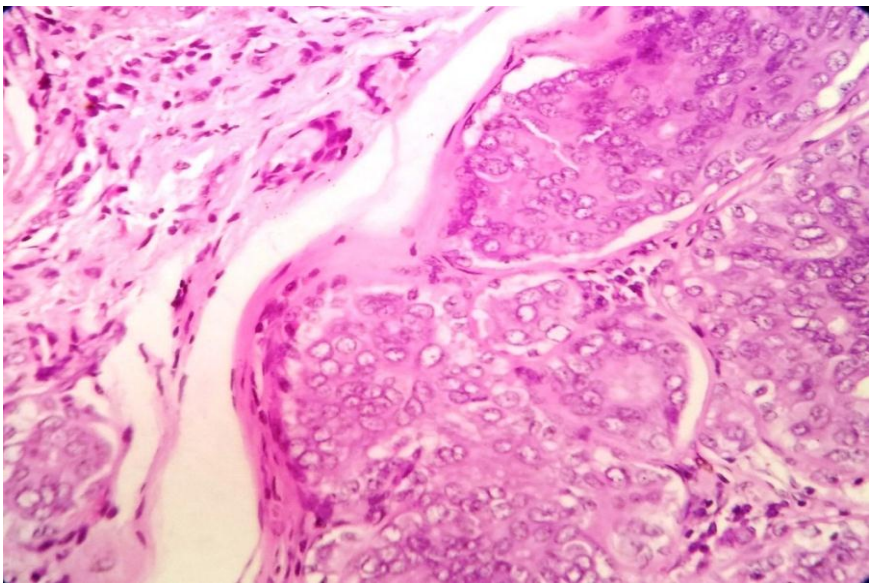


Figure 46 -Malignant epithelial in sheets with moderate nuclear pleomorphism (400 X)

ER

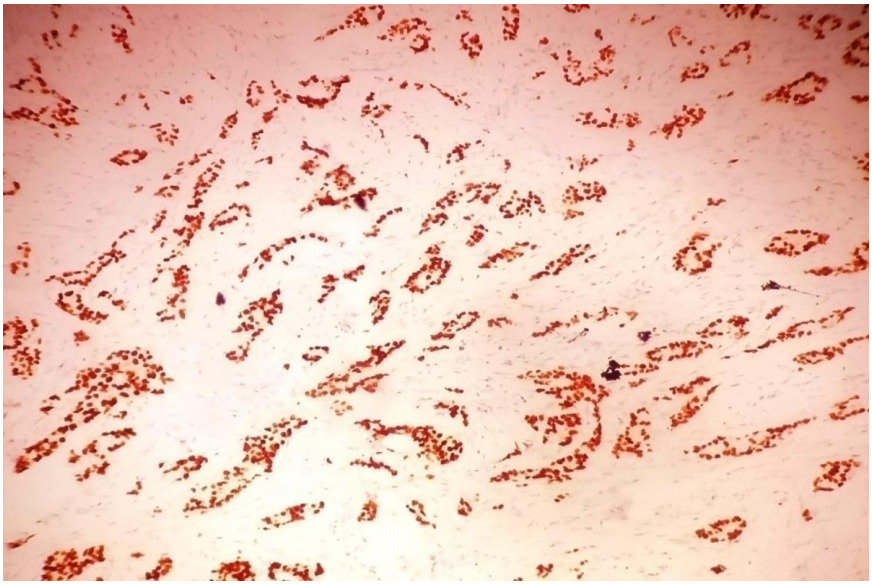


Figure 47:ER Positive(5+3)100X

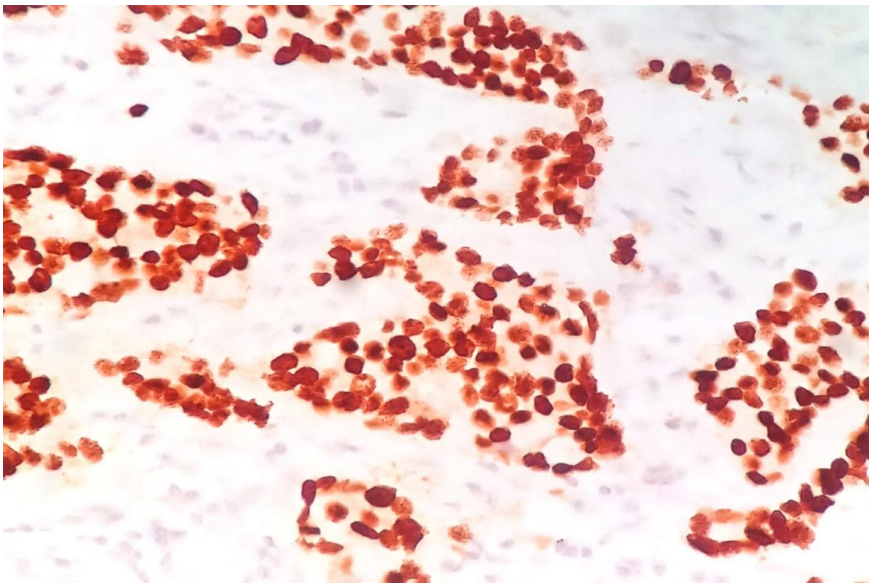


Figure 48:ER Positive(5+3)400X

PR

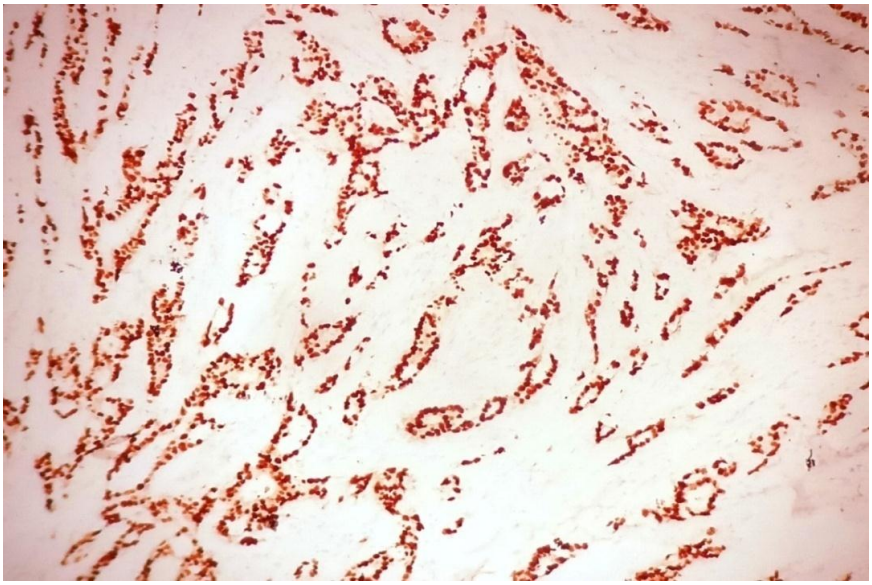


Figure 49:PR Positive(5+3)100X

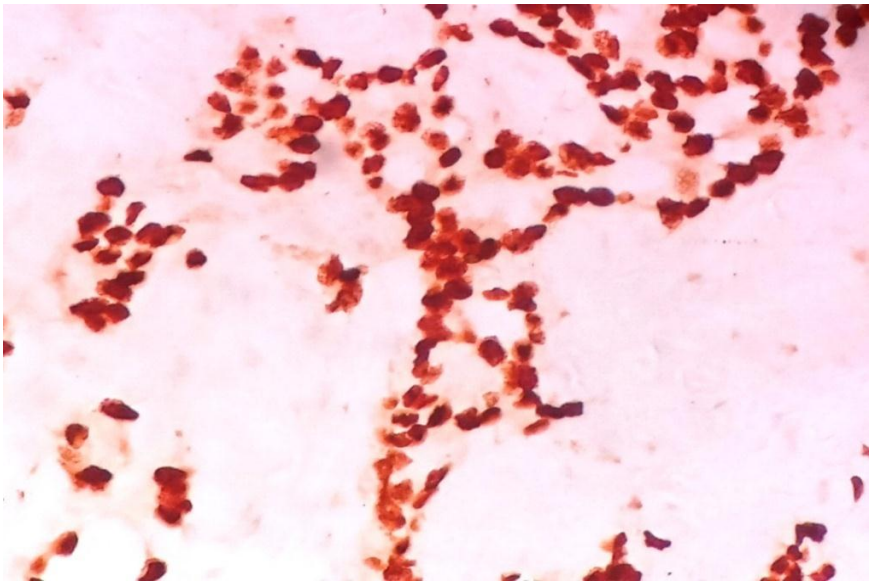


Figure 50:PR Positive(5+3)400X

DISCUSSION

DISCUSSION

Breast carcinoma is the most common cancer in the urban women and the second common cancer in rural women. It is a heterogeneous disease having varying clinical and pathological presentation.

We can reduce the mortality of breast carcinoma by early detection, appropriate management and targeted therapies. Many theories underlie the pathogenesis of breast carcinoma and there are many prognostic factors. The important prognostic factors of breast cancer include tumor size, histological grade and axillary lymph node status, lymphatic and vascular invasion, hormone receptor status and surface epithelial growth factors .

In this present study, Fine needle aspiration cytology was done in 50 cases of primary breast carcinoma and immunocytochemistry was done using cell blocks and the results were compared with IHC on corresponding tissue section for the same patients.

Madras Medical College being a tertiary care center, among the surgical specimens received breast specimens comprise 6.33 % of the total specimens. Malignant breast tumors constituted 62 % of all the breast specimens received.

The youngest age of presentation with invasive ductal carcinoma was 30 years and oldest age group reported was 75 years with 49 as median age of presentation. This is comparable with study done by Micello et al,Carreno et

al Hu et al, Honma et al which also showed that in India there is a rapid change in trend towards younger age group in the recent years. The highest incidence of breast carcinoma was reported in 41 to 50 years age group. This is in concurrence with the study done by Rajesh Singh Laishram et al.

Table-: 23:Comparison of Median age

	Median age of presentation
Micello et al.2010	58.7
Carreno et al.2007	61
Hu et al.2011	61
Honma et al.2012	56
Current study	49

Among the histological types, Invasive ductal carcinoma NOS type is the most common with 93.3%.This correlated with the study done by Albrektsen et al, Shirley SE et al and AM Dauda et al. [87] The incidence of IDC NOS type is higher in Indian women (89.62%) compared to that of western women accounting for the poor prognosis

Table 24: Comparison of distribution of histological subtypes of breast cancers

Histological subtypes	AM Dauda et al	Shirley SE et al	Albrektsen et al	Current study
Invasive ductal carcinoma NOS	78.8%	69.3%	81.4%	100 %

Table-: 25 Comparison of size of tumors (%)

Size	Christine L. Carter et al [92]	E F S Al-Joudi et al [93]	Lakmini et al [94]	Current study
<2cm	33.6	3.14	14.5	22
2-5 cm	55.4	19.37	74	16
> 5 cm	11	77.49	11.5	62

The tumors of > 5 cm in size were more common than smaller size lesions. This observation coincides with the study done by E F S Al-Joudi et al and did not coincide with Christine L. carter et al and Lakmini et al, where the most common tumour size are between 2-5 cm .

Table : Comparison of grade of tumor (%)

Grade	Qiu J et al [98]	Carey et al [99]	G G Vanden Eyndenetal [100]	Current study
Grade I	33.3	25	32.63	0
Grade II	54	26	36.84	90.0
Grade III	12.7	49	30.53	10.0

Grading of tumor was done by Scarff-Bloom-Richardson grading system and the grade II tumors were more common than other grades of breast cancers. This observation coincides with the study done by Qiu J et al and G G Vanden Eynden et al and does not coincide with carey et al.

In the present study we have taken only primary breast carcinoma without adjuvant chemotherapy treatment which was similar to study done by Vameşu et al, S Rossi et al and L Pusztai et al.

The upper outer quadrant is more involved (30 cases) compared to other quadrants of breast which is similar to the study done by Seth Rummel et al ,Matthew T Hueman et al, Craig D. Shriver and Nick costantino et al.

38% of the cases showed lymph node metastasis and 12 % cases with >3 nodes positive. These data did not coincide with the study done by Jun Qiu et al and S E Shirley et al who have reported nodal metastasis in 60.32% and

75.7% of their cases. This may be due to incomplete nodal clearance during the surgery and incomplete nodal dissection in the specimen.

In our study we used cell blocks for immunocytochemistry which was similar to the study done by keykhosro mardanpour et al , Mahtab Rahbar et al, C Garbar et al,,Peterson M et al and Weidner et al .In the study done by K Radhika et al, U Handa et al and K Geethamala et al they used FNAC smears for ICC.

In the present study we used cold acetone for cell block preparation which was similar to the study done by U Handa et al, SL Williams et al,A Shabaik et al and KZ Hanley et al .In the study done by S Khan et al, GM Varsegi et aland Z Yang formalin was used and the results when compared with the present study showed less sensitivity and specificity.

Tumor location and the side of the breast involved had no correlation with ER and PR expression which is similar to the study done by Adedayo A.onitilo et al, N.Mukesh et al, T.Green lee et al and Jessica .M. engel et al.

In the present study microwave oven was used for antigen retrieval which was similar to the study done by Cuevas et al, PE Swanson et al and KR Vinod et al .In the study done by W Ding et al, K Geethamala et al and AJ Norton et al they used pressure cooker for antigen retrieval which yielded good results .

In the present study Tris buffer was used for antigen retrieval which was similar to the study done by U Handa et al, Adalberto Merighi et al Giorgio and Carmignoto et al. In the study done by SR Shi et al and JM Morganet al they used sodium citrate and EDTA for antigen retrieval and the results when compared with the present study showed high sensitivity and specificity.

In the present study ER and PR expression in core needle biopsy showed 16 and 5 positive cases compared to 13 and 3 positive cases in MRM specimens which was similar to the study done by Gemma B.Uyet al, Adriano V.Laudico et al and Jose M.Carnate et al

In the present study only ER and PR markers were used which was similar to the study done by K Radhika et al, U Handa et al and K Geethamala et al. In the study done by DS Dede et al, Cornfield et al and AS Glas et al they used multiple markers which includes ER,PR,p53, HER-2/neu, Ki-67, p21, and bcl-2.

In our study ER sensitivity for ICC correlates well with the study done by Uma Handa et al, Amit Kumar et al , Reetu Kundu et al, Usha Dalal,et al and Harsh Mohanet al and the specificity did not correlate with the study done by TJA Dekker et al and L Seymouret al.

The positive predictive value for ER in our study is 100% which correlates well with the study done by by keykhosro mardanpour et al, Mahtab

Rahbar et al ,C Garbar et al and did not correlate with the study done byK Radhika et al and AK Prayaga et al in which PPV was 67%

In our study the negative predictive study for ER is 86.11% which correlates with the study done by Shabaik A et al, Lin G, Peterson M et al, Hasteh F et al and Tipps A et al,

In our study PR sensitivity for ICC is 63.64% which correlates well with the study done by Uma Handa et al, Amit Kumar et al , Reetu Kundu et al, Usha Dalal,et al and Harsh Mohanet al and the specificity 100% which did not correlate with the study done by TJA Dekker et al and L Seymouret a in which the specificity was 95%.

The positive predictive value for PR in the present study is 100% which correlates well with the study done by by keykhosro mardanpour et al , Mahtab Rahbar et al ,C Garbar et al and did not correlate with the study done by K Radhika et al and AK Prayaga et al in which was PPV 33%..

In the present study the negative predictive value for PR is 90.70% which correlates with the study done by Shabaik A et al, Lin G, Peterson M et al, Hasteh F et al and Tipps A et al,

SUMMARY

SUMMARY

A total of 379 breast biopsy specimens were received in surgical pathology department institute of pathology, Madras medical college during 1 year period(March 2016-.April 2017). Out of which 238 were malignant. Of these 58 cases were diagnosed as malignancy in mastectomy specimens and rest of them were excision and trucut biopsy specimens from the Department of General Surgery and surgical Oncology, Madras Medical College and Rajiv Gandhi Government General Hospital.

- Detailed history regarding Patient's age, sex, side of the breast involved, Grade and Lymph node involvement were taken in 50 cases. ER and PR expression estimation was done only in 50 cases of Invasive ductal carcinoma-NST.
- Immunocytochemistry was done using FNAC cell blocks and Immunohistochemistry was done using trucut or tissue bits from modified radical mastectomy specimens for the same patients. Both the slides were evaluated and scoring was done using Allred scoring system and results were compared.
- Breast carcinoma (infiltrating ductal carcinoma NST) had a peak incidence in the age group of 51-60 years. The median age of the patient in this study was 49. The youngest age of presentation of breast cancer was 30 years in this study.
- Left breast was involved in 38% of cases and Right breast in 62% cases.
- 60% of tumours were located in the upper outer quadrant .

- 62% of the tumors were > 5 cm and 38% of tumors showed > lymph nodes involved by tumour.
- 45 cases (90%) were grade II, followed by 5 cases of grade III (10).
- In tissue IHC out of 50 cases, ER was found positive in 19 cases (38%) and PR positive in 11 cases (22%).
- In cell block IHC out of 50 cases, ER positive in 14 cases (28%) and PR positive in 7 cases (14%)
- In the present study ER sensitivity is 73.68%, specificity- 100%, Positive predictive value- 100%, Negative predictive value -86.11%, Diagnostic accuracy -90% ,false positivity rate- 0%, false negativity rate -26.32%,Kappa value -0.776% and P value <0.776 which is statistically significant.
- In the present study PR sensitivity is 63.64%, specificity- 100%, Positive predictive value -100%, Negative predictive value -90.70%, Diagnostic accuracy- 92%, false positivity rate- 0%,false negativity rate -36.36%,Kappa value -0.732% and P value <0.001 which is statistically significant.

CONCLUSION

CONCLUSION

- In the present study, among 379 breast specimens received 238 were malignant which constitutes 62% and the incidence of invasive ductal carcinoma –NST is high among the various histological types of breast Cancers
- Fine needle aspiration cytology is a suitable method for early detection of breast carcinoma among the patients presenting with breast mass
- Identifying suitable fixative and antigen retrieval processing plays a important role for better results.
- Immunocytochemistry on fine needle aspiration samples has a rapid turn around time with minimal processing time and antigen loss due to fixation.
- Hormone receptors can be studied using cell blocks/cytology slides and the patients can be treated with preoperative hormone therapy on the basis of FNAC /ICC and surgical procedure can be avoided for the diagnosis and hormone receptors study in a sizable number of cases.
- It helps in sequential receptor status determinations at different times during the course of treatment.
- It can be used in the evaluation of metastatic tumors of unknown origin without the need of invasive open surgical biopsy.

ICC is more advantageous in planning treatment for patients for whom surgery is contraindicated because of elderly age, advanced inoperable tumor, metastases, local disease recurrence, or malignant effusions

ANNEXURES

ANNEXURE I

NOTTINGHAM MODIFICATION OF SCARF BLOOM

RICHARDSON GRADING SYSTEM

TUBULE FORMATION	**SCORE**
Tubular formations in >75% of the tumor	1
Tubular formations in 10–75% of the tumor	2
Tubular formations in <10% of the tumor	3
NUCLEAR PLEOMORPHISM	**SCORE**
Nuclei with minimal variation in size and shape	1
Nuclei with Moderate variation in size and shape	2
Nuclei with marked variation in size and shape	3
MITOTIC RATE	**SCORE**
<10 mitosis / 10 high power field	1
10 – 20 mitosis / 10 high power field	2
>20 mitosis / 10 high power field	3

GRADE	SCORE
Grade I	3,4,5
Grade II	6,7
Grade III	8,9

ANNEXURE II

TNM classification of carcinomas of the breast:

T	-	Primary tumor
TX	-	Primary tumor cannot be assessed
T0	-	No evidence of primary tumor
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 tumor.

(Note- Carcinomas in the breast parenchyma associated with Paget disease are categorized based on the size and characteristics of the parenchymal disease)

T1	-	Tumor ≤ 20 mm in greatest dimension
T1mi	-	Tumor ≤ 1 mm in greatest dimension
T1a	-	Tumor > 1 mm but ≤ 5 mm in greatest dimension
T1b	-	Tumor > 5 mm but ≤ 10 mm in greatest dimension
T1c	-	Tumor > 10 mm but ≤ 20 mm in greatest dimension
T2	-	Tumor > 20 mm but ≤ 50 mm in greatest dimension
T3	-	Tumor > 50 mm in greatest dimension
T4	-	Tumor of any size with direct extension to the chest wall and/or to the skin (ulceration or skin nodules)
T4a	-	Extension to chest wall, not including only pectoralis muscle adherence/invasion
T4b	-	Ulceration and/or ipsilateral satellite nodules and/or edema (including peau d'orange) of the skin
T4c	-	Both T4a and T4b

T4d - Inflammatory carcinoma

ANNEXURE III

PROFORMA

Case number : Name :

HPE number : Age :

IP number : Sex :

Clinical diagnosis: Menstrual status :

Risk factors if any :

Side of breast : Right/Left

Specimen : Simple Mastectomy /Modified radical Mastectomy / Radical Mastectomy/ Toilet Mastectomy /others

GROSS

Specimen size :

Nipple areola : Skin :

Tumor size : Tumor margin :

Appearance :

Resected margins : Superior : Inferior :

Medial : Lateral :

Posterior :

Associated findings :

Total number of nodes dissected :

Largest node size :

MICROSCOPY

Histological subtype :

Histological score : Nuclear score : Mitotic score :

Modified Scarf Bloom Richardson GRADE :I / II / III

Skin : Free / involved

Nipple & Areola : Free/ Involved

Margins : Superior : Free / Involved

Inferior : Free / Involved

Medial : Free /Involved

Lateral : Free /Involved

Posterior : Free/Involved

Lymphatic invasion : Present /Absent

Vascular Invasion : Present /Absent

Lymphocytic infiltration : Present / Absent

Necrosis : Present /Absent

Associated breast lesions :

Total number of nodes dissected :

Number of nodes involved :

ANNEXURE IV

ALLRED SCORE FOR ESTROGEN AND PROGESTERONE RECEPTORS

PROPOTIONAL SCORE(PS):

0- No staining
1- Staining of < 1 % of tumor cells
2- Staining between 1% and 10 % of tumor cells
3- Staining between 1/10 and 1/3 of tumor cells
4- Staining between 1/3 and 2/3 of tumor cells
5- Staining of > 2/3 of tumor cells

INTENSITY SCORE (IS):

0- No staining
1- Average weak intensity
2- Average moderate intensity
3- Average strong intensity

Allred score (range, 0 to 8) = PS+ IS

Possible Allred scores are

1) 0 = negative,

2) 2-8 = diffusely & strongly positive tumor.

ANNEXURE V

MOLECULAR CLASSIFICATION OF BREAST CANCER

Luminal A

- This phenotype is observed in 40% - 50% of the IDC NOS type of breast carcinoma.
- Includes ER positive and HER2-neu negative tumor.
- Most of these tumors are moderately to well differentiated with increased occurence among post menopausal women.
- The tumors in this subtype responds well to hormonal treatment.

Luminal-B

- This phenotype is observed in 15% to 20% of IDC-NOS type of breast cancer.
- They are triple positive receptor tumors with expression of ER, PR & HER2neu.
- They are of tumors of higher grade with increased proliferating potential.
- Increased frequency of metastasis to lymph nodes .
- These tumors responds well to chemotherapy.

Normal Breast Like

- This phenotype observed in about 6% - 10% of IDC NOS type of breast carcinoma.
- This group contains well differentiated ER positive & HER2neu negative tumors which show similar gene expression pattern like that of normal breast tissue.

Basal Like

- This phenotype observed in 13% to 25% of IDC NOS type tumors.
- This type of breast carcinomas are characterized by the absence of PR, ER & HER2neu receptor expression ,but expressing basal myoepithelial markers like P-Cadherin, P63, and of progenitor cells / putative stem cells (CK 5/6)
- This group is termed as "TRIPLE NEGATIVE" carcinomas.[63, 64]
- Medullary & Metaplastic carcinomas belongs in this category.
- Breast carcinomas harboring BRCA1 mutations come in this category.
- These are high grade tumors with increased proliferating potential and aggressive clinical behaviour.
- They are frequently associated with brain and Visceral metastasis.
- Complete response following chemotherapy is seen only 15-20% of cases.

HER2neu Positive

- This phenotype is observed in about 7% - 12% of IDC NOS type of breast cancers.

- This group includes carcinomas exhibiting HER2neu over expression and ER / PR negativity.

- The overexpression of HER2neu in more than ninety percent of these cancers is due to the amplification of the DNA segment on chromosome 17q21 which harbours the HER2neu gene .

- They belong to poorly differentiated tumors generally with increased proliferative potential & associated with increased frequency of brain metastasis.

MASTER CHART

S.No	Biopsy No	Name	Age	Site of tumour	Size of tumour	quadrant	Type of Biopsy	Histological diagnosis	Grade	Lymph node status	Tissue IHC HORMONE STATUS				FNAC IHC HORMONE STATUS			
											ER	Score	PR	Score	ER	Score	PR	Score
1	2367/17	Saroja	70	Lt breast	4x2	UOQ	Lt MRM	IBC-NST	II	o/8 nodes	Positive	4+3/8	Positive	4+3/8	Positive	4+3/8	Positive	4+3/8
2	2090/17	Valarmathi	31	Lt breast	3x1	Central	Lt MRM	IBC-NST	II	5/11 nodes	Positive	4+2/8	Positive	2+2/8	Positive	4+2/8	Positive	2+2/8
3	2659/17	Vijaya	40	Rt breast	2x2	UOQ	Trucut Biopsy	IBC-NST	II		Negative		Negative		Negative		Negative	
4	2765/17	Pappathy	61	Lt breast	2x2	UIQ	Trucut Biopsy	IBC-NST	II		Negative		Negative		Negative		Negative	
5	2759/17	Manimegalai	32	Rt breast	2x1	UOQ	Trucut Biopsy	IBC-NST	II		Positive	4+3/8	Positive	3+2/8	Positive	4+3/8	Positive	3+2/8
6	2802/17	Angamma	61	Rt breast	4x3	LIQ	Trucut Biopsy	IBC-NST	II		Positive	5+3/8	Positive	2+2/8	Positive	5+3/8	Positive	2+2/8
7	2805/17	Jayammal	70	Rt breast	6x4	UOQ	Trucut Biopsy	IBC-NST	III		Positive	5+3/8	Positive	2+2/8	Positive	5+3/8	Positive	2+2/8
8	2549/17	Pandiammal	36	Lt breast	2x2	UIQ	Trucut Biopsy	IBC-NST	II		Positive	3+2/8	Negative		Positive	3+2/8	Negative	
9	2253/17	Manorama	55	Lt breast	4x3	UOQ	Trucut Biopsy	IBC-NST	II		Positive	5+3/8	Positive	4+3/8	Negative		Negative	
10	4183/17	shafira	55	Rt breast	2x2	UIQ	Trucut Biopsy	IBC-NST	III		Negative		Negative		Negative		Negative	
11	3489/17	Rosamma	75	Rt breast	2x1	UOQ	Rt MRM	IBC-NST	II	0/6 nodes	Negative		Negative		Negative		Negative	
13	3882/17	Malliga begam	65	Rt breast	3x1	UOQ	Trucut Biopsy	IBC-NST	II		Positive	5+3/8	Positive	5+3/8	Positive	5+3/8	Positive	5+3/8
14	2873/17	Saroja	55	Rt breast	4x2	UOQ	Rt MRM	IBC-NST	II	2/15 nodes	Negative		Negative		Negative		Negative	
15	3414/17	Desamma	52	Rt breast	5x5	UOQ	Trucut Biopsy	IBC-NST	II		Positive	2+2/8	Negative		Positive	2+2/8	Negative	
16	2550/17	Gomathi	36	Lt breast	2.5x2	UOQ	Trucut Biopsy	IBC-NST	II		Positive	4+3/8	Negative		Positive	4+3/8	Negative	
17	1208/17	Muniyammal	60	Rt breast	6x4	UIQ	Trucut Biopsy	IBC-NST	II		Negative		Negative		Negative		Negative	
18	5562/17	Vasanthi	45	Rt breast	6x3	UOQ	Rt MRM	IBC-NST	II	9/12 nodes	Positive	2+2/8	Negative		Positive	2+2/8	Negative	
19	5139/17	jasmine	82	Lt breast	4x3	UOQ	Trucut Biopsy	IBC-NST	II		Positive	4+2/8	Negative		Positive	4+2/8	Negative	
20	5411/17	Lakshmi	55	Lt breast	3x2	UOQ	Lt MRM	IBC-NST	II	0/13 nodes	Negative		Negative		Negative		Negative	
21	6431/17	Meebonisha	57	Rt breast	6x4	UIQ	Wide local excision	IBC-NST	III		Negative		Negative		Negative		Negative	
22	6807/17	manomani	45	Rt breast	1x1	Central	Wide local excision	IBC-NST	II		Negative		Negative		Negative		Negative	
23	7097/17	Valliammal	59	Rt breast	3x3	Central	Trucut Biopsy	IBC-NST	II		Negative		Negative		Negative		Negative	
24	4441/17	Dhanammal	50	Rt breast	3x2.5	UIQ	Rt MRM	IBC-NST	II	2/12 nodes	Negative		Negative		Negative		Negative	
25	3052/17	Malliyammal	55	Rt breast	5x3	UOQ	Rt MRM	IBC-NST	II	0/10 nodes	Negative		Negative		Negative		Negative	
26	3518/17	Angoaratham	64	Rt breast	3x3	UOQ	Rt MRM	IBC-NST	II	0/16 nodes	Positive	3+2/8	Negative		Positive	3+2/8	Negative	
27	3572/17	Nambikkai	60	Rt breast	3x2	UOQ	Rt MRM	IBC-NST	II	0/4 nodes	Negative		Negative		Negative		Negative	
28	3146/17	Maharani	36	Rt breast	1.5x1	LOQ	RT MRM	IBC-NST	II	11/17 nodes	Negative		Negative		Negative		Negative	
29	3428/17	Aijthrusha	60	Rt breast	4x4	UOQ	Rt MRM	IBC-NST	II	4/11 nodes	Negative		Negative		Negative		Negative	

30	3572/17	Nagalakshmi	60	Rt breast	4x3.5	UIQ	Rt MRM	IBC-NST	II	0/4 nodes	Negative		Negative		Negative		Negative	
31	3518/17	Angoorbala	64	Rt breast	3x2	UIQ	Rt MRM	IBC-NST	II	0/16 nodes	Positive	3+2/8	Negative		Positive	3+2/8	Negative	
32	2199/17	chandra	35	Lt breast	2x1	UOQ	Lt MRM	IBC-NST	II	13/13nodes	Negative		Negative		Negative		Negative	
33	3042/17	Rani	80	Lt breast	1x1	UOQ	Lt MRM	IBC-NST	III	3/11 nodes	Negative		Negative		Negative		Negaivet	
34	2873/17	Saroja	55	Rt breast	2.5x2	UIQ	Rt MRM	IBC-NST	II	2/15 nodes	Negative		Negative		Negative		Negative	
35	3819/17	Neelabai	65	Lt breast	2x2	UIQ	Trucut Biopsy	IBC-NST	II		Negative		Negative		Negative		Negative	
36	7549/17	Sivagami	36	Rt breast	3x2	Central	Trucut Biopsy	IBC-NST	II		Negative		Negative		Negative		Negative	
37	6807/17	Manimegalai	45	Rt breast	1x0.5	Central	Wide local excision	IBC-NST	II		Negative		Negative		Negative		Negative	
38	6388/17	Anjalakshmi	60	Rt breast	2x1	UOQ	Trucut Biopsy	IBC-NST	II		Negative		Negative		Negative		Negative	
39	5616/17	Shaburnisha	75	Lt breast	4x4	UOQ	Trucut Biopsy	IBC-NST	II		Positive	4+3/8	Positive	4+3/8	Negative		Negative	
40	7369/17	vasantha	56	Rt breast	5x5	UOQ	Trucut Biopsy	IBC-NST	III		Negative		Negative		Negative		Negative	
41	6533/17	Sathya	50	Rt breast	1.5x1	Central	Trucut Biopsy	IBC-NST	II		Negative		Negative		Negative		Negative	
42	6769/17	Tharamani	44	Lt breast	2x2	UOQ	Trucut Biopsy	IBC-NST	II		Negative		Negative		Negative		Negative	
43	4646/17	Banumathi	40	Lt breast	3x2	LOQ	Trucut Biopsy	IBC-NST	II		Positive	4+3/8	Positive	4+2/8	Negative		Negative	
44	6772/17	Gowri	56	Rt breast	4x4	UOQ	Lt MRM	IBC-NST	II	2/5 nodes	Positive	3+2/8	Negative		Negative		Negative	
45	4121/17	Banumathi	63	Rt breast	3x3	LOQ	Trucut Biopsy	IBC-NST	II		Negative		Negative		Negative		Negative	
46	2199/17	Indra	35	Lt breast	2.5x2.5	UIQ	Lt MRM	IBC-NST	II	13/13 nodes	Negative		Negative		Negative		Negative	
47	4153/17	Lakshmi	38	Lt breast	5x5	LIQ	Trucut Biopsy	IBC-NST	II		Negative		Negative		Negative		Negative	
48	4183/17	Shajira	55	Rt breast	4x4	UOQ	Trucut Biopsy	IBC-NST	II		Negative		Negative		Negative		Negative	
49	3951/17	karija	50	Lt breast	3.5x2.5	LIQ	Trucut Biopsy	IBC-NST	II		Positive	4+3/8	Positive	2+2/8	Positive	4+3/8	Positive	2+2/8
50	6644/17	Kala	52	Lt breast	4x4	LIQ	Trucut Biopsy	IBC-NST	II		negative		Negative		Negative		Negative	

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INFORMATION SHEET

- We are conducting a study on breast cancer among patients attending Government General Hospital, Chennai and for that your specimen may be valuable to us.
- The purpose of this study is to aid in prognostic value of Tumor Infiltrating Lymphocytes with the help of Immunohistochemical markers.
- We are selecting certain cases and if your specimen is found eligible, we may be using your specimen to perform extra tests and special studies which in any way do not affect your final report or management.
- The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.
- Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.
- The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of investigator Signature of participant

Date:

INFORMED CONSENT FORM

Title of the study : **A study on Estrogen and progesterone receptor status in FNAC breast carcinoma: comparison of immunocytochemistry and immunohistochemistry**

name of the participant :

Name of the Principal (Co-Investigator) :

Name of the Institution : Madras Medical College

Name and address of the sponsor / agency (ies) (if any) :

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in **"A study on Estrogen and progesterone receptor status in FNAC breast carcinoma: comparison of immunocytochemistry and immune histochemistry**

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study in which the resected Mastectomy specimens will be subjected to immunohistochemistry and histopathological examination.
4. I have been explained about my rights and responsibilities by the investigator. I have the right to withdraw from the study at any time.
5. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.
6. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.
7. I have understood that my identity will be kept confidential if my data are publicly presented
8. I have had my questions answered to my satisfaction.
9. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For adult participants:

Name and signature / thumb impression of the participant (or legal representative if participant
incompetent)

Name _____ Signature_____ Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____ Signature_____ Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____ Signature_____ Date_____