“A STUDY ON IMMUNOHISTOCHEMICAL EXPRESSION OF C-KIT IN INVASIVE BREAST CARCINOMA AND ITS CLINICOPATHOLOGICAL CORRELATION”

Dissertation submitted in partial fulfillment of the
requirements for the degree of

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BRANCH-III

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MADRAS MEDICAL COLLEGE
CHENNAI-600003

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY
CHENNAI

APRIL 2017

CERTIFICATE

This is to certify that this Dissertation entitled "**A STUDY ON IMMUNOHISTOCHEMICAL EXPRESSION OF C-KIT IN INVASIVE BREAST CARCINOMA AND ITS CLINICOPATHOLOGICAL CORRELATION"** is the bonafide original work of **Dr. N. LAVANYA**, in partial fulfillment of the requirement for M.D., (Branch III) in Pathology Examination of the Tamil Nadu Dr. M.G.R. Medical University to be held in April 2017.

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DECLARATION

I, **Dr. N. LAVANYA**, solemnly declare that the dissertation titled "**A STUDY ON IMMUNOHISTOCHEMICAL EXPRESSION OF C-KIT IN INVASIVE BREAST CARCINOMA AND ITS CLINICOPATHOLOGICAL CORRELATION**" is the bonafide work done by me at Institute of Pathology, Madras Medical College under the expert guidance and supervision of **Prof**. **Dr. GEETHA DEVADAS, M.D.**,**D.C.P.,** Professor of Pathology, Institute of Pathology, Madras Medical College. The dissertation is submitted to the Tamil Nadu Dr. M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch III) in Pathology.

Place: Chennai

Date: **Dr. N. LAVANYA**

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CERTIFICATE OF APPROVAL

To
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Dear Dr.N.Lavanya,

The Institutional Ethics Committee has considered your request and approved your study titled **"A study on Immunohistochemical expression of c-Kit (CD117) in Invasive breast cancer and its clinicopathological correlation" No.28082015.**

The following members of Ethics Committee were present in the meeting held on 04.08.2015 conducted at Madras Medical College, Chennai-3.

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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.

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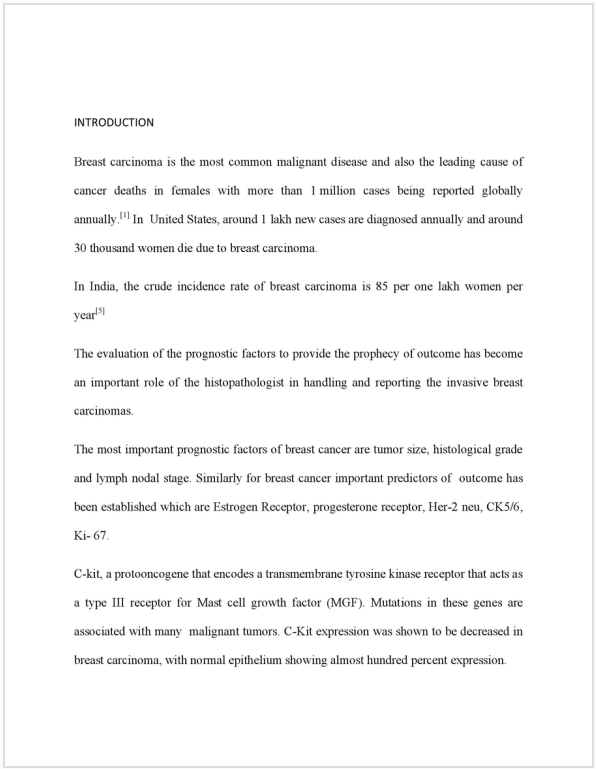

INTRODUCTION

Breast carcinoma is the most common malignant disease and also the leading cause of cancer deaths in females with more than 1 million cases being reported globally annually.[1] In United States, around 1 lakh new cases are diagnosed annually and around 30 thousand women die due to breast carcinoma.

In India, the crude incidence rate of breast carcinoma is 85 per one lakh women per year[5]

The evaluation of the prognostic factors to provide the prophecy of outcome has become an important role of the histopathologist in handling and reporting the invasive breast carcinomas.

The most important prognostic factors of breast cancer are tumor size, histological grade and lymph nodal stage. Similarly for breast cancer important predictors of outcome has been established which are Estrogen Receptor, progesterone receptor, Her-2 neu, CK5/6, Ki- 67.

C-kit, a protooncogene that encodes a transmembrane tyrosine kinase receptor that acts as a type III receptor for Mast cell growth factor (MGF). Mutations in these genes are associated with many malignant tumors. C-Kit expression was shown to be decreased in breast carcinoma, with normal epithelium showing almost hundred percent expression.

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18

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ABBREVIATIONS

MGF	:	Mast Cell Growth Factor
ER	:	Estorgen Receptor
PR	:	Progesterone Receptor
HER-2	:	Human Epidermal Growth Factor Receptor-2
ICMR	:	Indian Council of Medical Research
HRT	:	Hormone Replacement Therapy
BRCA1	:	Breast Cancer Antigen
DCIS	:	Ductal Carcinoma Insitu
LCIS	:	Lobular Carcinoma Insitu
IDC NOS	:	Infiltrating Ductal Carcinoma- Not Otherwise Specified
CNS	:	Central Nervous System
HMWCK	:	High Molecular Weight Cytokeratin
LN	:	Lymph Node
CA	:	Carcinoma
EGFR	:	Epidermal Growth Factor Receptor
TNBC	:	Triple Negative Breast Cancer
GIST	:	Gastrointestinal Stromal Tumour
LVI	:	Lympho Vascular Invasion
UDH	:	Usual Ducta Hyperplasia
ADH	:	Atypical Ductal Hyperplasia
FA	:	Fibroadenoma

CONTENTS

INTRODUCTION

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In the recent years the c-kit role in the pathogenesis of preinvasive and invasive and in specific subtypes of breast carcinomas especially in triple receptor negative breast carcinomas are being investigated. C-kit appears to be an indicator of high grade breast carcinoma which has poor prognosis.

This study of 50 cases, aims at evaluating the expression of c-kit in breast carcinomas, to correlate with clinicopathological factors namely age, tumor size, location of the tumor, grade, lymph nodal status, lymphovascular invasion, margin, necrosis, lymphocytic infiltration, skin involvement, associated DCIS component, ER, PR, Her-2 neu status.

AIMS AND OBJECTIVES

1) To identify the relative frequency and distribution of breast carcinoma in the study group.

2) To study the histomorphological features of breast carcinoma including histological subtypes, grade, lymph node status, lymphovascular invasion, lymphocytic response, necrosis and skin infiltration.

3) To assess the expression of ER, PR, HER2-neu receptor in invasive breast carcinomas.

4) To assess the expression of c-Kit in these cases.

5) To compare the c-Kit expression and clinico-pathological parameters in breast carcinoma.

6) To assess the correlation between the expression of c-Kit and Estrogen, Progesterone, Her-2neu Receptors.

REVIEW OF LITERATURE

Breast cancer is the most common malignant neoplasm and also the most common cause for cancer deaths in females with more than 1 million cases being reported globally annually.[1] In United States, around 1 Lakh new cases are reported every year and approximately 30 thousand women die because of breast carcinoma. The new cases reported are high in Northern Europe and North America (91.4 new cases per 100 000 women per year), intermediate in Latin American and southern European countries, low in most of the Asian and African Nations (but rising rapidly in the recent years with increased affluence of some of these countries). In USA, there is a sharp increase in the diagnosis of breast cancer, due to the widespread use of mammography.[2]

According to Globacon 2012 (3), estimated breast cancer incidence was 1.67 million in 2012, which constitutes 25% of all malignancies. It is the most common malignancy among females in both well and less developed countries. Incidence rates vary around 4 times across the globe, ranging from 27/100000 in middle Africa and eastern Asia to 92/ 100000 in Northern America. (Figure 1&2)

Breast carcinoma holds 5^{th} rank for the cause of death from cancer overall (around 522000) and this could be the most common

cause of death in cancer in less developed nations next to lung cancer. (Figure 3)

Figure 1. worldwide incidence and mortality rates of breast cancer according to GLOBACON 2012.

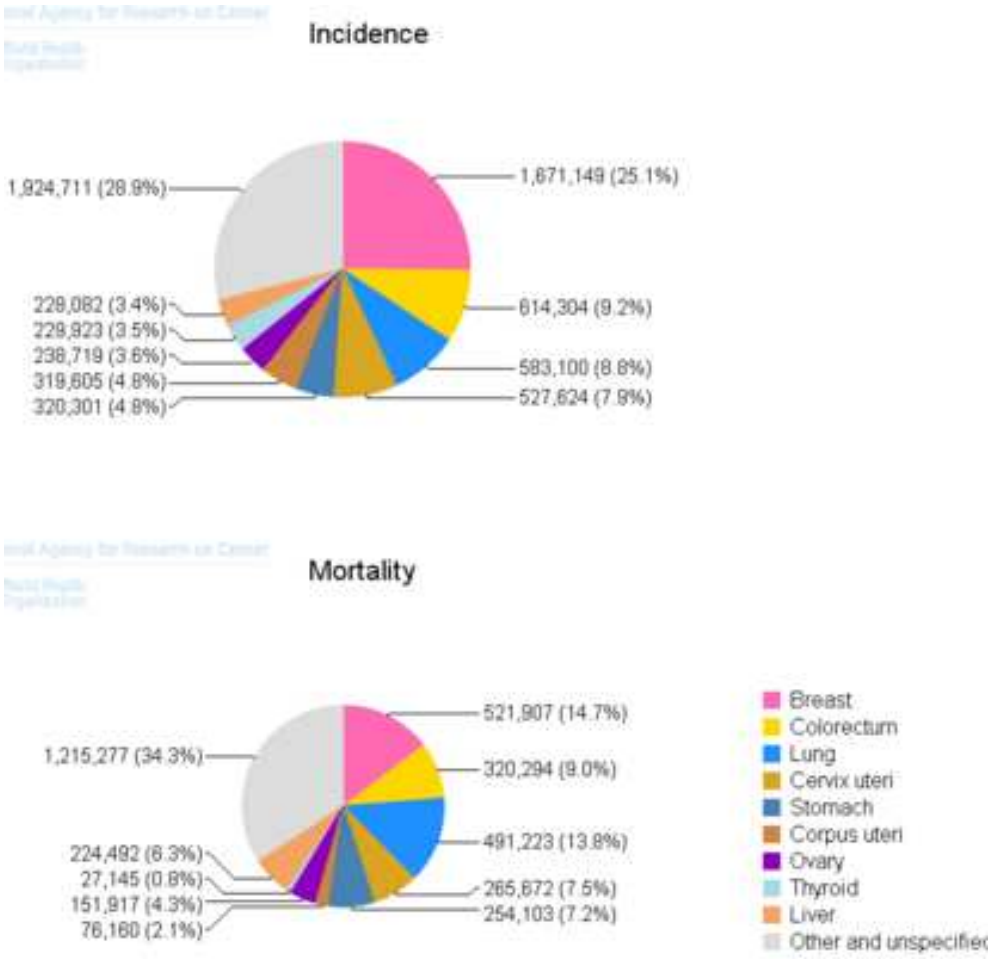


FIGURE 2. ESTIMATED BREAST CANCER INCIDENCE WORLDWIDE IN 2012

FIGURE 3. ESTIMATED BREAST CANCER MORTALITY WORLDWIDE IN 2012

IN INDIA

According to National Cancer Registry Programme ICMR (2009-2011), the most common malignancy in many cities of India is breast carcinoma which accounts for twenty five percent to thirty percent of

all cancers in women and is the second most common cancer in suburban(4).

In India, the crude incidence rate of breast cancer is 85 per one lakh women per year. [5] The death per incident ratio in India is highest with 50%, compared to 30% in China and 18% in United States.

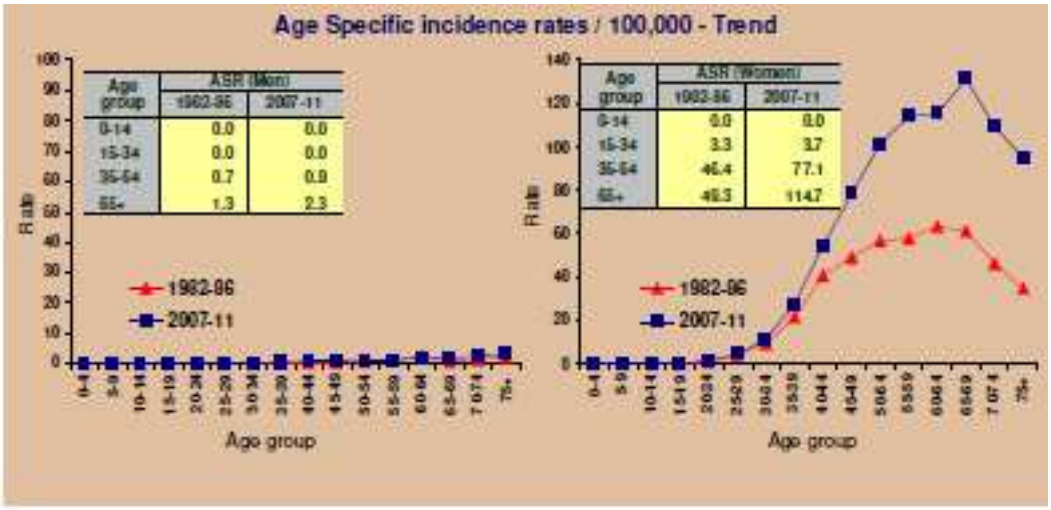
Breast cancer is more common in 50-60 years of age group constituting 69% of breast cancer. India is rapidly moving towards industrialization which results in lifestyle changes. This is probably the reason for increase in breast cancer incidence in India.

The annual age-adjusted rate is 30 to 33/ 1 lakh in urban and 8.6 / 1, 00,000 in rural women. [6]

According to Madras Metropolitan Tumor Registry (MMTR)

In 1982-86, Breast cancer was ranked second and became first since 2002 among women. The histological verification of breast cancer diagnosis rose from 68% in 1982-86 to 86% in 2007-11 as also diagnosis by any imaging from 1% to 4% while diagnosis by clinical evaluation only decreased from 25% to 8% in the corresponding period.(7) (Figure 4)

FIGURE 4: 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 2015 was 11402. Among that, the total number of breast disease specimens was 844, including 380 Breast cancer specimens.

RISK FACTORS

1) ***Family History*** Females who have 1º relative with breast cancer have a risk of 2-3 times that of the general population, and if the relative was affected at an early age the risk further increases[8]

2) ***Menstrual and reproductive history.*** Increased risk is correlated with early menarche, late age at first birth, nulliparity and late

menopause. [9, 10] Breast carcinoma is rare in women who have undergone oophorectomy before the age of 35 years reduces the risk to one-third. Women who have their first child before the age of 18 years have only 1/3rd the risk of those whose first child is delayed until age 30.[11] A reduction in the risk of breast carcinoma among premenopausal women who have lactated has been documented, but no such effect was detected among postmenopausal women with breast cancer.[12] Breast carcinoma risk is increased in postmenopausal women with a increased androgen in plasma .[13]

3) ***Fibrocystic disease and epithelial hyperplasia.*** These changes in the breast have an increased risk of invasive carcinoma. In some older series, there has been an 2.5-fold overall risk increased [14] whereas in others 2 to 9-fold increased risk was observed only in patients with a previous diagnosis of fibrocystic disease.[15]

4) ***Exogenous estrogens.*** More recently, a large cohort study and a large case-control study have provided strong evidence for a greater risk in women using hormone replacement therapy (HRT) than in those using estrogens alone.[16] Very recently, studies have added that recent long-term use of hormone replacement therapy is associated with an increased risk of breast carcinoma, particularly of the lobular type.[17] In December 2002, the

hormone estrogen was declared a known human carcinogen by the National Toxicology Program.

5) ***Contraceptive agents.*** The various epidemiologic studies have shown no increased risk, or at most a very low increase among young long-term users.[18]

6) ***Ionizing radiation.*** An increased risk of breast carcinoma has been documented with exposure to ionizing radiation, particularly if this exposure occurred at the time of breast development. For example, those who have received irradiation to the mediastinum for Hodgkin lymphoma at early age.[19]

7) ***Breast augmentation.*** Breast carcinomas are sometimes detected in women who have undergone augmentation mammoplasty.[20] However, the re-analysis of a previously published studies has shown that the incidence of breast carcinoma was neither higher nor lower than that among the general population.[21]

8) ***Others.*** An interesting association between breast carcinoma and Meningioma is noted. [22] Even more peculiar is that fact that the breast carcinoma may be found to metastasize within the Meningioma. Ataxia–telangiectasia syndrome and Cowden syndrome have an increased risk of breast cancer.[23]

GENETIC PREDISPOSITION

Approximately 5 to 10% of all breast cancers are familial.[24] There are 2 high-penetrance susceptibility genes, when affected by germline mutations, are associated with an increased life-time risk of occurrence of breast cancer as well as few other cancers like ovarian carcinoma identified. They are *BRCA1*, located on chromosome 17q21, and *BRCA2*, sited on 13q12.3 chromosome (Table 20.1).[25] Mutations of these genes are present in around 2% of Ashkenazi Jews; it has been calculated that among carriers the risk for breast carcinoma is 70 to 80% by the age of seventy years.[26] Study of the breast carcinomas occurring in carriers of *BRCA1* mutations has found a increased percentage of carcinomas with features of medullary carcinoma, i.e., carcinomas are of higher grade, mitotically very active, pushing margins,with a syncytial growth pattern, confluent necrosis, negativity for hormone receptors , HER2neu ('triple negative'), basal-like gene expression profile and with *TP53* mutation.[27] *BRCA2*-associated cancers are a heterogeneous group without a specific morphological feature or phenotype and mostly positive for ER, PR (hormone receptors).[28]

Other known susceptibility genes account for less than 10% of hereditary breast cancers. The tumor suppressor genes like *LKBI/STK11* (Peutz-Jeghers syndrome), *PTEN* (Cowden syndrome), *ATM* (ataxia

telangiectasia), are seem to be mutated in lesser than 1% of all breast carcinomas and are described elsewhere [29, 30]

SPORADIC BREAST CANCER

It is well established that the risk factors for sporadic breast cancers are related to hormone exposure, sex, age at menarche and age at menopause, exogenous estrogens.

LOCATION

About half of the breast cancers are located in the upper outer quandrant, fifteen percent are in the upper medial quadrant, ten percent are in the lower lateral quadrant, seventeen percent are in the central region , five percent are in the lower inner quadrant and three percent breast cancers are diffuse (massive or multifocal).

MULTICENTRICITY

Definition of multicentricity is the presence of tumor in a breast quadrant other than the quandrant containing dominant mass. Multicentricity is more commonly seen in invasive lobular carcinomas than in invasive ductal carcinomas. A recent study states that multicentric cancers are associated with a lower survival rate than unicentric cancers of the same aggregate volume.

The chance that a woman with invasive breast cancer in one side to develop carcinoma in the contralateral breast is around 5 times that

of the general population, and is even greater if a positive family history of breast carcinoma is present. In cases of lobular carcinoma, the number can be as high as 25–50%.

CARCINOGENESIS AND TUMOR PROGRESSION

The Cell of origin is resident breast tissue stem cells. Most common driver mutations involve the protooncogenes PIK3CA, MYC, HER2, and CCND1 and the tumor suppressor genes TP53, in familial breast cancers (BRCA1 & BRCA2). Once the process is initiated in such cells with a driver mutation, there appear 3 major genetic pathways of carcinogenesis.

1) The ER positive, HER-2 negative cancers arise via dominant pathway of breast cancer development constituting 50-65% of cases. This subtype is the most common breast cancer subtype in BRCA2 germline mutation. ER POSITIVE cancers are called as LUMINAL as these cancers closely resemble normal breast luminal epithelail cells in terms of mRNA expression which is dominated by genes that are regulated by estrogen. Depending upon the proliferation rate (Ki 67) and the response to therapy LUMINAL cancers are subdivided into LUMINAL A and LUMINAL B. Putative precursor lesions of this subtype of breast carcinoma is atypical ductal hyperplasia and flat epithelial atypia.

2) The HER-2 positive cancers arise through the pathway which is strongly associated with amplifications of HER-2 gene and chromosome number 17q. They constitute around 20 percent of all breast cancers and may either ER positive or negative. This subtype is the most common breast cancer in p53 germ line mutation (Li-Fraumeni syndrome). Putative precursor lesion of this subtype is termed as atypical apocrine adenosis .

3) ER negative and HER-2 negative cancers arise through a distinct pathway which is independent of ER mediated changes in the gene expression and HER-2 gene amplification. Precursor lesion of this subtype is yet to be described and so this is the least understood pathway. These cancers comprise about 15% of overall breast cancers, mostly observed in BRCA1 mutation. Sporadic type often has a loss of functional mutations in TP53. These cancers have a “basal-like” pattern of mRNA expression that includes many genes which are expressed in normal myoepithelial cells.

Neoplastic epithelial cells do not develop in isolation and are dependent on interactions with the stromal cells in local microenvironment.

The transition of carcinoma in situ to invasive carcinoma is the final step in carcinogenesis. The molecular events that occur in the

normal formation of new ductal branch points , lobules during puberty and pregnancy, abrogation of the basement membrane, escape from growth inhibition, new blood vessel formation,stromal invasion may be seen in the progression of carcinogenesis.[31] The inflammatory and "wound healing like" tissue reactions that occur during the remodeling of the breast explain the transient increase in breast cancers incidence during and soon after pregnancy, because these changes can facilitate the carcinoma insitu to transform into invasive carcinoma.[32]

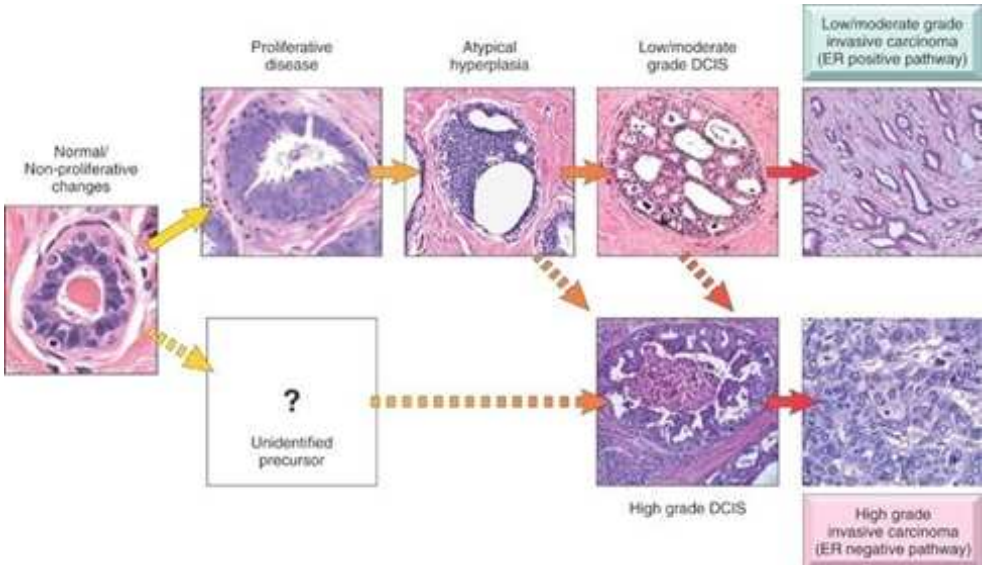


Figure 5. carcinogenesis of breast cancer

CLINICAL EXAMINATION

Screening for breast diseases are done by triple assessment test which includes clinical examination, imaging and tissue sampling.

PALPATION

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

RADIOLOGICAL IMAGING

1. Mammogram:

- The widespread use of mammography brought about a dramatic change in the diagnosis of breast neoplasm.
- Mammographic screening used for detecting small non palpable carcinoma that was asymptomatic.
- The primary signs of mammographically detected carcinomas include density and calcification.

Mammographic Density

- Mammographic density is produced frequently by invasive carcinoma, fibroadenoma or cyst.
- Most tumors are denser radiologically compared to the adjacent normal breast parenchyma.

Calcification

- Calcification forms in the areas of necrosis, hyalinised stroma or secretion.

- Hyalinised fibroadenomas,apocrine cysts and sclerosing adenosis are associated with benign calcification.

- Calcification in malignancy are usually tiny, numerous, irregular and clustered.

- DCIS is most frequently detected as calcification in mammogram. They are deposited as a linear branching pattern.

- Small sized Infiltrating Ductal adenocarcinomas rarely present with calcification unaccompanied by mammographic radiodensity. Lymph node metastasis is rare in such cases.

USG

- It can distinguish between solid and cystic lesions.

- It can delineate the borders more accurately in case of solid masses.

MRI

- It detects breast carcinomas by uptake of contrast agents owing to increased vascularity of the mass.

- It is helpful for screening the high risk women and those with dense breast.

- For evaluating cases of breast implants with rupture.

TISSUE SAMPLING METHODS

- Core needle Biopsy
- Excision Biopsy (lumpectomy)
- Incision Biopsy
- Radical and Modified Radical Mastectomy
- Fine needle aspiration cytology

CLASSIFICATION OF BREAST CANCER

More than ninety five percent of breast carcinomas are Adenocarcinomas. They are classified into carcinoma insitu and invasive carcinomas. Insitu Carcinoma refers to proliferation of neoplastic cells that are limited within ducts and lobules by the basement membrane. Invasive carcinoma ("infiltrating" carcinoma) refers to those cancers which have breached the basement membrane and invaded into the stromal tissue. Also the neoplastic cells can invade into the vessels and reach regional lymph nodes and distant sites.

CARCINOMA INSITU

Ductal carcinoma insitu

With the advent of mammographic screening, diagnosis of DCIS rapidly raised from fewer than 5% of all carcinomas to 15% to 30% of carcinomas in the well-screened population.

DCIS is constituted by a malignant clonal proliferation of epithelial cells which are limited to ducts and lobules by basement membrane. There are preserved myoepithelial cells eventhough they are reduced in number.

Morphology: DCIS has been divided into five subtypes: comedocarcinoma, solid, cribriform, papillary, micropapillary.

LOBULAR CARCINOMA INSITU

Lobular carcinoma in situ is an incidental biopsy finding, as it is not associated with calcifications or stromal reactions which produce mammographic densities. The LCIS incidence is 1% to 6% of all carcinomas. When biopsy is done on both breasts, the percentage of LCIS in both breasts is twenty to forty percent.

Morphology: Atypical lobular hyperplasia, LCIS, invasive lobular carcinoma all consist of dyscohesive cells with round or oval nuclei and small nucleoli.

INVASIVE CARCINOMAS

INFILTRATING DUCTAL CARCINOMA NOS

IDC NOS constitutes about 75-80% of breast carcinomas and it is the most common type. [33] Grossly, it has irregular infiltrating borders that imparts stellate appearance and has firm to hard consistency. It has

abundant elastotic stroma with small foci of calcification that gives grating sound while cutting.

Microscopically, it is composed of tubules, solid sheets, nests, single cells in varying proportions depending on the degree of differentiation. Grading of the tumors is by Nottingham Modification of Richardson System. (Annexure III). (COLOUR ATLAS FIGURE 7, 8, 9)

INFILTRATING LOBULAR CARCINOMA

This tumor accounts for 10% of all breast carcinomas and also the second commonest type of breast cancer. It has greater incidence of multicentricity and bilaterality. Grossly it presents as a discrete mass or diffuse indurated area.

Microscopically there is poorly cohesive neoplastic cells that infiltrates the stroma in single file arrangement or in loosely arranged clusters or in sheets. Genetic profile is similar to Luminal A Breast carcinomas. [34]There is characteristic loss of E-cadherin that functions as tumor suppressor and there is biallelic loss of expression of CDH 1. [35,36, 37]

This tumor shows positivity for HMW keratin and lack of p53. [38] To these, p120 catenin has been recently added and lobular carcinoma shows a characteristic cytoplasmic staining with this marker. [38] Grading is similar to other breast carcinomas. [39]

MEDULLARY CARCINOMA

These tumors have basal like gene expression profile. [40] They are positive for CK7 and are triple negative. [41] They are more common in sixth decade and is associated with BRCA1 mutation. [42]

They are well circumscribed and slow growing thus they clinically mimic benign lesion. Grossly the tumor is well circumscribed, soft and fleshy. Microscopically, more than 75% of the tumor is composed of solid sheets of cells, pleomorphic vesicular nucleus with prominent nucleoli admixed with lymphoplasmacytic infiltrate, most of them being cytotoxic T cells. [43]

The tumor shows increased mitosis and has pushing borders due to the overexpression of E-cadherin and because of this, there is limited metastasis. [42] This tumor has a scant fibrous stroma with minimal glandular differentiation.

MUCINOUS CARCINOMA

These breast carcinomas are common in older age group (seventh decade) and have a variety of names like Mucoid carcinoma, Colloid carcinoma and Gelatinous carcinoma. Grossly, these tumors are well circumscribed and appear as a gelatinous mass held by fibrous septa. (COLOUR ATLAS FIGURE 10)

Microscopically, in this type there are clusters of tumor cells floating in pools of mucin.(COLOUR ATLAS FIGURE 11) The tumor cell clusters may exhibit acinar or micropapillary architecture or may be solid.[44] When more than 90% of tumor content is formed by the mucin, it is called as Pure mucinous carcinoma, Otherwise it is called as Mixed mucinous carcinoma.

The mucin present in the tumor is extracellular and may be acidic or neutral. Histochemically, they are O-acylated forms of Sialomucin. They show strong positivity for MUC2. The tumor cells are ER, PR positive and HER2neu negative.[45]

APOCRINE CARCINOMA: (COLOUR ATLAS FIGURE 12)

This is a rare type of tumor constituting 1-4% of breast carcinomas. In this type of breast carcinoma more than 90% of tumor cells are of apocrine cells.

Microscopically, two types of apocrine cells are present. Type A cells with abundant granular acidophilic cytoplasm and Type B cells showing clear foamy cytoplasm. **(COLOUR ATLAS FIGURE 13).** There is also glandular differentiation with characteristic apocrine snouts. These tumors are negative for ER, PR and BCl2.

METAPLASTIC CARCINOMA (COLOUR ATLAS FIGURE 14)

This subtype has a predominant component of tumor that have an appearance other than epithelial and glandular type.[46] Microscopically, it is composed of heterogeneous components like Squamous, spindle, mesenchymal elements like chondroid and osseous material in varying proportions. **(COLOUR ATLAS FIGURE 15)**

This subtype also includes Matrix producing carcinoma which shows overt transition from carcinoma to cartilaginous or osseous matrix without spindle transition zone. It has basal like gene profile with infrequent lymph node metastasis.

TUBULAR CARCINOMA

These breast carcinomas are common around 50 years of age. Grossly, they are small showing ill-defined margins with hard consistency. Microscopically irregular and angulated glands are arranged haphazardly in a desmoplastic stroma.

CRIBRIFORM CARCINOMA

These are rare breast carcinomas in which more than 90% of tumor cells are arranged in sieve like cribriform pattern similar to insitu counterpart but showing stromal invasion.

INVASIVE PAPILLARY CARCINOMA (COLOUR ATLAS FIGURE 16)

These tumors account for less than 1% of breast carcinoma. They are circumscribed tumors in which cells are arranged in delicate blunt papillae and myoepithelial cells are not present. They have better prognosis than conventional IDC NOS

INVASIVE MICROPAPILLARY CARCINOMA

This subtype constitutes less than 2% of breast carcinomas. Microscopically, these tumors have pseudopapillary structures without fibrovascular core. They are high grade tumors, highly invasive and can exhibit psammoma bodies.[47] (COLOUR ATLAS FIGURE 17)

INTRACYSTIC PAPILLARY CARCINOMA

This subtype is rare, usually seen in elderly women accounts for 0.5 to 1.0% of all breast cancers and carries favorable prognosis.

NEUROENDOCRINE CARCINOMA

This term indicates invasive tumors that have features of neuro endocrine differentiation. It includes Carcinoid, Large cell neuroendocrine and small cell neuroendocrine carcinoma. Microscopically, small cells arranged in solid nests separated by fibrous stroma are seen.

INFLAMMATORY CARCINOMA

This subtype is named so, because clinically it presents as a red warm breast with widespread edema. Pathologically, it presents as an undifferentiated carcinoma associated with lymphatic permeation.

Biopsy of skin demonstrates the presence of dermal lymphatic invasion. This is an ominous sign for occult inflammatory carcinoma.

MOLECULAR CLASSIFICATION OF BREAST CANCER: [48, 49, 50, 51]

Luminal A

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

Luminal-B

- This phenotype is seen in 15% to 20% of IDC-NOS type of breast cancer.
- They are triple receptor positive tumors with expression of ER, PR & HER2neu.

- They are of higher grade tumors with increased proliferating potential.
- Increased frequency of metastasis to lymph nodes is seen.
- These tumors respond well to chemotherapy.

Normal Breast Like

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

Basal Like

- This phenotype accounts for 13% to 25% of IDC NOS type tumors.
- This type of breast carcinomas are characterized by the absence of PR, ER & HER2neu expression ,but expressing basal myoepithelial markers like P63, P-Cadherin and of progenitor cells / putative stem cells (CK 5/6)
- This group is referred as “TRIPLE NEGATIVE” carcinomas.[52, 53]
- Medullary & Metaplastic carcinomas come in this category.

- Breast carcinomas harboring BRCA1 mutations belong to this category.
- They are of high grade tumors with increased proliferating potential and aggressive clinical behaviour.
- They are frequently associated with CNS and Visceral metastasis.
- Complete response following chemotherapy is observed in only 15-20% of cases.

HER2neu Positive

- This phenotype is seen in about 7% - 12% of IDC NOS type of breast cancers.
- This group includes carcinomas showing HER2neu over expression and ER / PR negativity.
- The overexpression of HER2neu in more than ninety percent of these cancers is because of the amplification of the DNA segment on chromosome 17q21 which harbours the HER2neu gene and varying number of adjacent genes.
- They are poorly differentiated tumors generally with increased proliferative potential & associated with increased frequency of CNS metastasis.

PROGNOSTIC FACTORS

In the counseling of the patients regarding the likely outcome of the disease and for the appropriate treatment ,the knowledge about the prognostic factors is important.

AGE OF THE PATIENT

Better prognosis is seen in women less than fifty years of age. Prognosis declines after the age of 50.

SIZE

Size is considered as an important prognostic factor and studies show good correlation between size of the tumor and survival rate.[54, 55] For the definition of minimal breast carcinoma, size is one of the two criteria, which includes all insitu carcinomas regardless of size and the invasive carcinomas of <1cm in diameter.

SITE

Tumors located in the upper inner and lower inner quadrants have greater risk of (50%) relapse and tumor related death than the laterally located tumors. [56]

CYTOARCHITECTURAL TYPE

There is no prognostically significant difference between ordinary infiltrating ductal and lobular carcinoma .[57] Morphological variants

like Mucinous, Medullary, Papillary, Tubular ,Cribriform ,secretory and Adenoid cystic carcinoma have good prognosis.[58]

Variants like Metaplastic, Squamous cell carcinoma, Neuroendocrine, Inflammatory and Signet ring cell carcinoma are aggressive tumors having bad prognosis. [59]

PRESENCE OR ABSENCE OF INVASIVENESS

In carcinomas of ductal type that have both in situ and invasive component, a significant relationship exists between the proportion of the invasive component and the probability of metastasis to lymph nodes.

The amount of insitu component correlates with incidence of multicentricity and indirectly with probability of occult invasion. [60].

Insitu ductal malignancies of the comedocarcinoma type can also be associated with metastases in the absence of a detectable invasion.

TUMOR NECROSIS

Tumor necrosis is associated with reduced survival rates and increased nodal metastases , [61] particularly if it is very extensive. This feature is usually associated with tumors having high histologic grade.[62]

TYPE OF MARGINS

Tumors with infiltrating margins have a worse prognosis than the tumors with pushing margins.[63, 64]

MICROSCOPIC GRADE

Grading is done based on Nottingham Modification of Scarff Bloom Richardson system (Annexure III).[44] Ellis et al established that there is an excellent correlation between the Nottingham grading system and patient's survival rate and metastasis.

SKIN INVASION

Breast carcinomas with overlying skin infiltration are associated with decreased survival rate.[65]

NIPPLE INVASION

Carcinomas involving the nipple areolar complex is associated with higher incidence of axillary metastasis.[66]

BLOOD VESSEL EMBOLI

Vascular emboli shows a high association with histological grade, size of the tumor, tumor type, lymph node status and distant metastasis. Tumors with vascular invasion is associated with poor prognosis.[67]

LYMPHATIC TUMOUR EMBOLI

There is increased risk of tumor recurrence if lymphovascular invasion is present.[68, 69]

LYMPH NODE STATUS

Metastatic deposit in the axillary lymph nodes is considered as a poor prognostic factor. Number of nodes involved, level of the nodes and amount of tumor cells present in the node, presence or absence of the tumor cells in the efferent blood vessels have an important implication in the patient's survival. [70]

METASTASIS

Locally advanced disease with distant metastasis have poor prognosis. The time of detection and location of metastasis is also influenced by the tumor type.[71, 72]

BRCA-1 STATUS

The carcinomas developing in BRCA 1 mutation carriers are associated with overall poor survival rate, if they have not received adjuvant chemotherapy.[73] Absent (or) reduced nuclear BRCA 1 expression measured using immunohistochemistry is associated with many microscopic unfavorable features and also shorter disease free intervals, whereas cytoplasmic expression of this specific marker is associated with the development of tumor recurrence.[74]

STAGING (TNM) (ANNEXURE VI)

PROLIFERATION RATE

The proliferation rate is measured with mitotic counts, IHC detection of cellular proteins like Ki67, Cyclins, and flow cytometry. Poor prognosis is observed in tumors with high proliferation rate but the response to chemotherapy is better. It is also be measured by S-Phase fraction (SPF) and with thymidine labeling index.

OTHER PROGNOSTIC FACTORS

Many factors like lymphocytic infiltration, Tumor necrosis, Skin involvement, association with pregnancy and lactation, [75] keratin, BRCA mutation[76] and vimentin expression[77] also have variable prognostic implications in breast cancer.

HORMONE RECEPTORS

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

IHC was discovered 30 years back which was used to classify breast carcinomas. Nuclear hormone receptors detected by IHC correlated with better outcome and also predict the response to hormonal therapy.[78, 79]

Earlier hormone receptor expression was measured with Dextran coated charcoal and sucrose gradient assay and it is now replaced by IHC and a very good correlation is established between these methods.[80, 81] ER positive tumor cells depend on estrogen for their growth and so the use of anti-estrogenic agents (eg. Tamoxifen) inhibit cell proliferation.[82, 83]

ER and PR are co-independent variables. Estrogen receptor, a better predictor of response to hormone therapy than Progesterone. [84]HER2neu positive carcinomas have worse prognosis in spite of showing good response to Transtuzumab, a monoclonal antibody. [85] It can be measured by IHC or FISH and a better correlation exists between these methods.[86, 87]

Fisher et al proposed that there is significant association between ER expression and older age group, high nuclear grade, absence of necrosis , marked tumor elastosis . [88]

Shorlie et al and Person et al found that the “Heat maps” generated by the microarray technique was used to find the specific expression pattern of 426 genes.[89] These lead to the sub classification of the breast carcinomas.[88]

Harvey et al in 1999 suggested the cut off values for ER/PR score for 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.

SIGNIFICANCE OF HER2NEU IN BREAST CANCER

Around one fourth of primary or metastatic breast carcinomas over express HER2neu. So some breast cancers that are ER positive also will be positive for HER2neu .

Recent observations demonstrated that both ER positive, HER2neu positive human breast carcinomas that have metastasized respond less to hormonal therapy than the carcinomas that show only ER positivity. This is consistent with in vitro observation that the MCF cells (ER positive) after they are transfected with HER2neu oncogene became resistant to tamoxifen.

HER2neu activation results in the alteration of the ER.

Treatment of the neoplastic cells that over express HER2neu with Estrogen, decreases HER2neu mRNA, as well as down regulate the HER2neu product.

The cross link between a polypeptide growth factor receptor activated pathway and the hormone receptor pathway appears to be the mechanism by which a cell can become hormone independent.

TRIPLE NEGATIVE BREAST CANCER

The carcinomas that are negative for Hormone receptors ER , PR and HER2neu are called Triple negative Breast carcinomas. This means that in these tumors, the growth is not supported by Estrogen and Progesterone Hormones and they do not have HER2neu receptors. So they do not show response to the treatment by Tamoxifen nor to therapies that are targeting on HER2neu receptors (Trastuzumab).

This category of breast cancers incites an interest to doctors and researchers in finding the therapies that interfere with the growth process of the HER2neu receptors. The triple negative tumors are generally more aggressive, have higher grade than other breast carcinomas and they express basal like markers like CK5/6, P53. These cancers are more common in younger age group especially before 40 years of age and more common in those patients with BRCA1 mutation.

Triple negative breast tumors are typically treated using multimodality therapy with surgery, Chemotherapy and Radiotherapy. Some researches observed that Triple negative breast cancers actually showed a better response to Chemotherapy than other types of breast cancers.

IMMUNOHISTOCHEMISTRY

In 1941 Dr.Albert Coons first described immunohistochemistry. Since then several advancements have been made in the technique .[90]The commonly used technique is the Peroxidase-antiperoxidase immune complex technique, developed by Sternberger in the year 1970. The newer, biotin-avidin immunoenzymatic technique which was developed by Heitzman and Richards in 1974.[91, 92]

USES OF IHC IN BREAST PATHOLOGY

1) 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 malignant lesions.Eg. Usual Ductal Hyperplasia and Ductal carcinoma insitu can be distinguished with the help of HMWCK.

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

5) To detect the sentinel lymphnode metastasis.

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

7) Evaluation of Metaplastic carcinoma from the mesenchymal lesions.

ANTIGEN RETRIEVAL

In 1991, Shi et al developed the antigen retrieval technique, in which high temperature was utilised to bring out the antigenicity of the tissues which was masked by the formalin fixation. Antigen retrieval is done either by the heat induced epitope retrieval or by the proteolytic epitope retrieval.

HEAT INDUCED

The tissue sections are placed in the retrieval solution and are subjected to heat for varying periods of time. This will breakdown the protein crosslinks and retrieves the antigenicity. [93]The heat can be applied with microwave oven, steamer, pressure cooker, autoclave or waterbath. The commonly used retrieval solutions are citrate buffer at the PH 6, TRIS EDTA at the PH 9, EDTA at the PH 8.

PROTEOLYTIC EPITOPE RETRIEVAL [94]

The Tissue antigenicity is also restored using proteases like Proteinase K, trypsin, pepsin and Chymotrypsin. The main disadvantage in this method is, it alters the tissue morphology and also destroys some epitopes.

TARGET ANTIGEN DETECTION METHODS

After retrieval of the tissue antigenicity, specific antibodies are added which forms the Antigen antibody complex. This can be visualized by using Direct and Indirect methods.

DIRECT METHOD

The antibodies used here are labeled antibodies which react with the antigens in tissue sections. Some of the labels used are fluorochrome, alkaline phosphatase and horse radish peroxidase . It is simple and also rapid but has low sensitivity.

INDIRECT METHOD

Here, unlabeled primary antibody is added in the first step, which binds with the target antigen. Then, a labeled secondary antibody is added in the second step, which react with the primary antibody. It is more sensitive and also it uses only a small number of secondary antibodies. [95]

C-KIT (CD 117)

CD 117, a proto oncogene is a transmembrane tyrosine kinase growth factor receptor which maps to chromosome 4 (q11-12) is structurally related to the platelet-derived growth factor/colony stimulating factor-1 receptor family of proteins[96].

Dimerization and autophosphorylation of CD117, upon binding to its specific ligand, known as stem-cell factor, is known to inhibit apoptosis and potentiate cell proliferation.[97, 98].

In the development of gastrointestinal stromal tumors, small-cell lung cancer, breast cancer and melanoma CD 117 has an important role.[99]

Studies by several researchers have shown that CD 117 expression is high in normal breast tissue, but is diminished or is completely not expressed in primary infiltrating breast carcinomas.[100, 101]

The CD 117 expression is found in normal mammary tissue and in tumor cells.[101] In breast carcinoma, the CD 117 expression represents a highly controversial subject, but most of the studies have found reduced CD 117 expression in invasive breast cancers.[106]

Kondi-Pafiti *et al*,[107] reported that CD117 was highly expressed in stromal cells of high grade breast carcinoma despite negative CD 117 expression in tumor cells.

Lennartsson *et al*,[108] described the loss of CD 117 expression during the progression of normal breast epithelium to breast carcinomas.The c-kit signaling pathway regulates the proliferation and differentiation of normal breast epithelium. [109] Prevalence of CD117 immunoreactivity in invasive breast carcinoma (28.6%-36%).[105, 106]

Reduction of CD 117 expression in malignant transformation in breast epithelium may suggest its carcinogenic role in the breast cancer[102] as the neoplastic cells escape from the regulatory mechanisms.[110]

Increased level of CD 117 expression is infrequent in breast carcinoma.[111] Eroğlu and Sari[112] found that CD 117 expression is increased in invasive breast carcinomas.

In high-grade invasive breast carcinoma groups that contain the carcinomas with mesenchymal and/or myoepithelial differentiation that is basal-like subtype which have a poor prognosis,CD 117 expression appeared to be an important prognostic indicator. [103]

Both membranous and cytoplasmic staining of CD117 were evaluated in tumor epithelial and stromal cells.The proposed scoring system from studies of Tsuda *et al*.[103] and Diallo *et al*.[104]

Score 0, no staining or staining < 10 percent of epithelial cells; score 1+, the cytoplasm was discretely and weakly to moderately stained in ten percent or more of the epithelial cells; score 2+, the cytoplasm was strongly stained with or without membranous staining in ten percent or more of the epithelial cells.

Cases with score 0 are taken as negative and cases with a score of 1+ and 2+ are taken as positive.

X Chui et al, [113] studied CD 117 expression in 57 breast carcinoma cases comparing with twenty normal breast tissues and fifty eight benign breast neoplasms. In normal breast tissues, the CD 117 expression was strong on cell membrane and/or cyoplasm of alveolar and ductal cells.

Abdallah et al, [114] have done a retrospective study in 50 cases of Invasive breast carcinoma. It includes 42 IDC NOS, 5 lobular carcinoma, 2 medullary and single mucinous carcinoma. C-kit found to be positive in 90.5% of IDC NOS, and 100% positivity in other subtypes. He concluded that there is overexpression of CD 117 in tumors with high nuclear grade.

Susruthan et al,[115] studied CD 117 expression in 62 infiltrating ductal carcinomas and found that 100% expression was seen in normal breast epithelium, reduced expression seen in insitu carcinomas and 52% of malignant epithelium showed c-Kit expression. In Grade 1 tumours, 36% showed positivity, 57% in grade 2 and 46%in grade 3 tumors. He concluded that CD 117 expression is high in triple negative cancers and did not correlate with age, size of the tumor, grade, lymph node status.

Maha M. Amin et al, [106] studied 126 cases of invasive breast cancers of different histological subtypes and grades. About 29% of invasive breast cancers showed CD117 expression. He found that the

age, tumor grade, size, lymph node metastasis and the c-kit expression had significant difference. Also there is significant correlation between c-kit expression and ER, PR positivity.

Palmu et al [116] investigated CD 117 expression of c-kit and HER2 neu receptors and their correlation with histopathological factors like cell proliferation, differentiation and apoptosis in poor prognostic breast cancers. He concluded that the C-kit expression was commonly present and did not correlate to other prognostic factors in poor prognosis breast cancer.

Ronald simon et al, [117] investigated 1654 breast carcinomas for CD117 expression and mutations.He concluded that normal breast epithelium showed 100% CD117 expression.But only 2.6% carcinomas showed CD117 expression. There was significant association between CD 117 expression and tumor grade ($p<0.0001$).

Paola ulivi et al [118] studied fourteen normal breast tissue, sixteen in situ breast carcinomas and seventy five percent invasive carcinomas of breast. He found that in normal breast tissue CD 117 expression is high, lower in carcinoma in situ and lost completely in infiltrating breast carcinomas.

Hitoshi Tsuda et al[119] studied thirty two undifferentiated type of breast cancers and 37 cases of differentiated breast cancers. He found

that CD 117 overexpression was seen more in undifferentiated breast cancers (34%).

Hitoshi Tsuda et al [120] studied CD 117 expression in 150 cases of breast carcinomas. He found that CD 117 expression was more in comedo type of DCIS and Infiltrating Ductal Carcinomas of the solid-tubular subtype.There was inverse correlation between CD 117 and ER expression.

S Tsutsui et al [121] analysed CD 117 expression in 217 Infiltrating Ductal Carcinomas of breast. He found CD 117 positivity in 59 (27%) cases, negative CD 117 expression in 158 (73%) cases. He concluded that there was significant association between negative expression of c-kit protein and metastatic deposits in lymph node. Also there was no significant association between CD 117 expression and the size of tumor, nuclear grade, ER expression.

Tahany M Shams et al [122] studied 72 triple negative breast cancers and found that 75% of breast cancers expressed CD 117 positivity, suggesting that CD 117 may be used as a therapeutic target.

Eroglu et al [123] analysed the CD 117 expression in 52 infiltrating breast carcinomas and sixteen benign breast neoplasms and found that in invasive breast carcinomas, there was high CD 117 expression and this was not significantly correlated with other clinico-pathological variables.

MATERIALSAND METHODS

This study is a descriptive prospective and retrospective 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 September 2014 to August 2016.

SOURCE OF DATA

A total of 22562 biopsy specimens were received in our surgical pathology department during this 2 year period. Out of which 1430 were breast specimens. Of these 580 were malignant. The invasive ductal carcinoma NOS and special subtype cases reported in mastectomy specimens received in the Institute of Pathology, Madras Medical College between September 2014 to August 2016 from the Department of Surgery, surgical Oncology, and Plastic surgery, Government General Hospital.

INCLUSION CRITERIA

- All modified radical mastectomy specimens of breast carcinomas.
- All invasive breast carcinomas no special type (ductal and lobular), medullary, mucinous, papillary, apocrine and metaplastic carcinomas.

EXCLUSION CRITERIA

- All small biopsies.
- Phyllodes tumors.
- Benign breast lesions.
- Tumors with preexisting premalignant conditions.
- Recurrent tumors.

METHOD OF DATA COLLECTION

Of the total cases reported during this study period, ER, PR and HER2neu expression was studied for 165 cases. 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 165 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 collected from slide filing and were reviewed and graded using the Nottingham modification of the Scarff Bloom Richardson Grading system (Annexure III) and they were further evaluated for the presence of necrosis, lymphocytic response, lymphovascular invasion and skin infiltration. 42 cases from Invasive ductal carcinoma NOS and 08 cases from special types such as

metaplastic, mucinous, apocrine, intracystic papillary carcinoma were randomly selected from the total cases and their representative formalin fixed paraffin embedded tissue samples were subjected to immunohistochemical analysis of C-KIT expression. Slides were evaluated and scoring was given. The results were recorded with photographs.

IMMUNOHISTOCHEMICAL EVALUATION

Immuno-histochemical analysis of ER, PR, H2N and c-kit were done in Paraffin embedded tissue samples using super sensitive polymer HRP system based on non-biotin polymeric technology.

Table 1: Immunohistochemical markers used in the current study

Antigen	Vendor	Clone	Dilution	Positive control
ER	Dako	Rabbit Monoclonal EP1	Ready to Use	Breast
PR	Dako	Mouse Monoclonal	Ready to Use	Breast
HER2neu	Dako	Rabbit Monoclonal SP-3	Ready to Use	Breast
C-KIT	PathnSitu	Rabbit Monoclonal	Ready to Use	GIST

4 µm thick sections from selected formalin fixed paraffin embedded tissue samples were transferred on to gelatin coated slides. Heat induced antigen retrieval was done using micro wave method. The ER and PR antigens are bound with mouse monoclonal antibodies (dako) and HER2neu (dako) and C-KIT (PathnSitu) antigens are bound with rabbit monoclonal antibody. Later antigen antibody complex are detected by the

addition of secondary antibody conjugated with horse radish peroxidase-polymer and Di-aminobenzidine substrate.The step by step procedure of Immunohistochemistry is given in Annexure IV.

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 V).

H2N

HER 2-neu expression is demonstrated in tumor cells as cytoplasmic expression is graded as 1+, 2+ and 3+.(Annexure V).

Table.2. C-KIT expression scoring (Tsuda et al) [103]

SCORE	STAINING PATTERN
Score 0	No staining was observed or staining was observed in less than 10% of epithelial cells
Score 1+	The cytoplasm was discretely and weakly to moderately stained in 10% or more of the epithelial cells
Score 2+	The cytoplasm was strongly stained with or without membrane staining in 10% or more of the epithelial cells
SCORE 0	NEGATIVE
SCORE 1+ and 2+	POSITIVE

STATISTICAL ANALYSIS

The Statistical analysis for this study was done using the software IBM Statistical Package for social science (SPSS) version 20. The correlation between C-KIT expression and different clinico-pathological parameters like age group, size, side, histopathology, grade, lymph node status, lymphovascular invasion, lymphocytic infiltration, necrosis, skin infiltration, Hormonal receptors like ER, PR and HER2neu was made and strength of association was calculated by Pearson Chi square test and P value less than 0.05 are considered statistically significant.

OBSERVATION AND RESULTS

In our Institute the total number of pathological specimens received from September 2014 to August 2016 was 22562, out of which the total number of breast specimens received was 1430. Out of 580 breast malignancies, the total number of breast carcinomas enrolled in this study period was 165 cases.

The age wise distribution of these 165 cases is given below (CHART 1)

Table- 3: Age Wise Distribution of Breast Cancer

Age Group in years	NO OF CASES(n)	Percent(%)
30-40	42	25.5
41-50	58	35.2
51-60	43	26.1
61-70	20	12.1
70 and above	2	1.2
Total	165	100.0

The highest incidence of breast cancers was found in the age group of 41-50 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.

SIDE

Table-4: Side of the breast involved

SIDE	NO OF CASES(n)	Percent
Right	88	53.3
Left	77	46.7
Total	165	100.0

77 cases of primary breast carcinomas were reported in left breast and 88 cases were reported in right breast (CHART 2)

TUMOR LOCATION

Table-5: Tumour Location Among The Breast Cancers

Tumour location	NO OF CASES(n)	Percent(%)
UOQ	66	40.0
UIQ	25	15.2
LOQ	22	13.3
LIQ	17	10.3
CQ	35	21.2
Total	165	100.0

66 cases of breast carcinoma were located in upper outer quandrant. (CHART 3)

TUMOR SIZE IN CM

Table-6. Distribution of Breast Cancers with Tumour Size

T SIZE	NO OF CASES(n)	Percent(%)
< 2	38	23.0
2-5	99	60.0
> 5	28	17.0
Total	165	100.0

38 cases (23%) had tumor size less than 2 cm,99 cases (60%) were of 2 to 5 cm in size and 28 cases (17 %) were more than 5 cm in size (CHART 4)

Table-7. Distribution Of Histological Sub Types Of Breast Cancers

HISTOPATHOLOGY	NO OF CASES(n)	Percent (%)
Invasive Ductal Carcinoma NOS	155	93.9
Metaplastic Carcinoma	3	1.8
IDC with Mucinous Carcinoma	5	3.0
Apocrine Carcinoma	1	0.6
Intracystic Papillary Carcinoma	1	0.6
Total	165	100.0

Commonest histopathological subtype in this study was IDC NOS which constituted 93.9% (n=155) of cases and other subtypes were metaplastic carcinoma 3 cases, mucinous carcinoma 5 cases,apocrine carcinoma (n=1) and intracystic papillary carcinoma (n=1).

GRADE

Table-8: Distribution Of Breast Cancers With Tumour Grade

GRADES	NO OF CASES(n)	Percent(%)
No grade	7	4.2
I	45	27.3
II	93	56.4
III	20	12.1
Total	165	100.0

Tumor grade was done according to modified SBR grading system,low grade (grade 1 and 2) includes 83.7%(n=138) and high grade (grade 3) seen in 12.1%(n=20) only.(CHART 6)

Table-9: Distribution Of Lymph Node Metastasis In Breast Cancers

LYMPH NODE STATUS	NO OF CASES(n)	Percent(%)
NEGATIVE	69	41.8
<= 3	62	37.6
4-9	25	15.2
>= 10	9	5.5
Total	165	100.0

62 cases (37.6%) had upto 3 nodes with metastatic carcinomatous deposits, 25 cases(15.2%) had 4 to 9 involved nodes,9 cases(5.5%) had more than 10 involved nodes,while 69 cases (41.8%) had no nodal involvement. (Colour atlas Figure 19.) (CHART 7)

MARGIN STATUS

Table-9: Distribution Of Breast Cancers And Its Margin Status

MARGIN STATUS	NO OF CASES(n)	Percent(%)
Present	13	7.9
Absent	152	92.1
Total	165	100.0

Positive margins were noted in 7.9% (n=13) and adequate margins wre noted in 92.1%(n=152) cases.

Table-10: Distribution Of Lympho Vascular Invasion In Breast Cancer

LVI STATUS	NO OF CASES(n)	Percent(%)
Present	95	57.6
Absent	70	42.4
Total	165	100.0

Lymphovascular invasion seen in 74.5% (n=123) and absent in 25.5% (n=42) cases. (Colour Atlas -Figure.19) (CHART 8)

Table-11: Distribution of lymphocytic infiltration in breast cancer

LYMPHOCYTIC INFILTRATION	NO OF CASES(n)	Percent
Present	123	74.5
Absent	42	25.5
Total	165	100.0

Lymphocytic infiltration seen in 123 cases (74.5%) .(CHART 9)

Table-12: Distribution of necrosis in breast cancer

NEROSIS	NO OF CASES(n)	Percent
Present	78	47.3
Absent	87	52.7
Total	165	100.0

78 out of 165 cases (47.3%) had necrosis. (CHART 10)

Table-13: Distribution of skin involvement in breast cancer

SKIN INVOLVEMENT	NO OF CASES(n)	Percent(%)
Present	10	6.1
Absent	155	93.9
Total	165	100.0

Skin infiltration was seen in 10 out of 165 cases, which constituted 6.1% (CHART 11)

Table-14: Distribution of associated breast lesions (chart 12)

ASSOCIATED LESIONS	NO OF CASES(n)	Percent(%)
Nil	24	14.5
FCD	95	57.6
DCIS	30	18.2
Adenosis	5	3.0
ADH	3	1.8
Epithelial Hyperplasia	2	1.2
FA	6	3.6
Total	165	100.0

ER, PR and Her-2 neu receptors were done in all 165 cases.IHC scoring of ER and PR was done using Alred scoring system based on intensity and proportion of cells exhibiting positivity.ER positive in 47.3%(n=78) cases (colour atlas figure 21) ,PR positive in 38.8% (n=64) (colour atlas figure 22) and Her-2 positive in 37% (n=61%)(colour atlas figure 23) . Both ER and PR positive in 16 cases and both negative in 23 cases. ER positive in 9 cases and PR alone positive in 2 cases.

Table- 15: Distribution of ER in breast cancers (chart 13)

ER STATUS	NO OF CASES(n)	Percent(%)
Positive	78	47.3
Negative	87	52.7
Total	165	100.0

Table- 16: Distribution of PR in breast cancers (chart 14)

PR STATUS	NO OF CASES(n)	Percent(%)
Positive	64	38.8
Negative	101	61.2
Total	165	100.0

Table-17:Distribution Of HER2neu Receptors In Breast Cancers (Chart 15)

HER-2 STATUS	NO OF CASES(n)	Percent(%)
Positive	61	37.0
Negative	104	63.0
Total	165	100.0

Table- 18: molecular subtypes of tumors (chart 16)

Molecular sub types	Number of cases	Percent(%)
Luminal A	56	33.9
Luminal B	28	17.0
HER-2 Enriched	33	20.0
Basal like	48	29.1
Total	165	100.0

Molecular subclassification of tumours in our study are Luminal A 33.9%(N=56), Luminal B 17%(n=28), Her-2 enriched 20 (n=33) and basal like 29.1% (n=48).

EXPRESSION OF C-KIT (CD117)

Out of 165 invasive breast carcinoma, 50 cases were evaluated for CD117 (C-KIT) expression using Rabbit Polyclonal Antibody (Pathnsitu- Ready to use). CD117 expression was graded according to proportion and intensity of positivity in membrane and cytoplasm of tumor cells using Tsuda et al study. Gastrointestinal stromal tumor and normal breast epithelium in the same slide were taken as control.

Intense 2+ staining was noted in GIST and Normal breast epithelium (COLOUR ATLAS FIGURE 24). In our study, 40% of invasive breast carcinomas had expressed c-kit . (COLOR ATLAS FIGURE 25, 26)

Table-18: Distribution of c-kit expression in breast cancers (chart 17)

CD117 EXPRESSION	Frequency	Percent
Negative	30	60.0%
1+	17	34.0%
2+	3	06.0%
Total	50	100.0%

Table-19: Correlation Of C-Kit Expression And Age Of The Patients (Chart 18)

Age Group in years	CD117 Scoring		Total	P value
	Positive	Negative		
30-40	5	7	12	0.881
41-50	6	11	17	
51-60	5	7	12	
61-70	4	4	8	
70 and above	0	1	1	
Total	20	30	50	

Expression of c-kit was seen more in the age group of 41-50 years. There is no significant correlation between age and C-KIT expression

Table-20: Correlation Of C-Kit Expression And Side Of Breast Involvement (Chart 19)

SIDE DISTRIBUTION	CD117 Scoring		Total	P value
	Positive	Negative		
Right	9(40.9%)	13(59.1%)	22	0.907
Left	11(39.3%)	17(60.7%)	28	
TOTAL	20	30	50	

CKIT positivity is more in left sided breast cancers than right side, but it is not statistically significant.

Table-21: Tumor Location And CD117 Distribution (Chart 20)

Tumour location	CD117 Scoring		Total	P value
	Positive	Negative		
UOQ	10 (41.7%)	14 (58.3%)	24 (100.0%)	0.201
UIQ	2 (28.6%)	5 (71.4%)	7 (100.0%)	
LOQ	3 (37.5%)	5 (62.5%)	8 (100.0%)	
LIQ	5 (71.4%)	2 (28.6%)	7 (100.0%)	
CQ	0 (.0%)	4 (100.0%)	4 (100.0%)	
Total	20	30	50	

There is no statistically significant correlation found between tumor location and the C-kit expression.

Table-22: Tumour Size and CD117 Expression (Chart 21)

Tumor size in cm	CD117 Scoring		Total	P value
	Positive	Negative		
< 2	9(56.3%)	7(43.8%)	16	0.150
2-5	10(37.0%)	17(63%)	27	
> 5	1(14.3%)	6(85.7%)	7	
Total	20	30	50	

C-kit expression was decreased in higher T stage group patient but it is not statistically significant.

Table-23: Correlation Of Histological subtype And CD117 Expression (Chart 22)

Histopathology	CD117 Scoring		Total	P VALUE
	Positive	Negative		
Invasive Ductal Carcinoma NOS	15(35.7%)	28(64.3%)	43	0.08%
Metaplastic Carcinoma	0(0.0%)	2(100%)	2	
Mucinous Carcinoma	3(100%)	0(0%)	3	
Apocrine Carcinoma	1(100%)	0(0%)	1	
Intracystic Papillary Carcinoma	1(100%)	0(0%)	1	
Total	20	30	50	

Ckit expression was more expressed in Infiltrating Ductal Carcinoma NOS (color atlas figure 25, 26) which is the commonest sub type of breast cancer. Special sub types like Mucinous (color atlas figure 27), Apocrine (color atlas figure 28) and Intracystic Papillary carcinoma (color atlas figure 29) showed 100% expression. CD117 was not expressed in Metaplastic carcinoma (color atlas figure 30).

Table-24: CD117 Expression Correlation With Grades (Chart 23)

GRADES	CD117 Scoring		Total	P value
	Positive	Negative		
No grade	4(80%)	1(20%)	5	0.028
I	3(18.8%)	13(81.3%)	16	
II	12(52.2%)	11(47.8%)	23	
III	1(16.7%)	5(83.3%)	6	
Total	20	30	50	

There is loss of c-kit expression in high grade tumors (Grade III) and it is statistically significant. Lower grade tumors (Grade I & II) has more of c-kit expression i.e 61% (n=15).

Table-25: Lymph Nodal Status And CD117 Expression (Chart 24)

Lymph nodal Status	CD117 Scoring		Total	P value
	Positive	Negative		
NEGATIVE	9(60%)	6(40%)	15	0.219
<= 3	8(30.8%)	18(69.2%)	26	
4-9	1(20%)	4(80%)	5	
>= 10	2(50%)	2(50%)	4	
Total	20	30	50	

There is no statistically significant correlation between the lymph node involvement status and CD117 expression in our study.

Table -26: Margin Status And CD117 Expression

MARGIN STATUS	CD117 Scoring		Total	P VALUE
	Positive	Negative		
Present	0(0%)	4(100%)	4	0.119
Absent	20(43.5%)	26(56.5%)	46	
TOTAL	20	30	50	

There is no statistically significant correlation between the margin status of the tumor and CD 117 expression

Table--27: Correlation Of CD117 Expression And Lymphovascular Involvement (Chart 25)

LVI STATUS	CD117 Scoring		Total	P value
	Positive	Negative		
Present	14(43.8%)	18(56.3%)	32	0.470
Absent	6(33.3%)	12(66.7%)	18	
TOTAL	20	30	50	

There is no statistically significant correlation between the lymphovascular invasion and C-kit expression

Table-28: Lymphocytic Infiltration And CD117 Expression (Chart 26)

Lymphocytic Infiltration	CD117 Scoring		Total	P VALUE
	Positive	Negative		
Present	14(35.9%)	25(64.1%)	39	0.221
Absent	6(54.5%)	5(45.5%)	11	
TOTAL	20	30	50	

14 patients (35.9%) who presented with Lymphocytic infiltration showed positivity in comparison with lymphocytic infiltration absent group 54.5% (n=6)} and there is no significant association between the lymphocytic response and CD 117 Expression.

Table -29: Necrosis And CD117 Expression (Chart 27)

NECROSIS	CD117 Scoring		Total	P VALUE
	Positive	Negative		
Present	8(34.8%)	15(65.2%)	23	0.487
Absent	12(44.4%)	15(55.6%)	27	
TOTAL	20	30	50	

There was loss of C-kit expression in tumors associated with necrosis i.e 65.2% (n=15)

Table- 30: skin involvement and CD 117 expression (chart 28)

Skin Involvement	CD117 Scoring		Total	P value
	Positive	Negative		
Present	6(60%)	4(40%)	10	0.149
Absent	14(35%)	26(65%)	40	
TOTAL	20	30	50	

CD 117 is expressed in 60% of tumors with skin infiltration, though not significantly associated.

Table-31: CD117 Expression In DCIS Component (Chart 29)

DCIS	CD 117 SCORING		TOTAL	P VALUE
	Positive	Negative		
PRESENT	11 (61.1%)	7(38.9%)	18	0.493

Out of 18 tumors with DCIS component , 11 (61.1%) showed CD 117 expression ,even though not significantly associated (color atlas figure 31).

Table-32: Correlation Of CD117 Expression With ER Expression (Chart 30)

ER STATUS	CD117 Scoring		Total	P VALUE
	Positive	Negative		
Positive	11(44%)	14(56%)	25	0.564
Negative	9(36%)	16(64%)	25	
TOTAL	20	30	50	

11 (44%) out of 25 ER positive cases showed CD117 expression,when compared to 9(36%) out of 25 ER negative cases showed cd 117 expression ,though not statistically associated.

Table-33: Correlation Of CD117 Expression With PR Expression (Chart 31)

PR STATUS	CD117 Scoring		Total	P VALUE
	Positive	Negative		
Positive	8(44.4%)	10(55.6%)	18	0.630
Negative	12(37.5%)	20(62.5%)	32	
TOTAL	20	30	50	

8 (44.4%) out of 18 PR positive cases showed CD 117 expression, but 12(37.5%) out of 32 PR negative cases showed CD 117 expression. It is not statistically significant.

Table-34: Correlation Of HER2neu Expression And C-Kit Expression (Chart 32)

HER-2 Status	CD117 Scoring		TOTAL	P value
	Positive	Negative		
Positive	7(35%)	13(65%)	20	.386
Negative	13(43.3%)	17(56.7%)	30	
Total	20	30	100	

Seven (35%) out of 20 HER2neu positive cases showed CD117 expression, but 13(43.3%) out of 30 HER2neu negative cases showed C-KIT expression. hence, it is not statistically significant.

Table-35: Correlation Of CD117 With ER And PR Expression Profile (Chart33)

Hormone Receptor	CD117 Scoring		Total	P VALUE
	Positive	Negative		
ER + and PR +	7(43.8%)	9(56.3%)	16	0.916
ER + and PR -	4(44.4%)	5(55.6%)	9	
ER - and PR +	1(50%)	1(50%)	2	
ER - and PR -	8(34.8%)	15(65.2%)	23	
TOTAL	20	30	50	

Among positively expressed CD 117 cases (n=20) ,40% (n=8) were ER and PR negative. There is no significant correlation between CD 117 expression and any of the ER and PR combination.

Table-36: Correlation Of CD117 Expression With Molecular Subtypes Of Breast Cancer (Chart 34)

Molecular Classification	CD117 Scoring		Total	P VALUE
	Positive	Negative		
Luminal A	6(35.3%)	11(64.7%)	17	0.085
Luminal B	6(60%)	4(40%)	10	
HER-2 Enriched	1(10%)	9(90%)	10	
Basal like	7(53.8%)	6(46.2%)	13	
Total	20	30	50	

Seven (53.8%) out of 13 cases of basal like breast carcinomas showed CD 117 expression, though not statistically sigfnificant.

CHART 1

AGE WISE DISTRIBUTION OF BREAST CANCERS

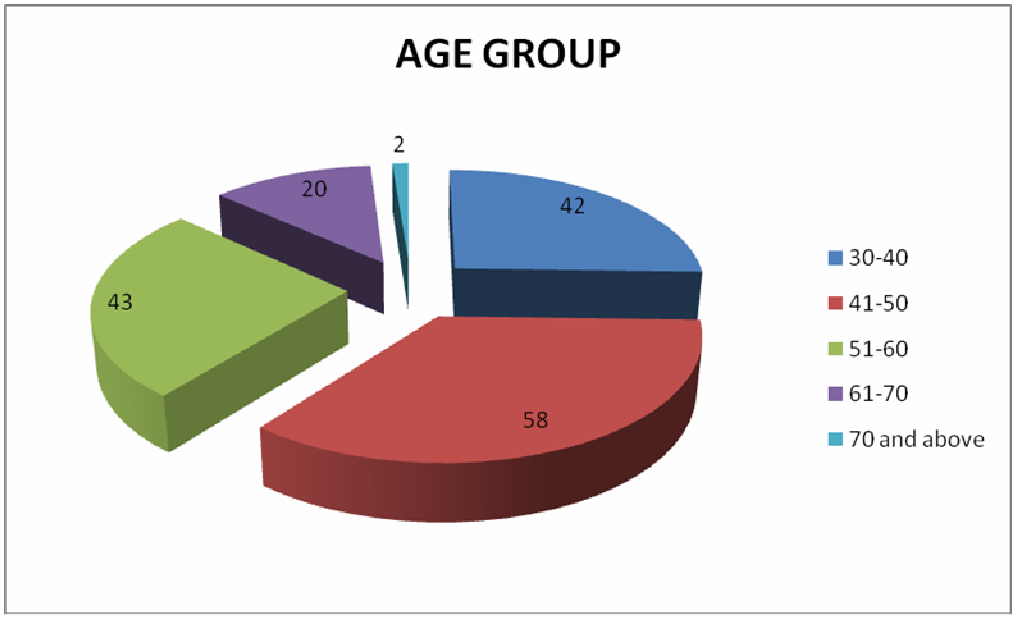


CHART 2

DISTRIBUTION OF SIDE OF INVOLVEMENT IN BREAST

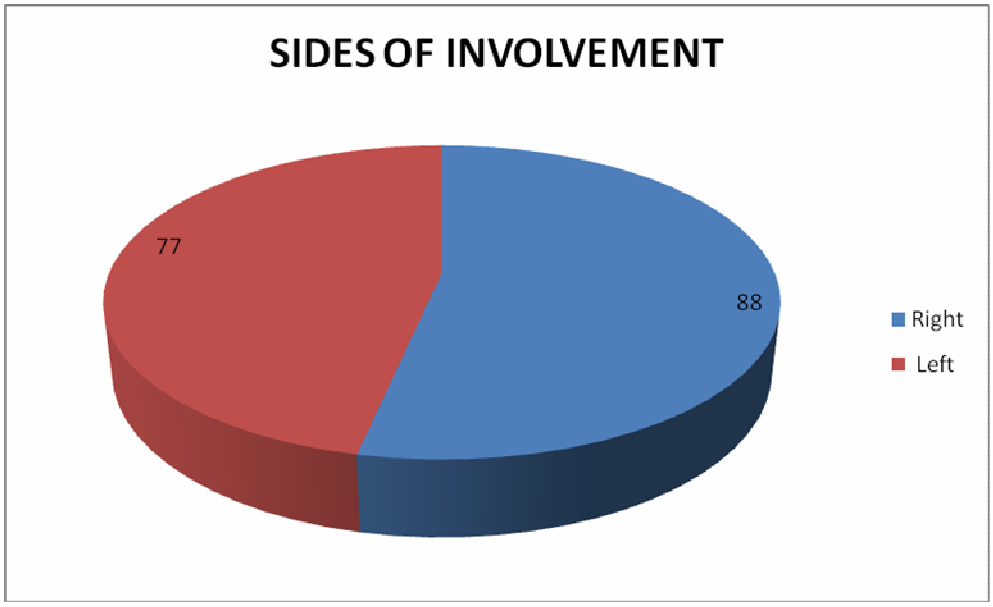


CHART 3

DISTRIBUTION OF TUMOR LOCATION IN BREAST CANCERS

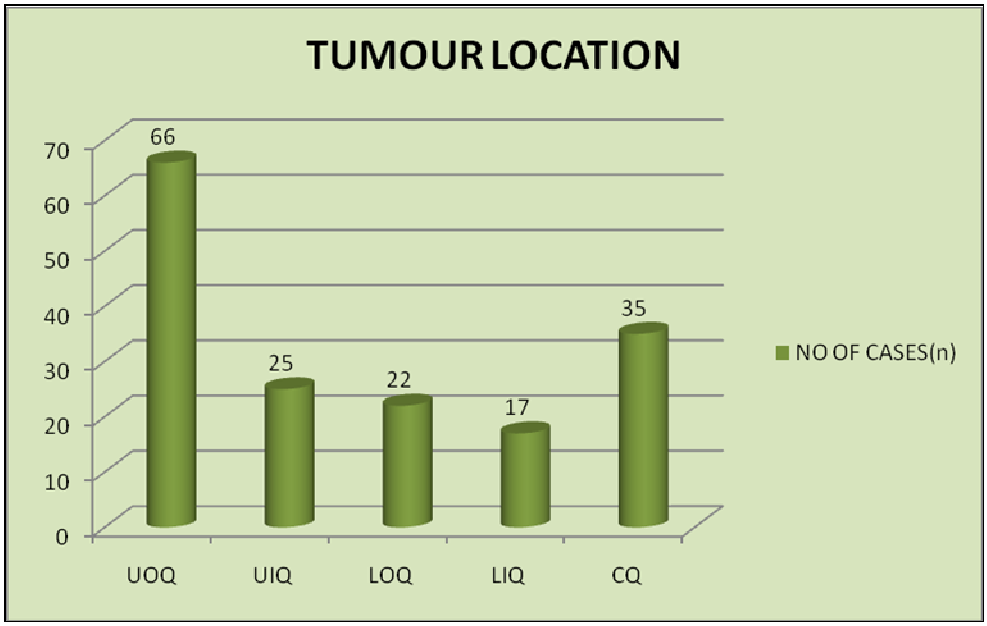


CHART 4

DISTRIBUTION OF SIZE OF THE TUMOR

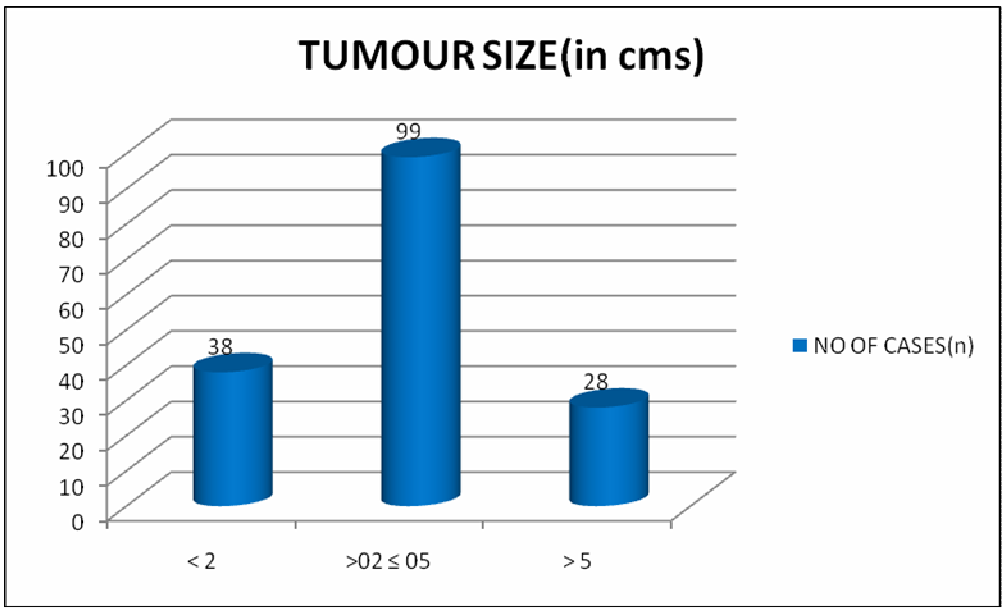


CHART 5

DISTRIBUTION OF HISTOLOGICAL TYPE

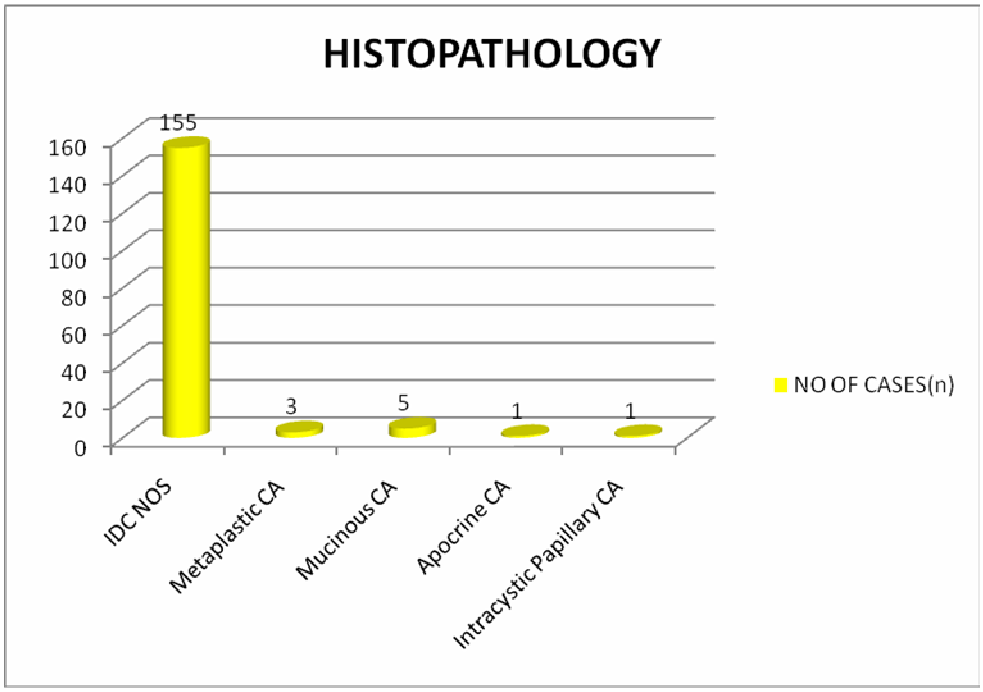


CHART 6

DISTRIBUTION OF HISTOLOGICAL GRADE

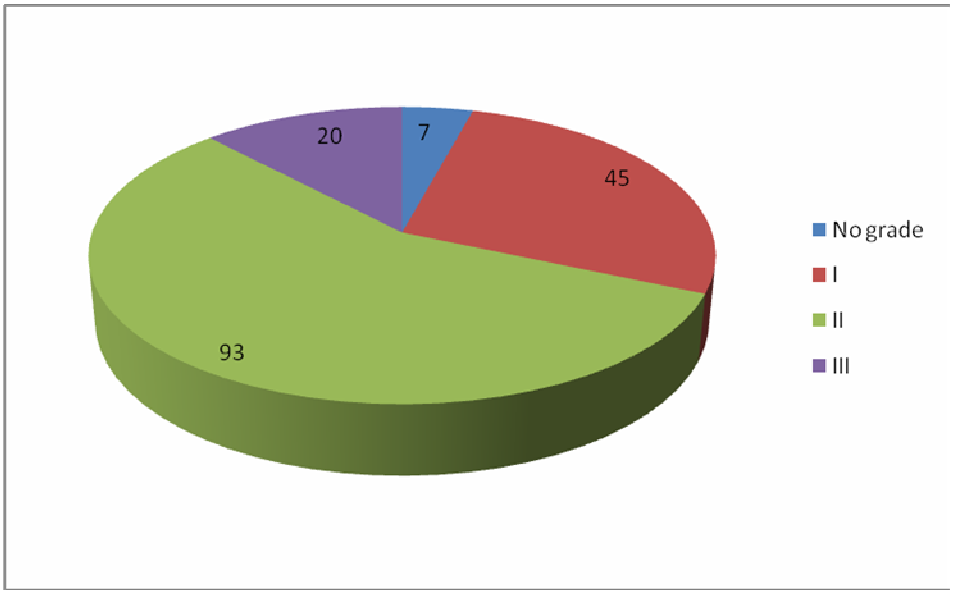


CHART 7

DISTRIBUTION OF LYMPH NODE METASTASIS IN BREAST CANCERS

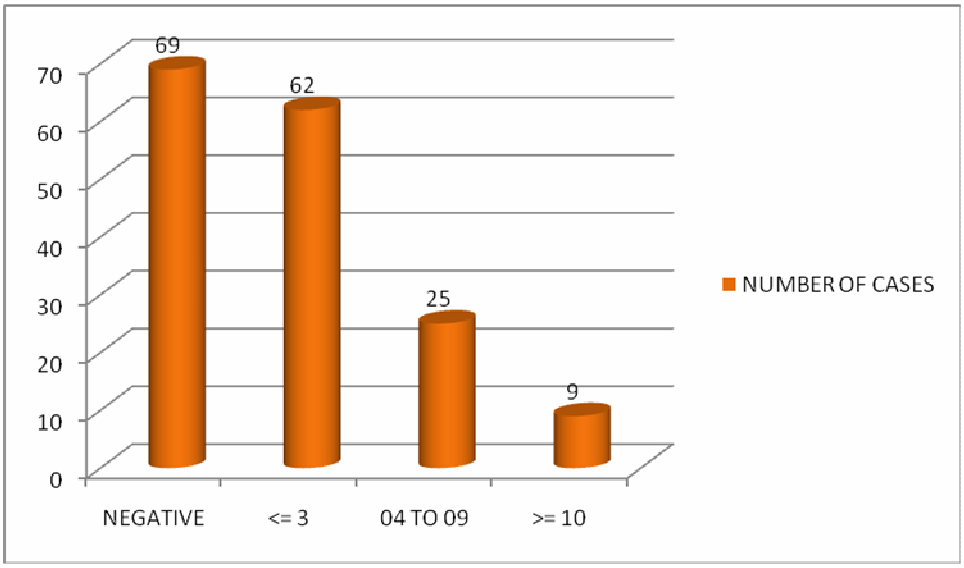


CHART 8

DISTRIBUTION OF LYMPHO VASCULAR INVASION IN BREAST CANCERS

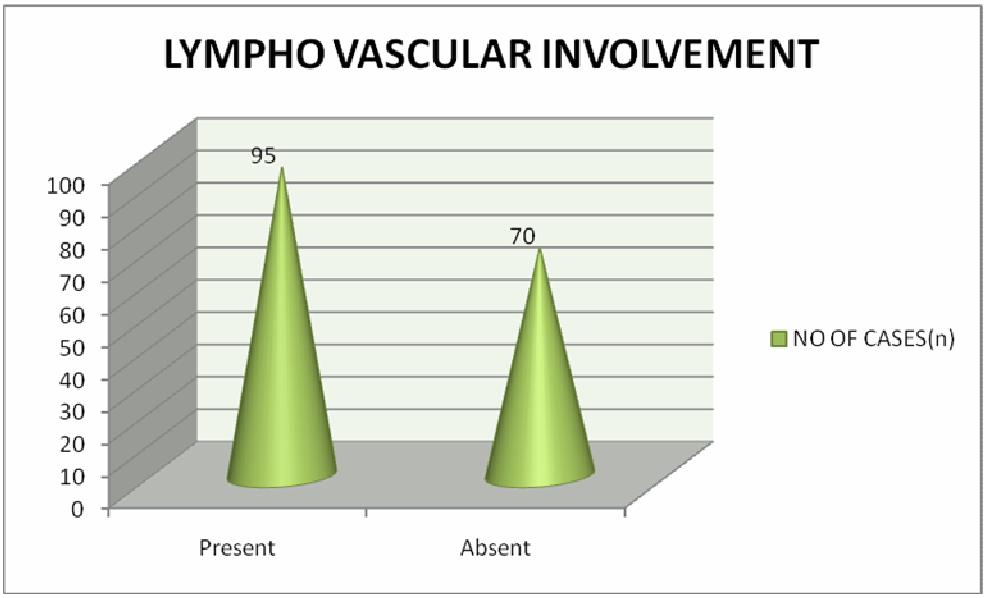


CHART 9

DISTRIBUTION OF LYMPHOCYTIC INFILTRATION IN BREAST CANCERS

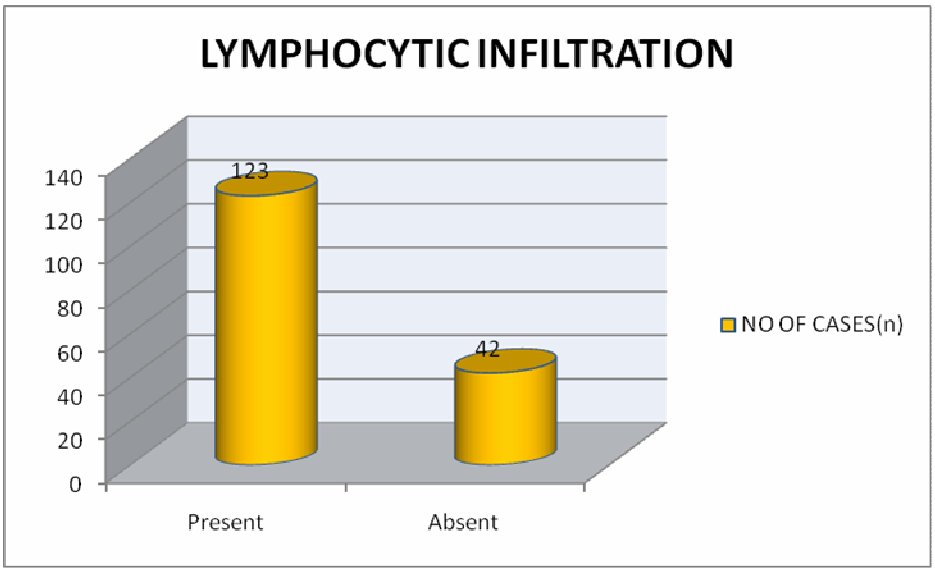


CHART 10

DISTRIBUTION OF NECROSIS IN BREAST CANCERS

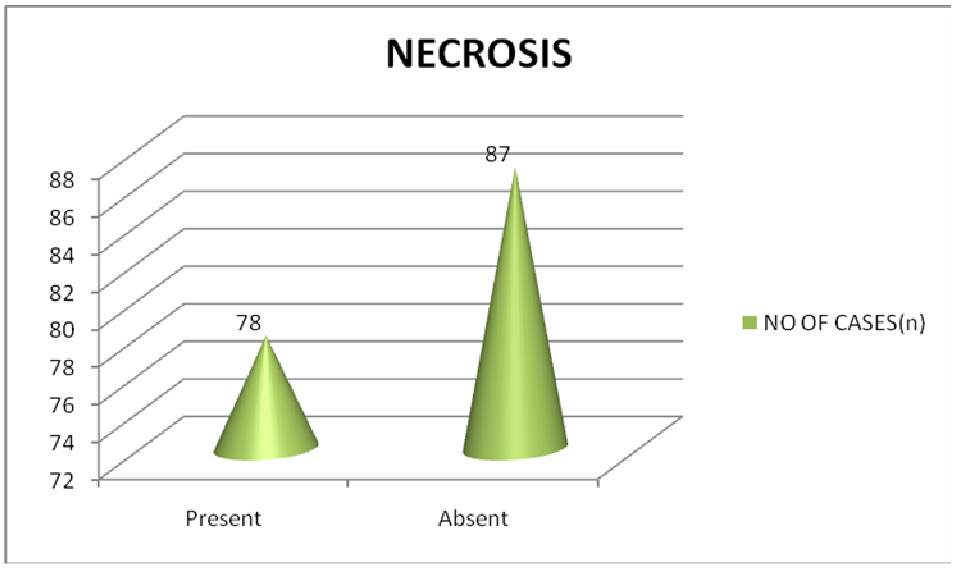


CHART 11

DISTRIBUTION OF SKIN INVOLVEMENT IN BREAST CANCERS

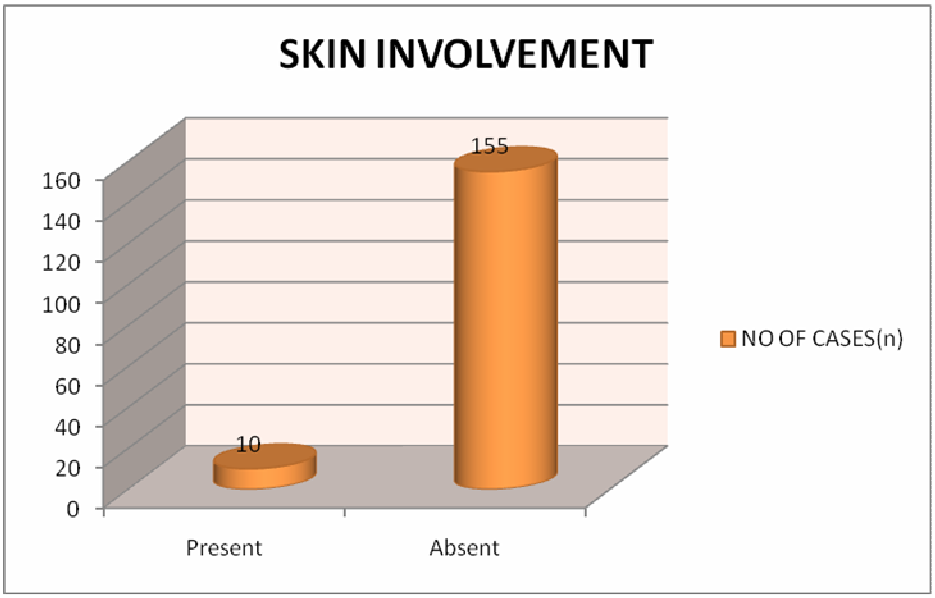


CHART 12

DISTRIBUTION OF ASSOCIATED BREAST LESIONS

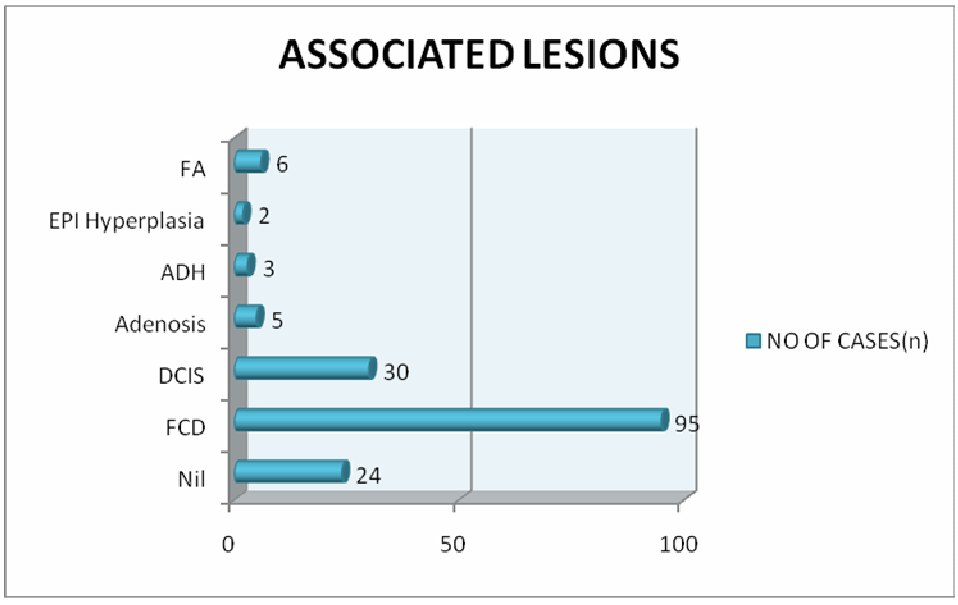


CHART 13

DISTRIBUTION OF ER EXPRESSION IN BREAST CANCERS

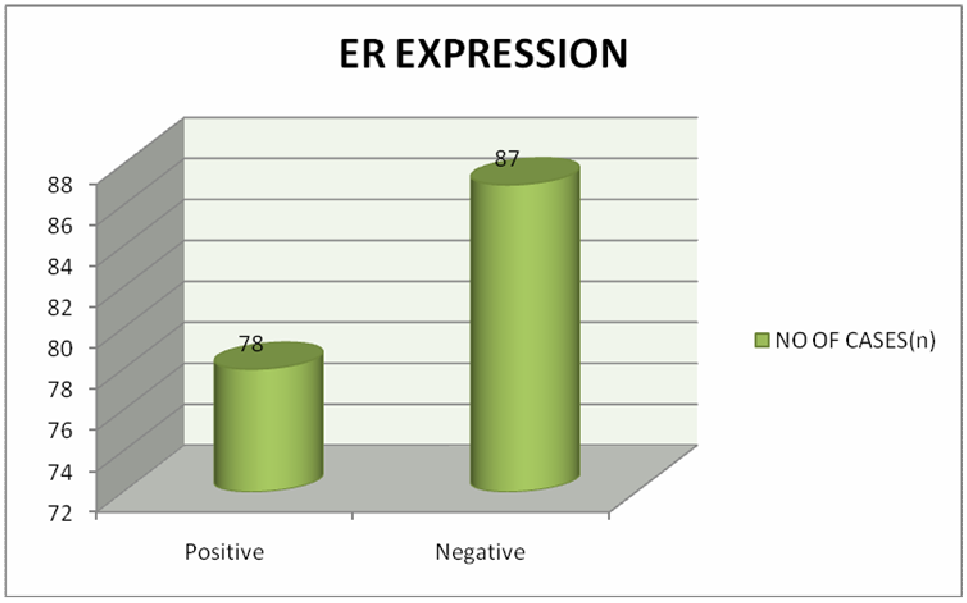


CHART 14

DISTRIBUTION OF PR EXPRESSION IN BREAST CANCERS

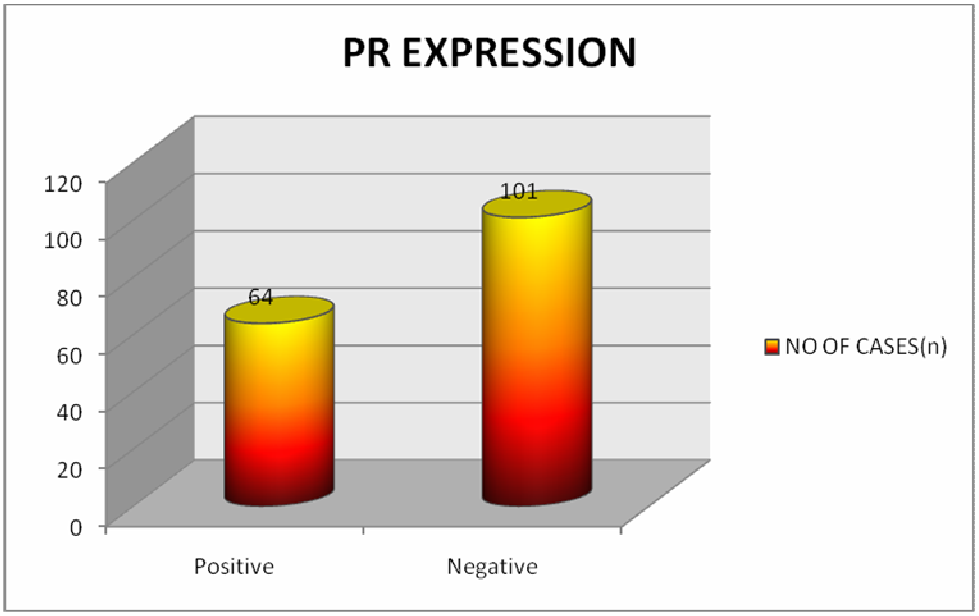


CHART 15

DISTRIBUTION OF HER-2 RECEPTORS IN BREAST CANCERS

CHART 16

MOLECULAR SUBTYPE DISTRIBUTION IN BREAST CANCERS

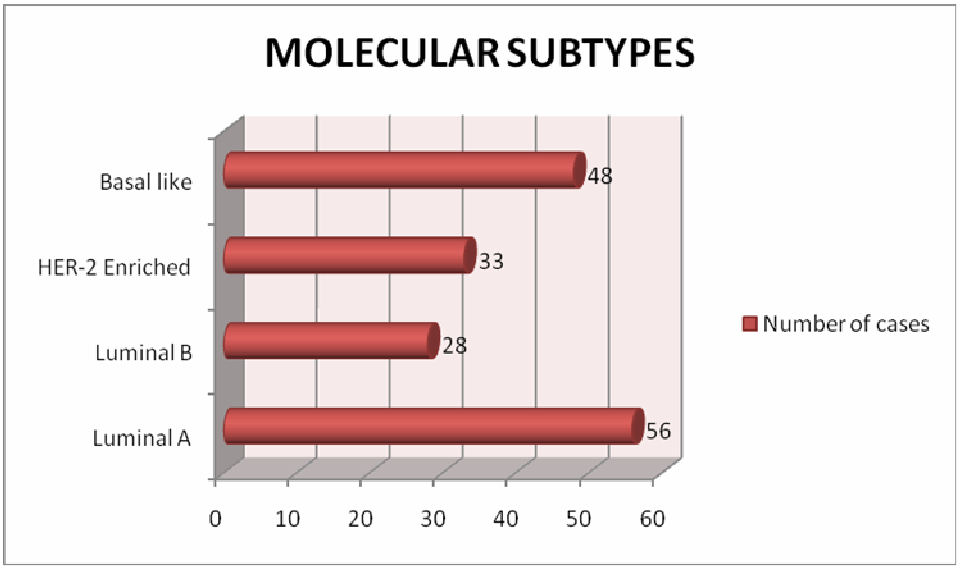
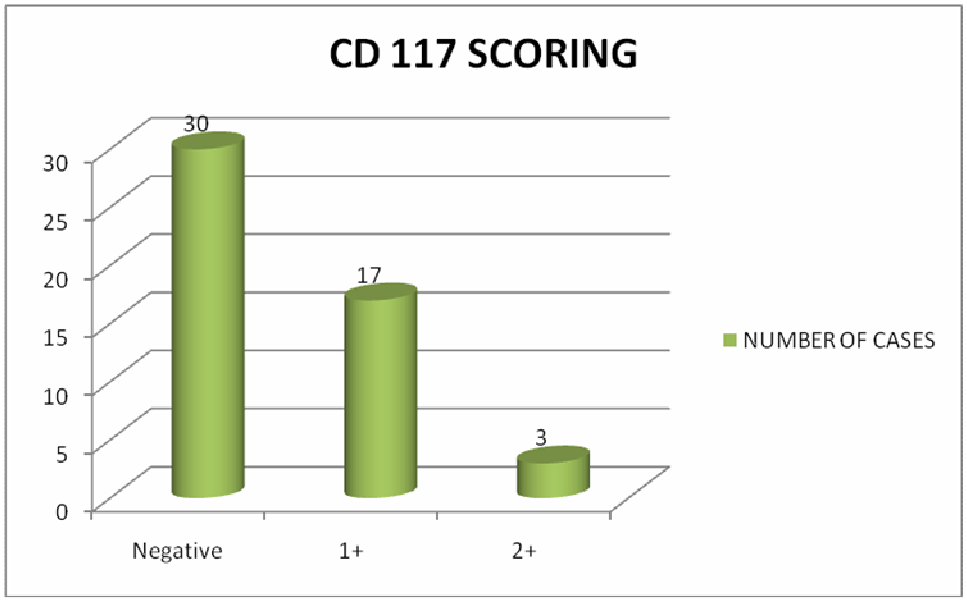


CHART17

CD 117 EXPRESSION SCORING IN BREAST CANCERS



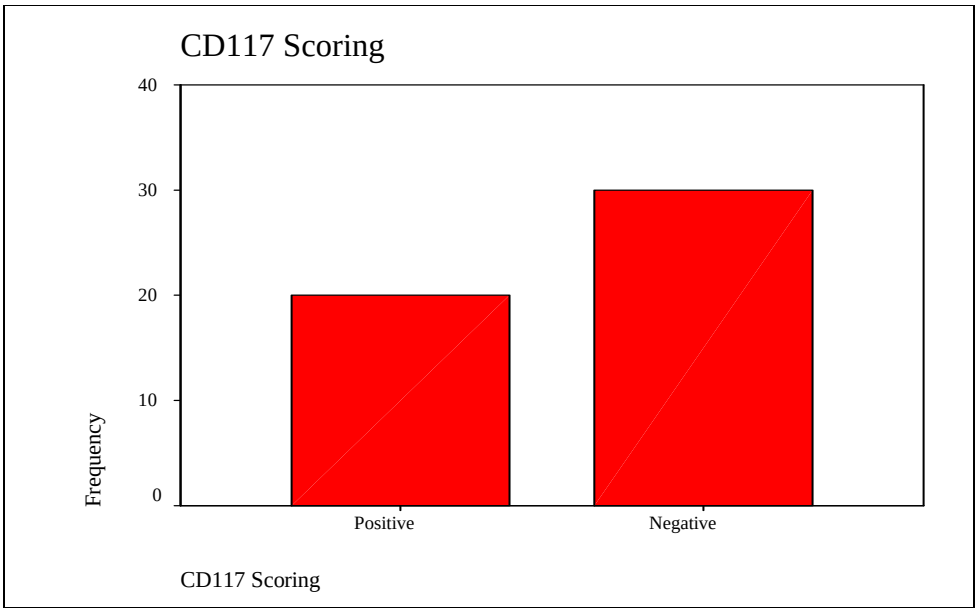


CHART 18

CORRELATION OF C-KIT EXPRESSION AND AGE OF THE PATIENTS

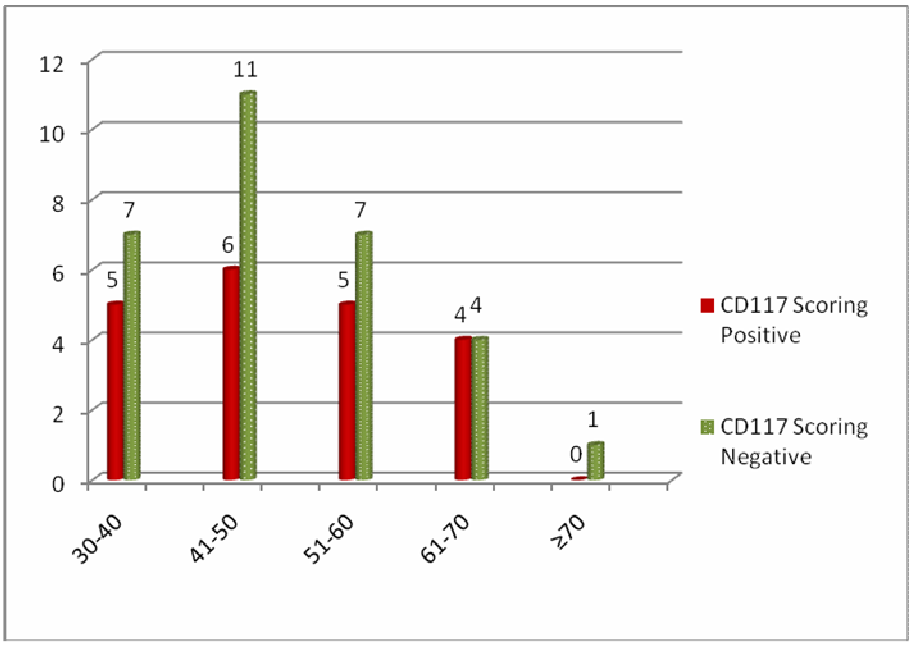


CHART 19

CORRELATION OF C-KIT EXPRESSION AND SIDE OF BREAST INVOLVEMENT

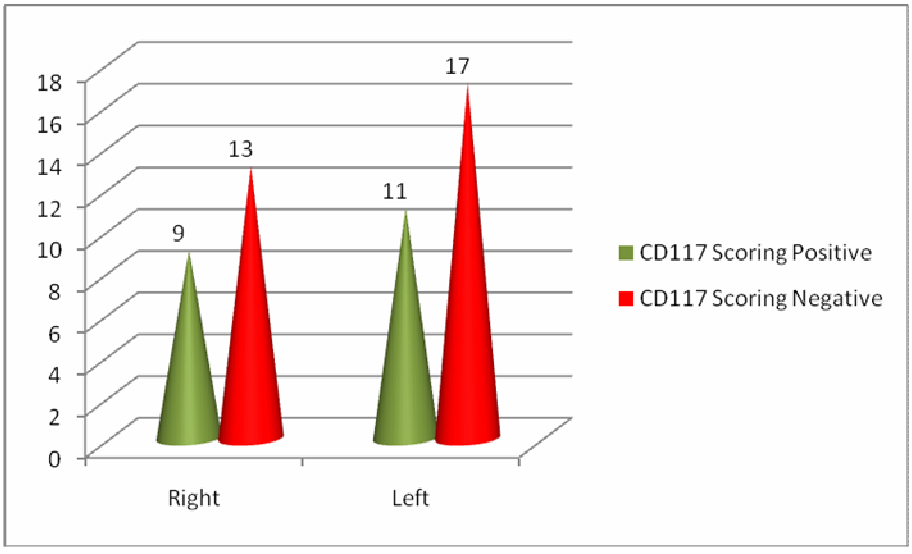


CHART 20

TUMOR LOCATION AND CD117 DISTRIBUTION

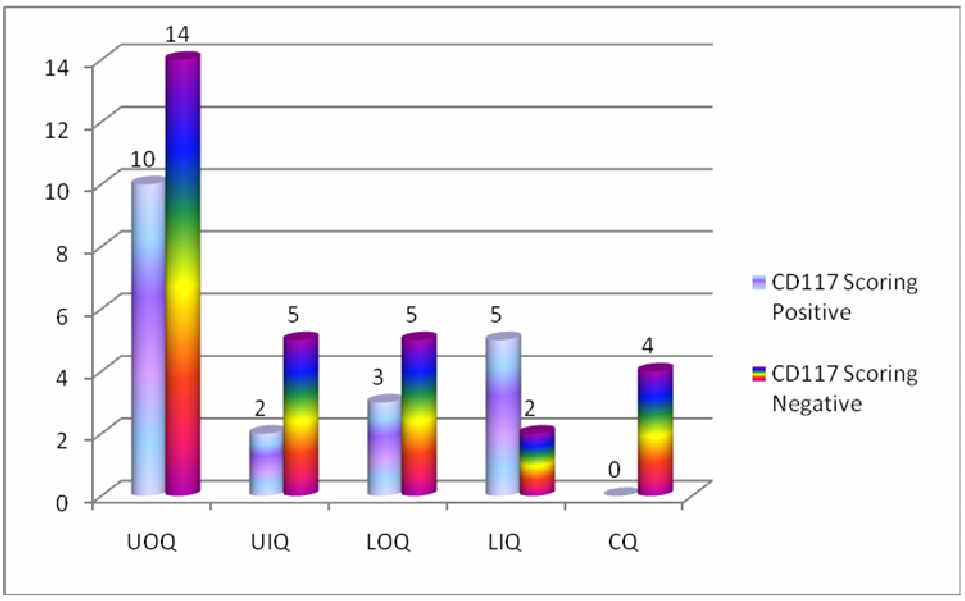


CHART 21

TUMOUR SIZE AND CD117 EXPRESSION

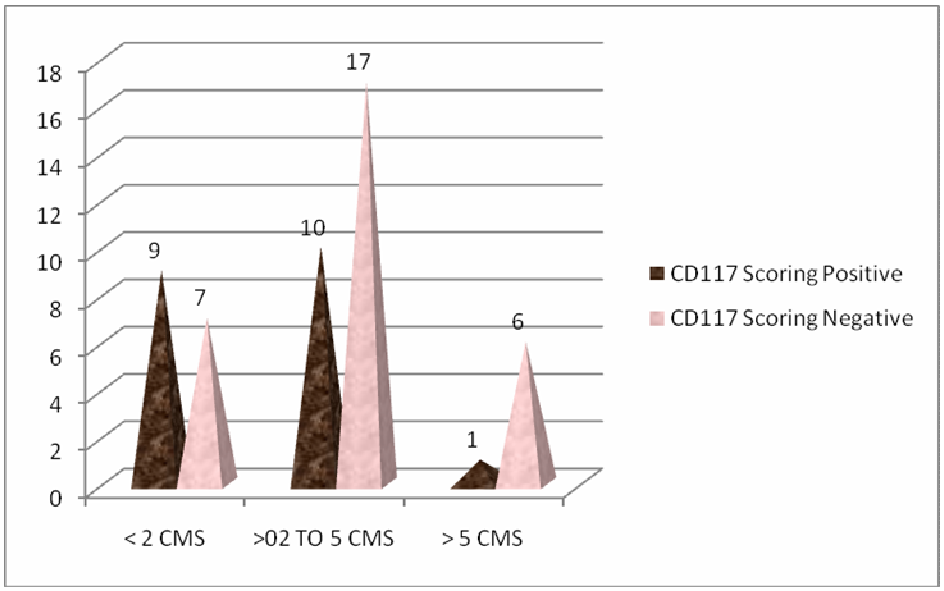


CHART 22

CORRELATION OF HISTOLOGY AND CD117 EXPRESSION

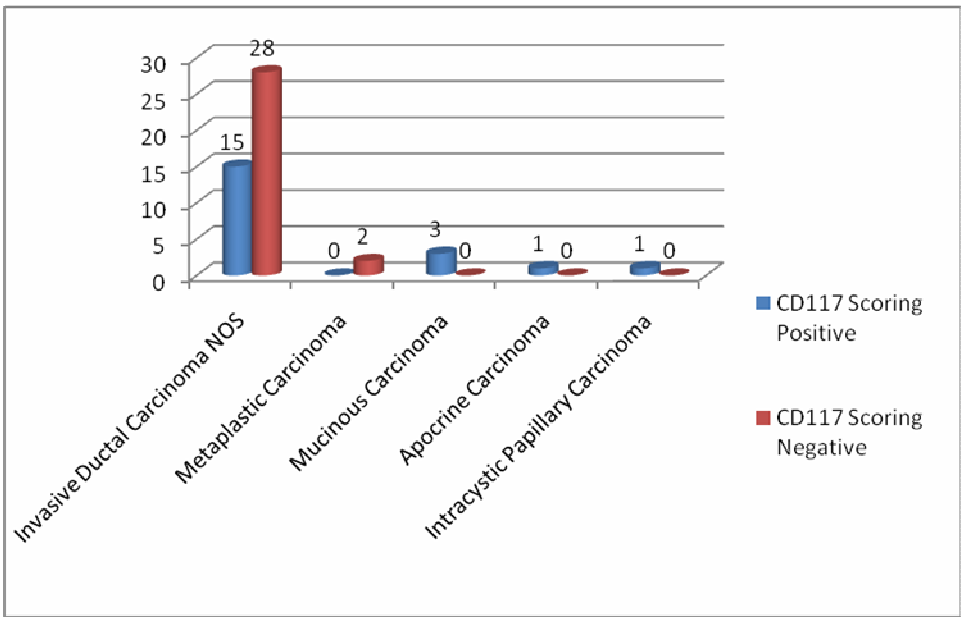


CHART 23

CD117 EXPRESSION CORRELATION WITH GRADES

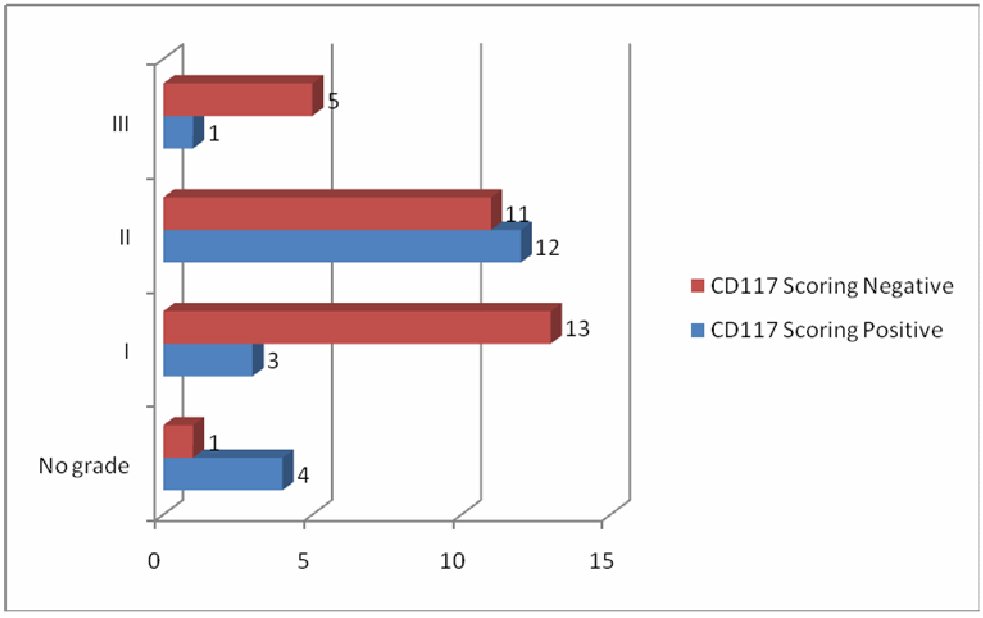


CHART 24

LYMPH NODAL STATUS AND CD117 EXPRESSION

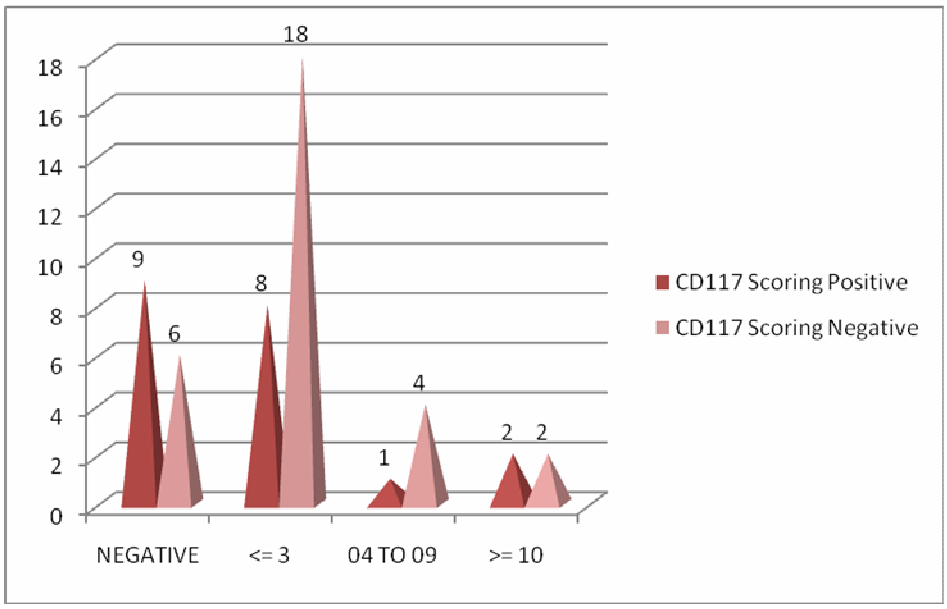


CHART 25

CORRELATION OF CD117 EXPRESSION AND LYMPHOVASCULAR INVOLVEMENT

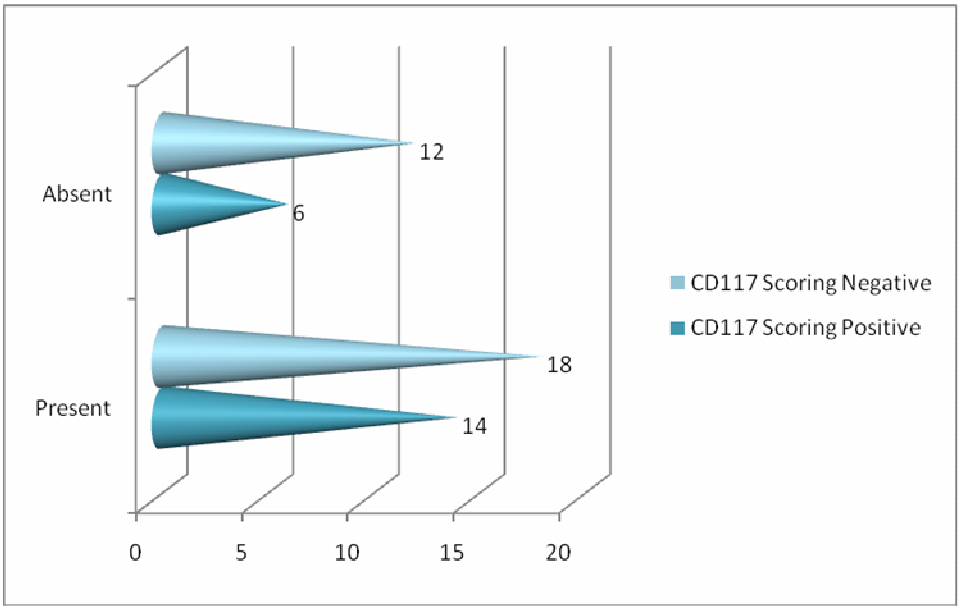


CHART 26

LYMPHOCYTIC INFILTRATION AND CD117 EXPRESSION

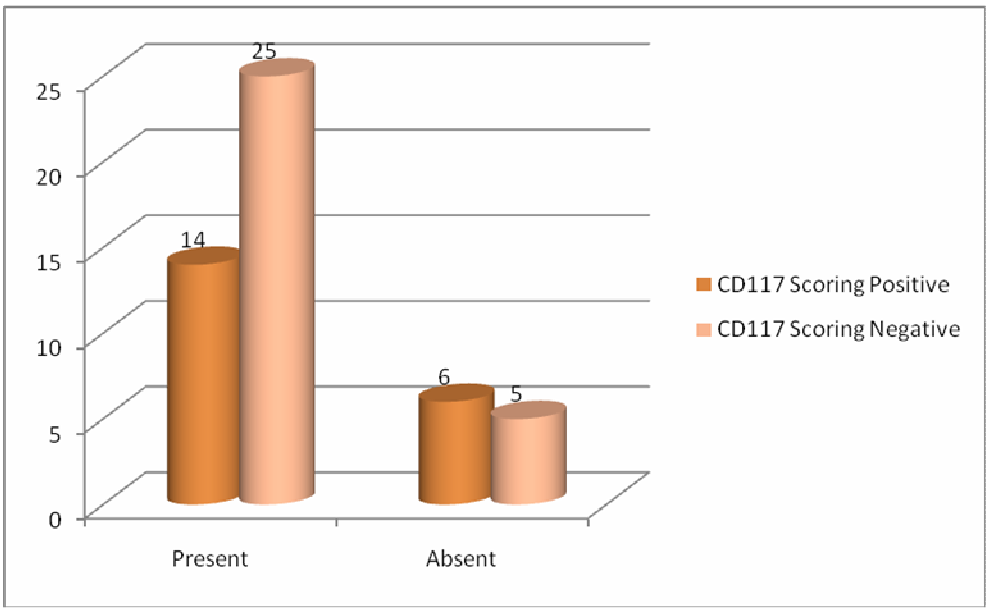


CHART 27

NECROSIS AND CD117 EXPRESSION

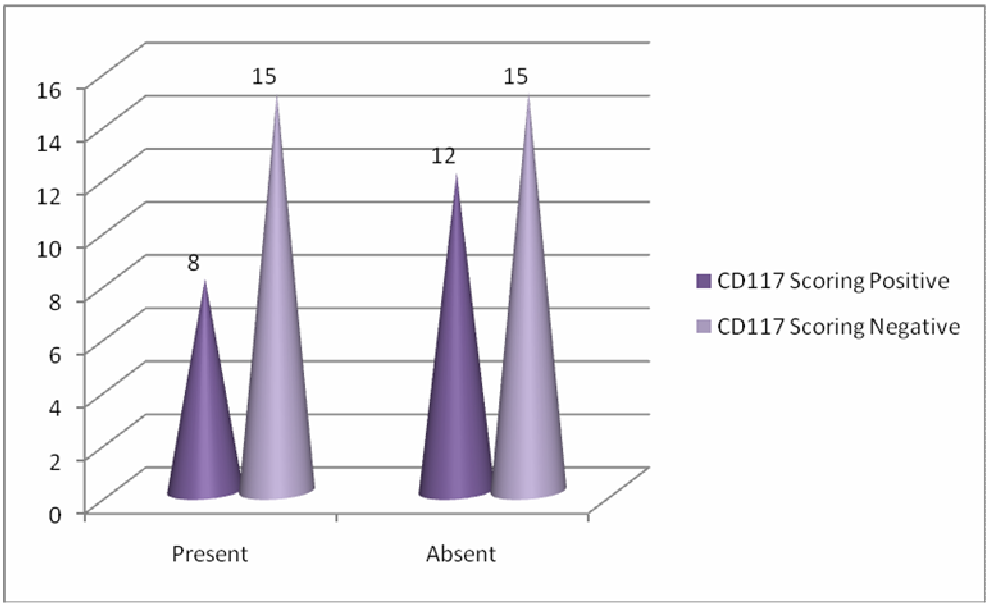


CHART 28

SKIN INVOLVEMENT AND CD 117 EXPRESSION

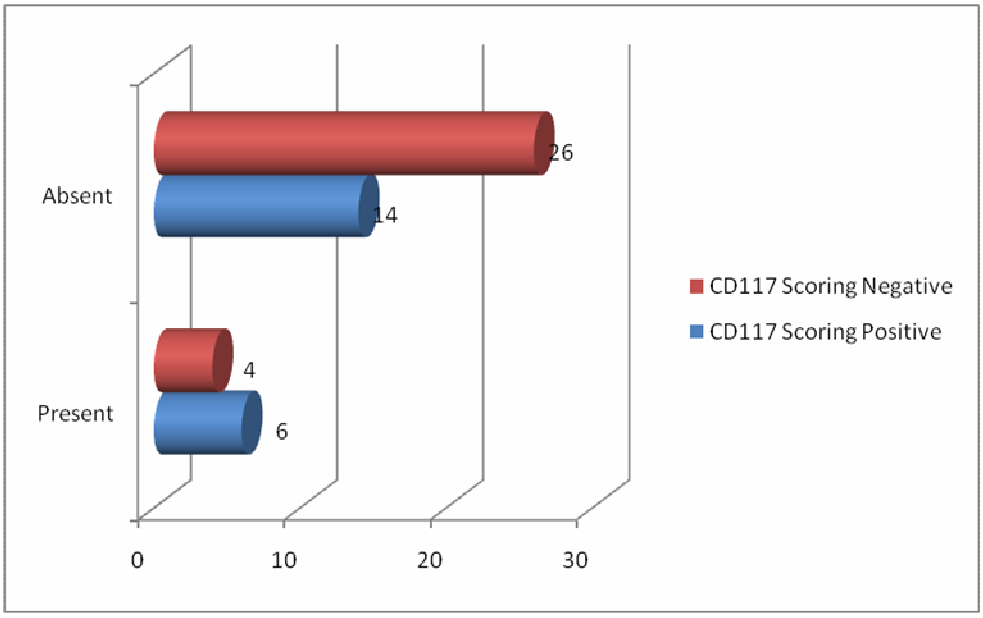


CHART 29

CDII7 EXPRESSIONS IN DCIS COMPONENTS

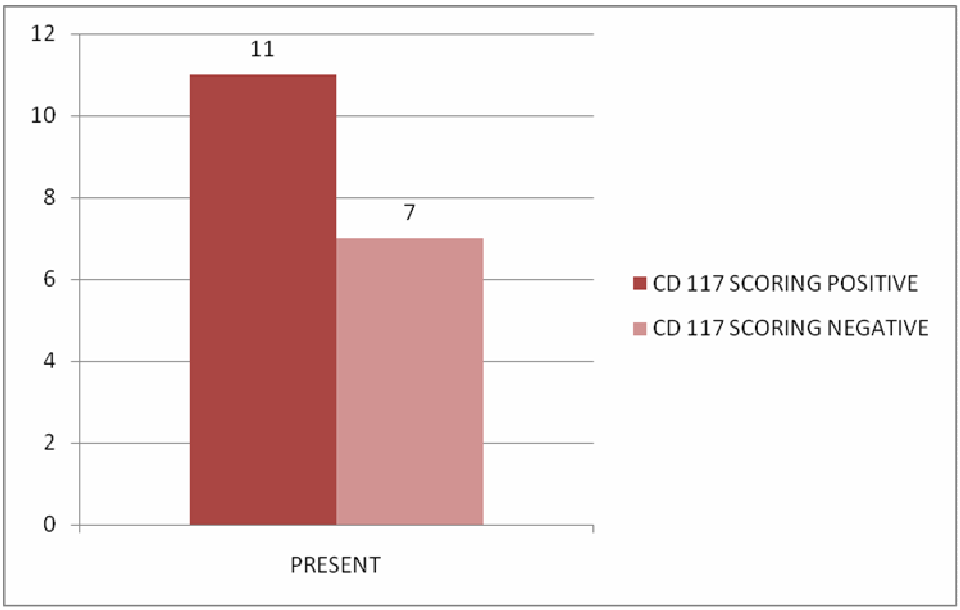


CHART 30

CORRELATION OF CDII7 EXPRESSION WITH ER EXPRESSION

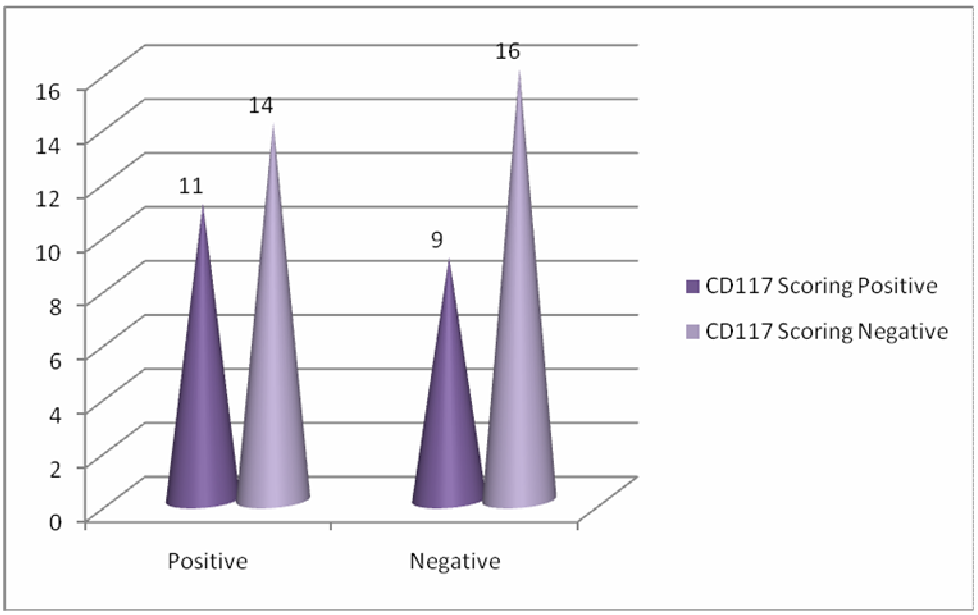


CHART 31

CORRELATION OF CD117 EXPRESSION WITH PR EXPRESSION

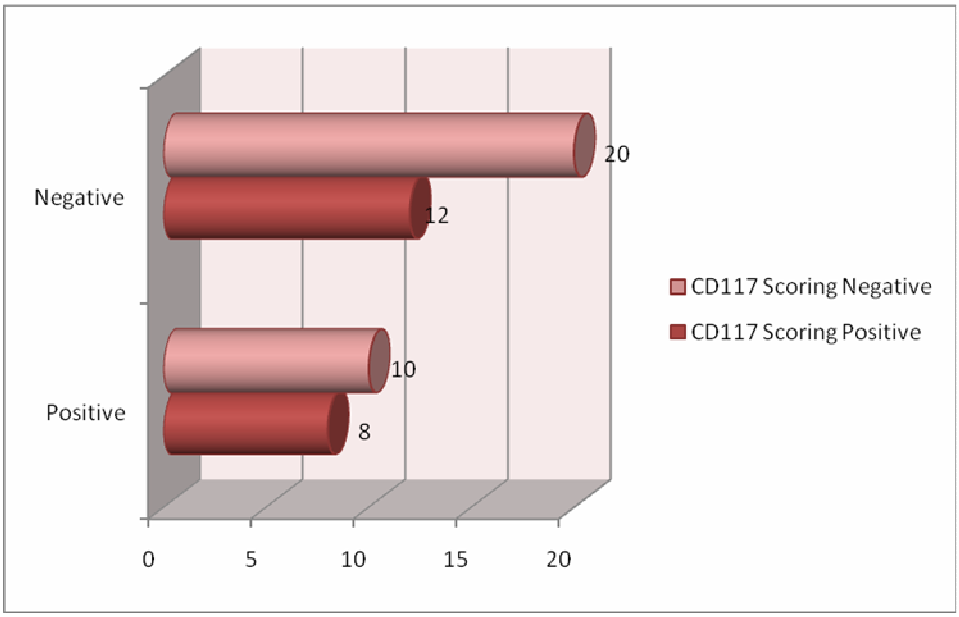


CHART 32

CORRELATION OF HER-2 EXPRESSION AND C-KIT EXPRESSION

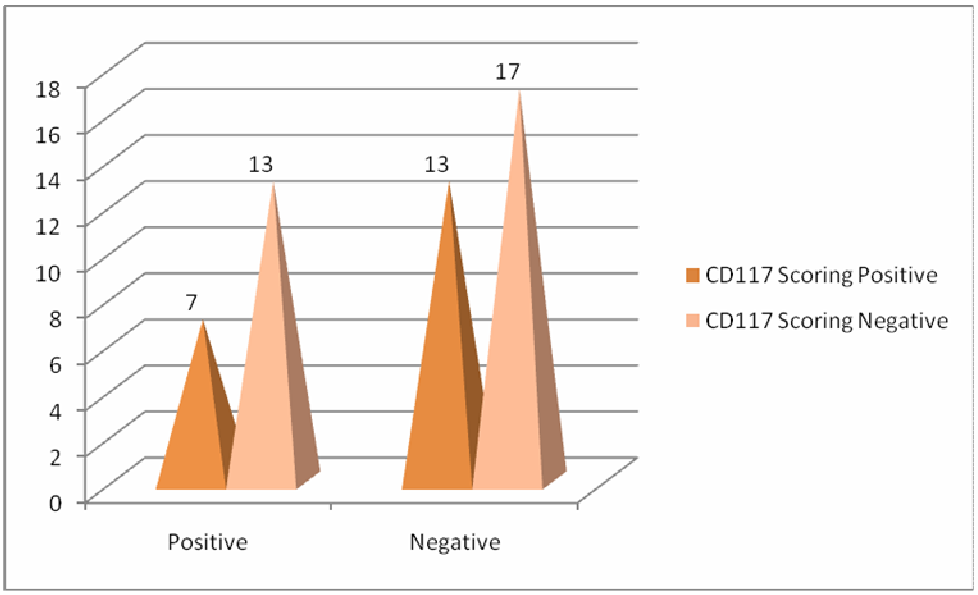


CHART 33

CORRELATION OF CD117 WITH ER AND PR EXPRESSION PROFILE

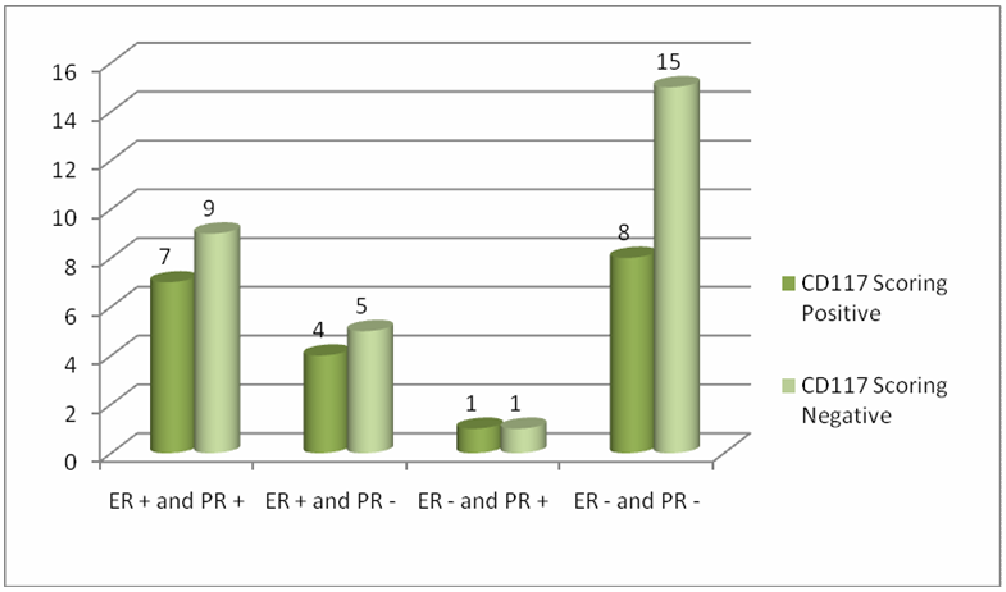
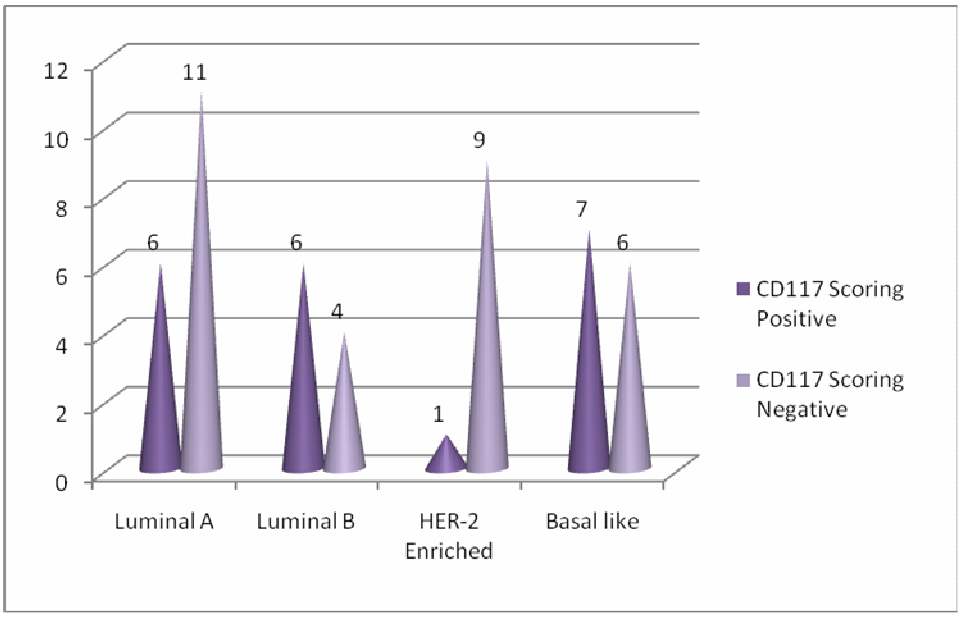


CHART 34

CORRELATION OF CD117 EXPRESSION WITH MOLECULAR SUBTYPES OF BREAST CANCER



COLOUR ATLAS

DUCTAL CARCINOMA BREAST

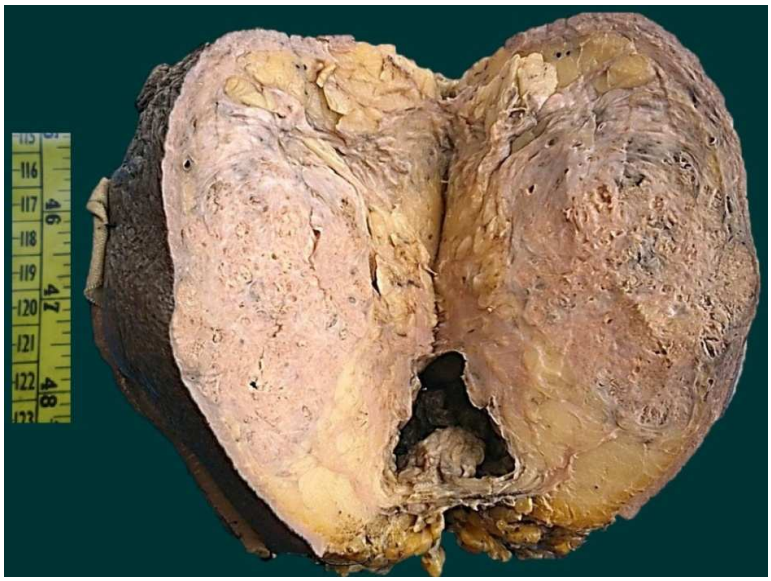


Figure.6. Grey white firm growth with irregular margins

IDC GRADE 1

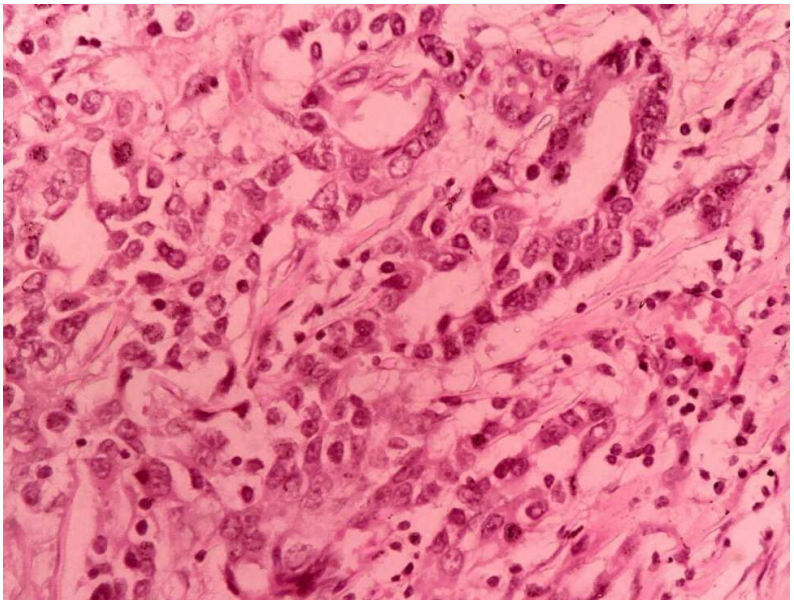


Figure.7.malignant duct epithelial cells arranged in tubules with mild nuclear pleomorphism (400x)

IDC GRADE 2

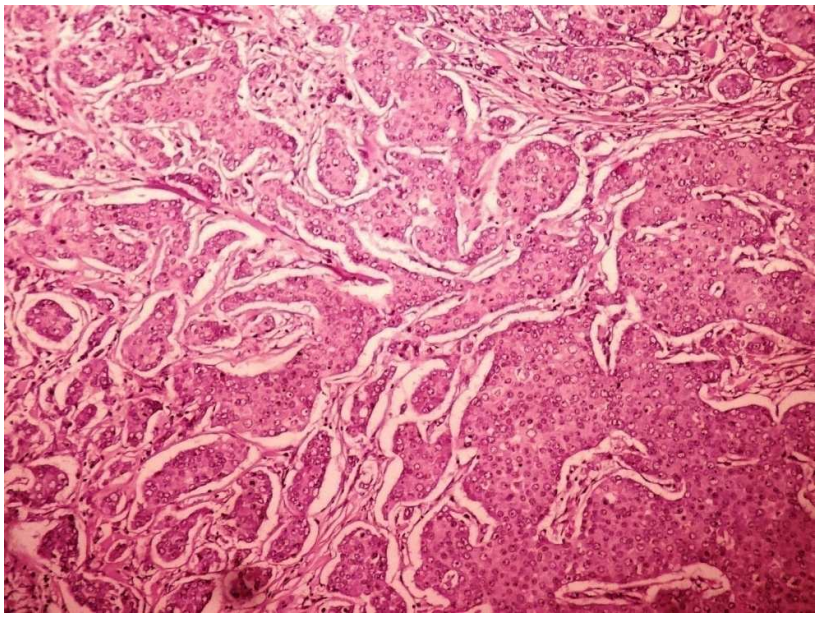


Figure.8.malignant duct epithelial cells in tubules and sheets with moderate nuclear pleomorphism(100X)

IDC GRADE 3

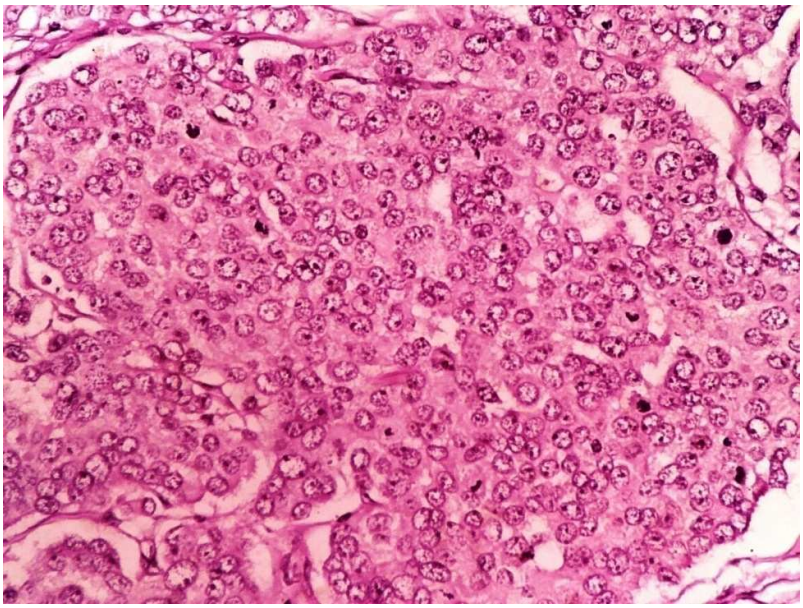


Figure.9.malignant duct epithelial cells in sheets with marked nuclear pleomorphism and increased mitosis(400x)

MUCINOUS CARCINOMA

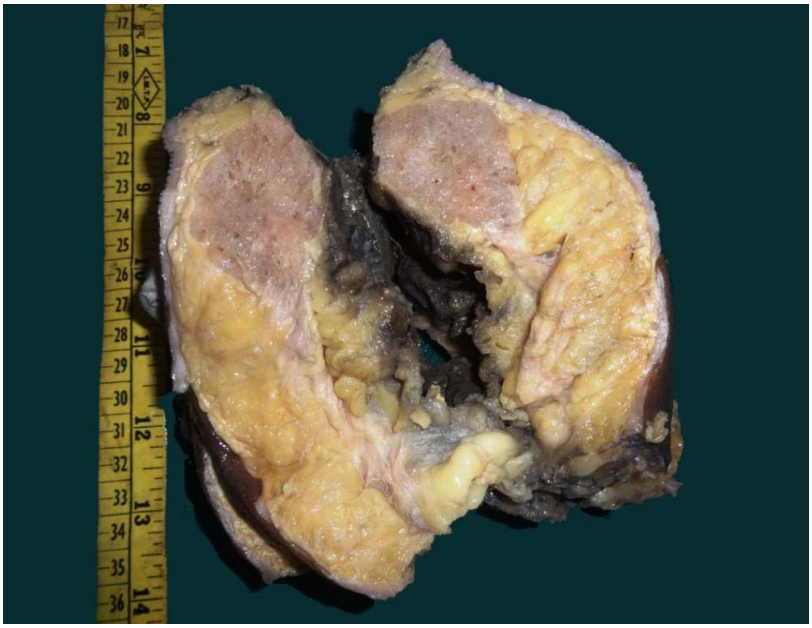


Figure.10. Well circumscribed glistening gelatinous growth

MUCINOUS CARCINOMA

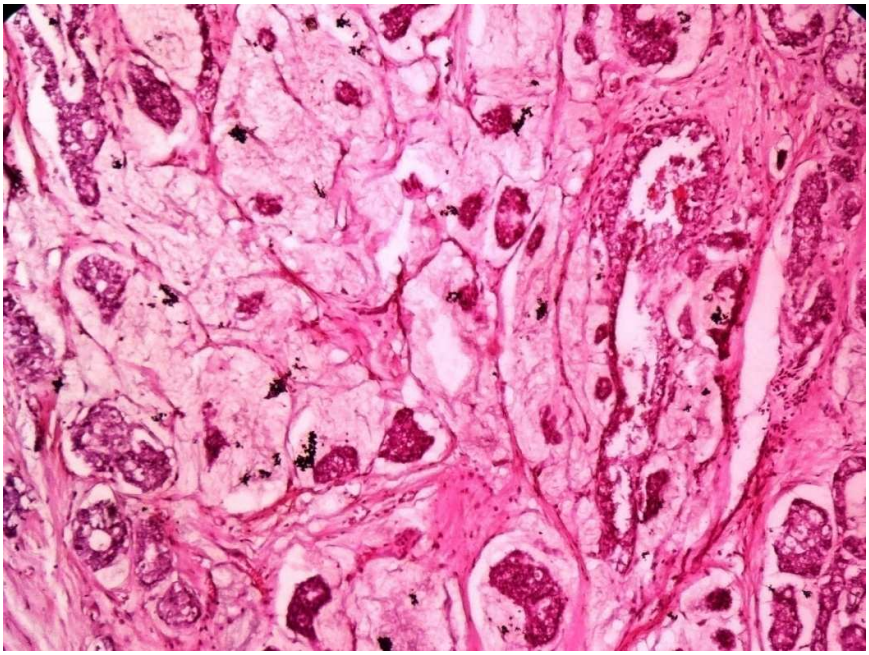


Figure 11. Tumor nests floating in pools of mucin(400x)

APOCRINE CARCINOMA

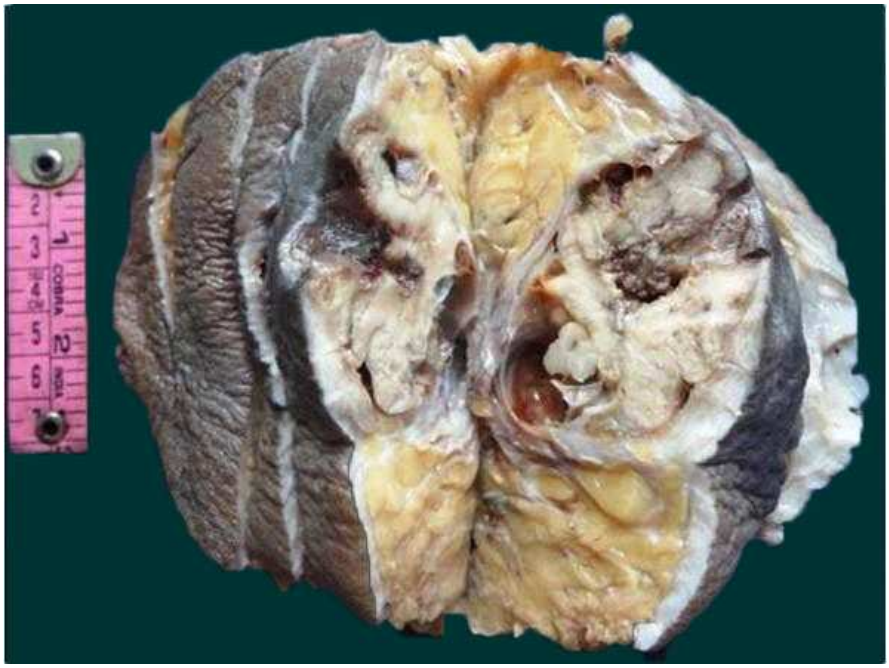


Figure. 12.Well circumscribed grey white growth with cystic degeneration and hemorrhage

APOCRINE CARCINOMA

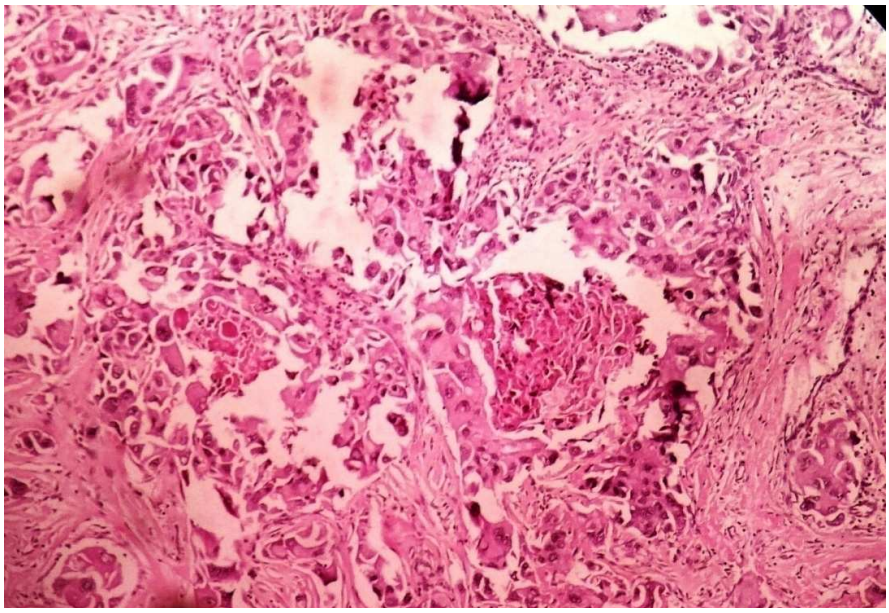


Figure.13. Apocrine cells with abundant granular eosinophilic cytoplasm (100x)

METAPLASTIC CARCINOMA

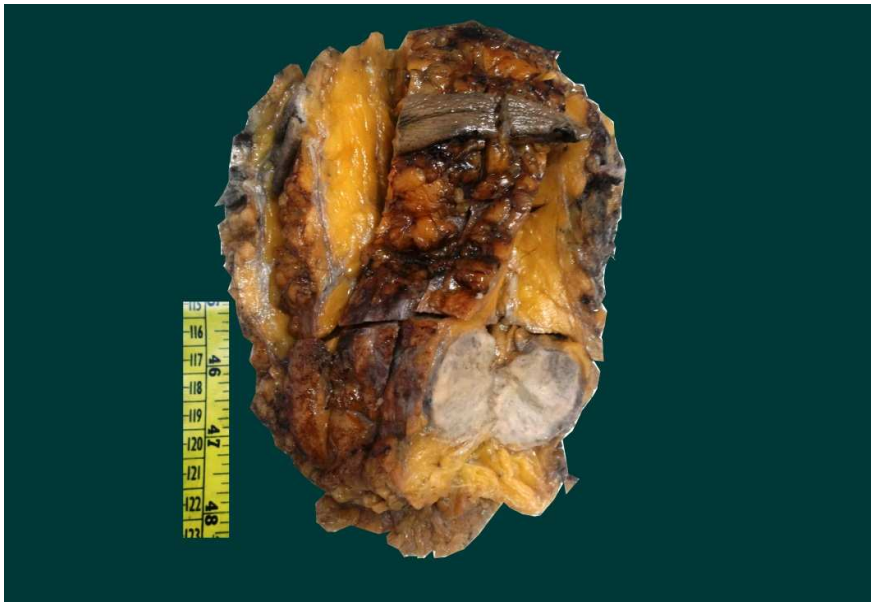


Figure .14.Well circumscribed grey white firm growth

METAPLASTIC CARCINOMA

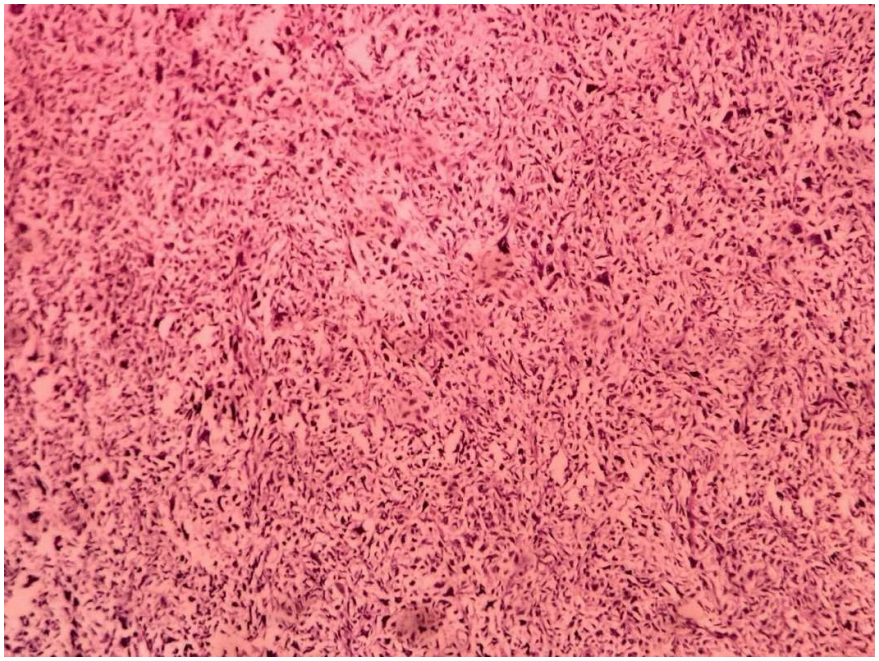


Figure.15.malignant spindle cells with multinucleated giant cells(100x)

PAPILLARY CARCINOMA

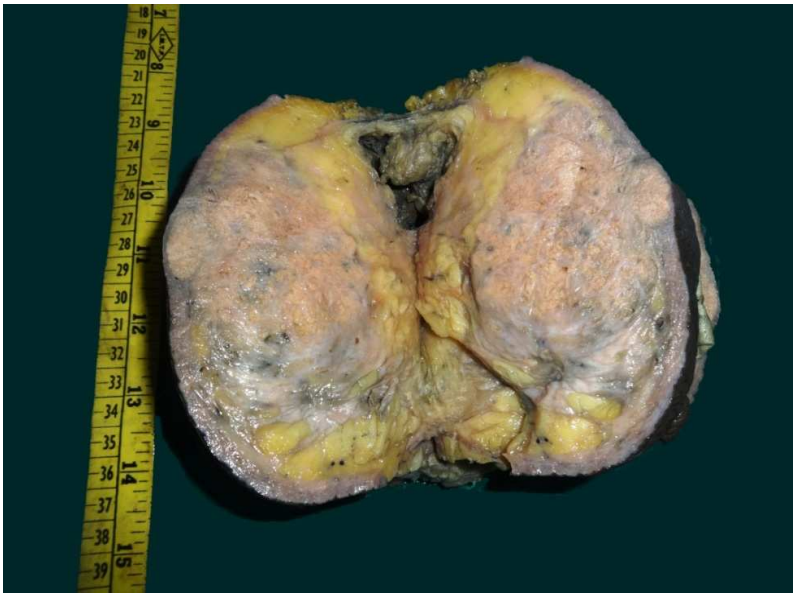


Figure 16. Well circumscribed grey white growth with granular surface

INTRA CYSTIC PAPILLARY CARCINOMA

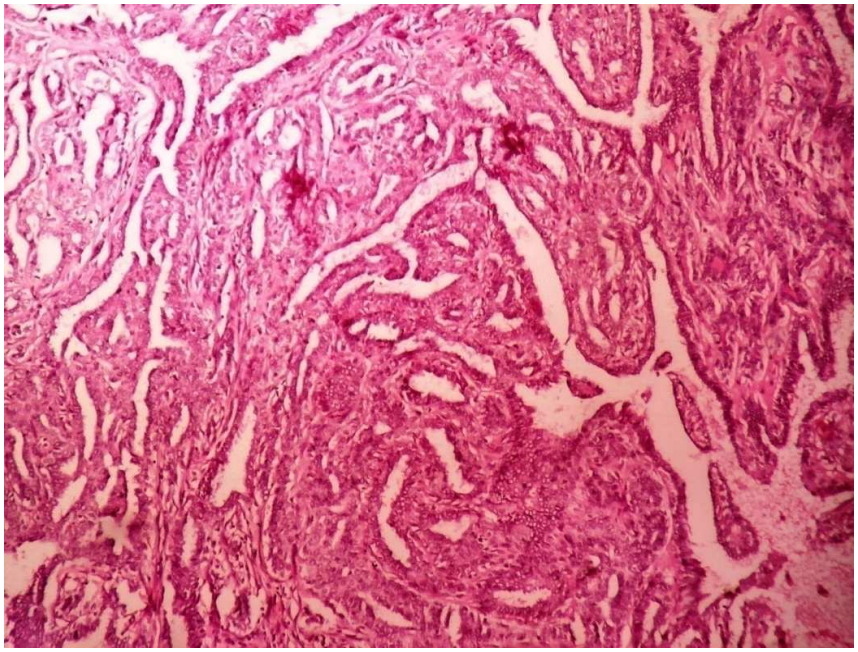


Figure 17.microscopy -100x

INTRACYSTIC PAPILLARY CARCINOMA

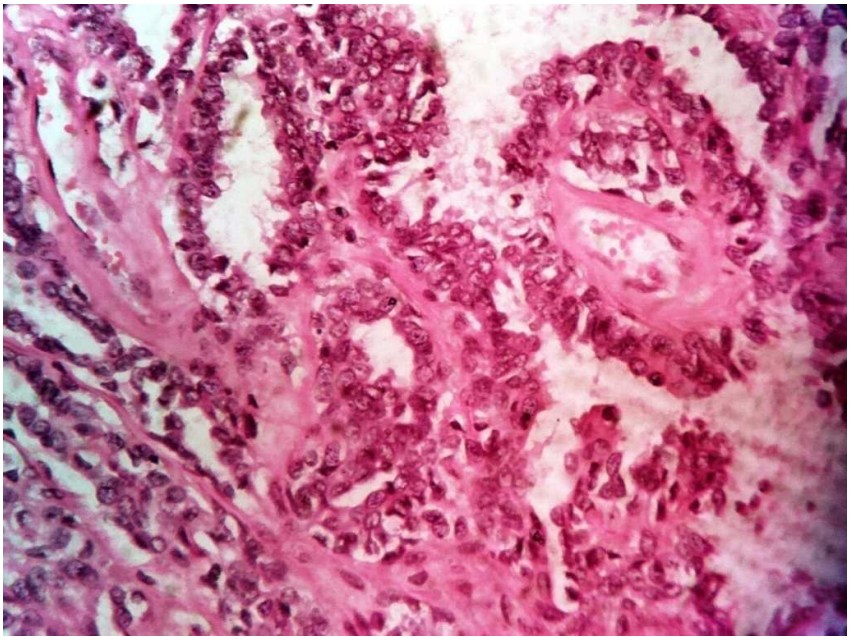


Figure 17. Tumor cells in delicate papillary pattern(400x)

METASTATIC DEPOSIT IN LYMPH NODE

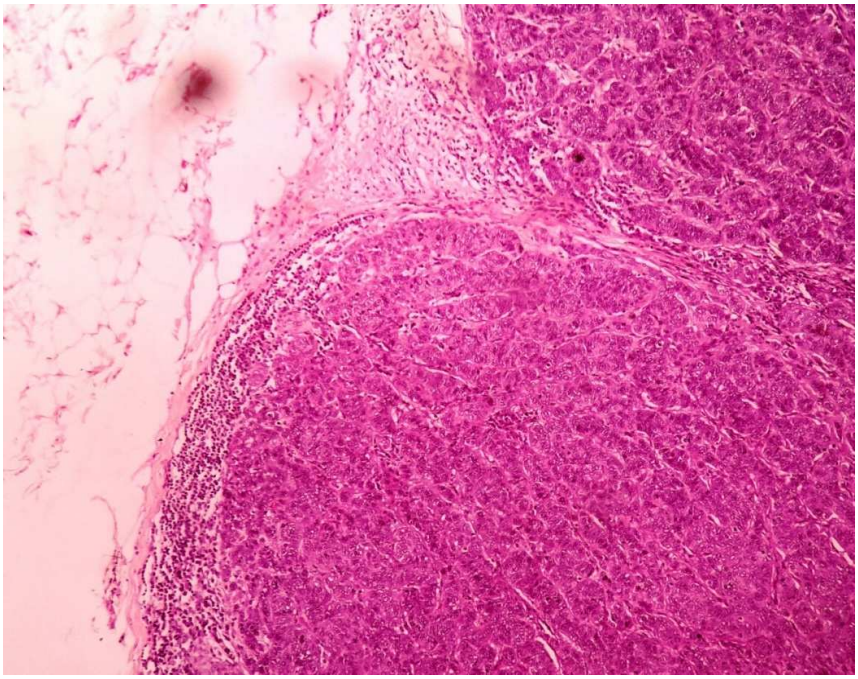


Figure 18. microscopy 100X

LYMPHOVASCULAR INVASION

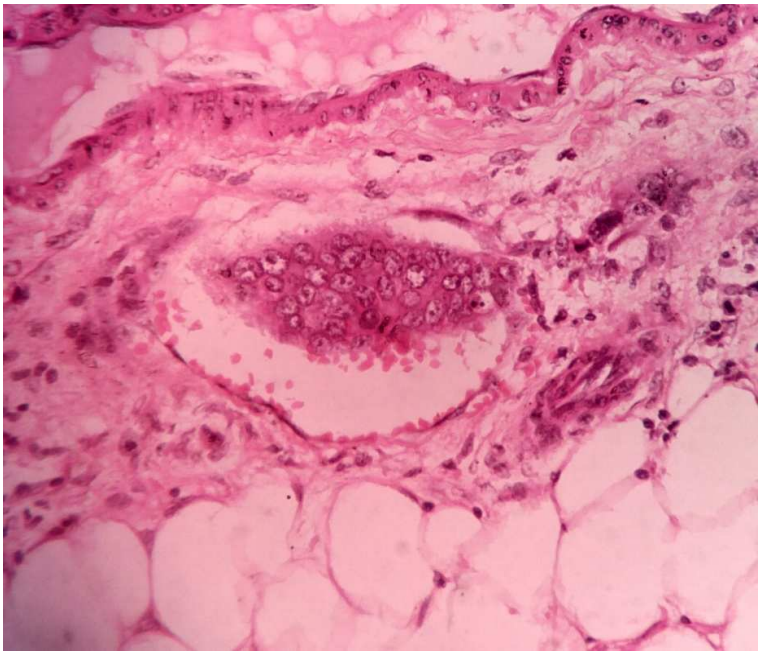


Figure 19.microscopy 400X

LYMPHOCYTIC INFILTRATION

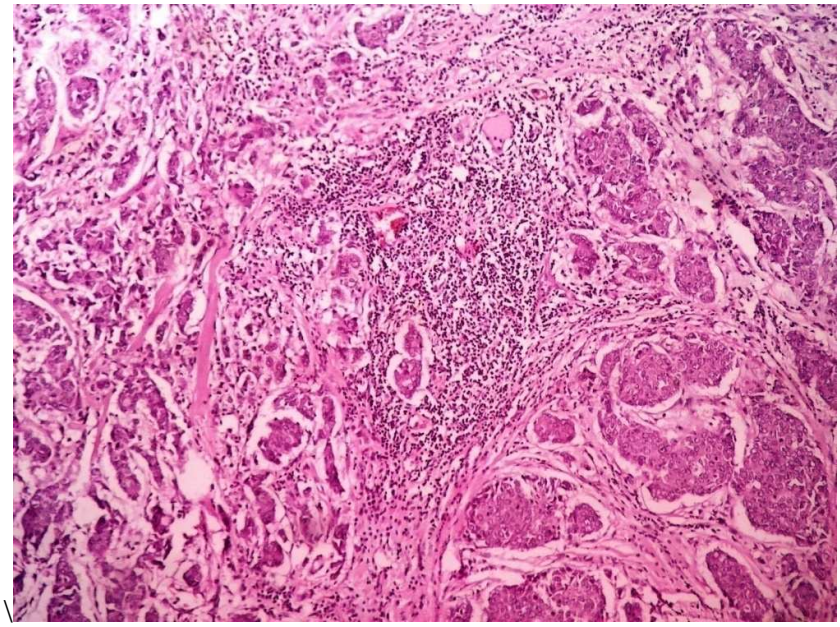


Figure 20.microscopy 100X

ESTROGEN RECEPTOR EXPRESSION

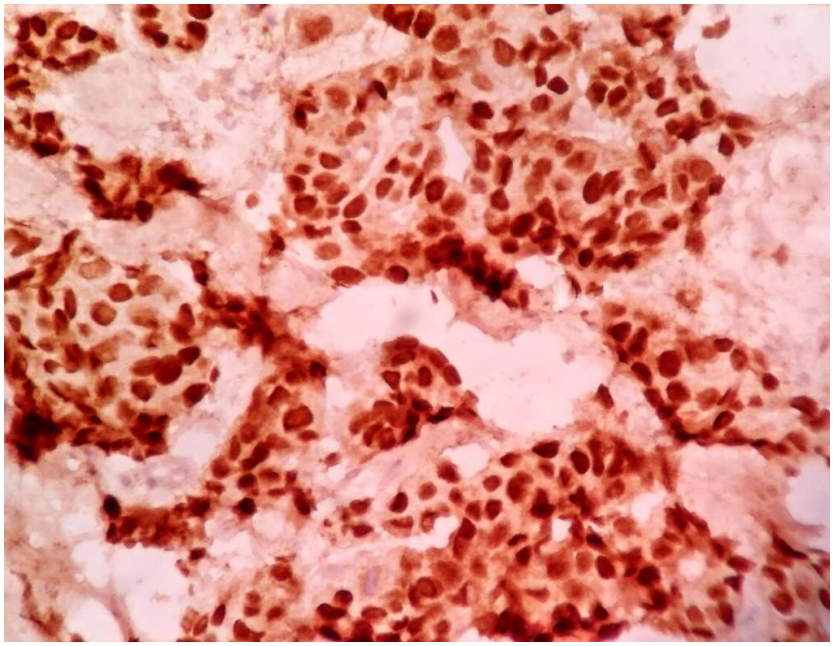


Figure 21.Invasive ductal carcinoma nos. Positive nuclear staining (5+3) for ER

PROGESTERONE RECEPTOR EXPRESSION

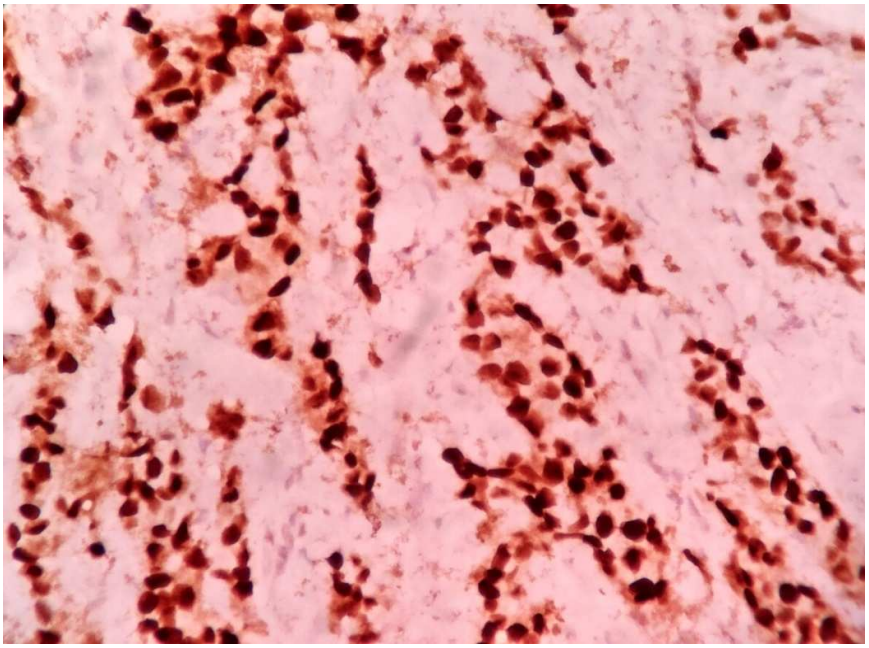


Figure 22.Invasive ductal carcinoma nos. Positive nuclear staining (5+3) for PR

INVASIVE DUCTAL CARCINOMA-NOS

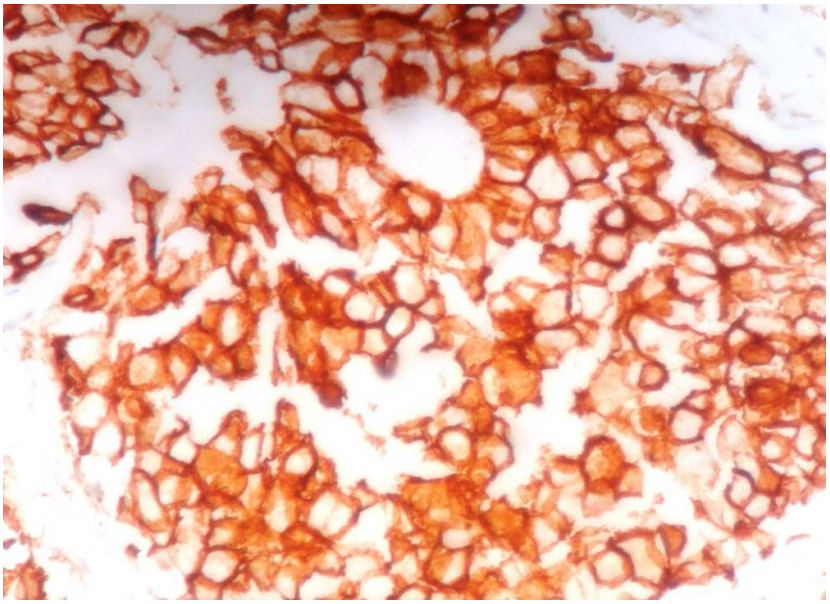
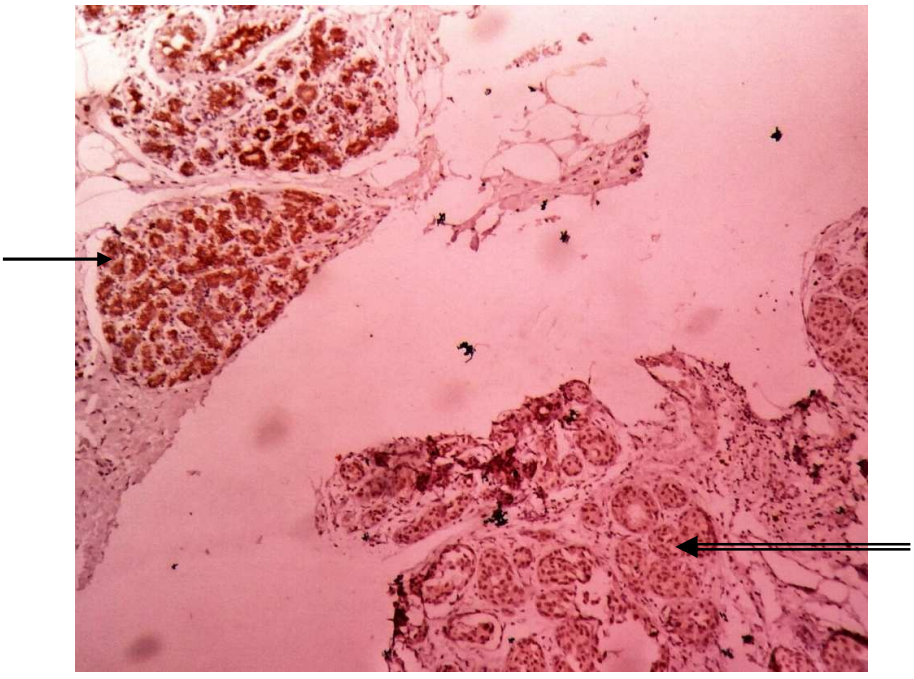


Figure-23. Cytoplasmic positivity (3+) of HER2neu



→ Normal Breast Epithelium

⟹ IDC NOS

FIGURE 24. C-kit expression in normal breast epithelium and IDC NOS

INVASIVEDUCTALCARCINOMANOS.

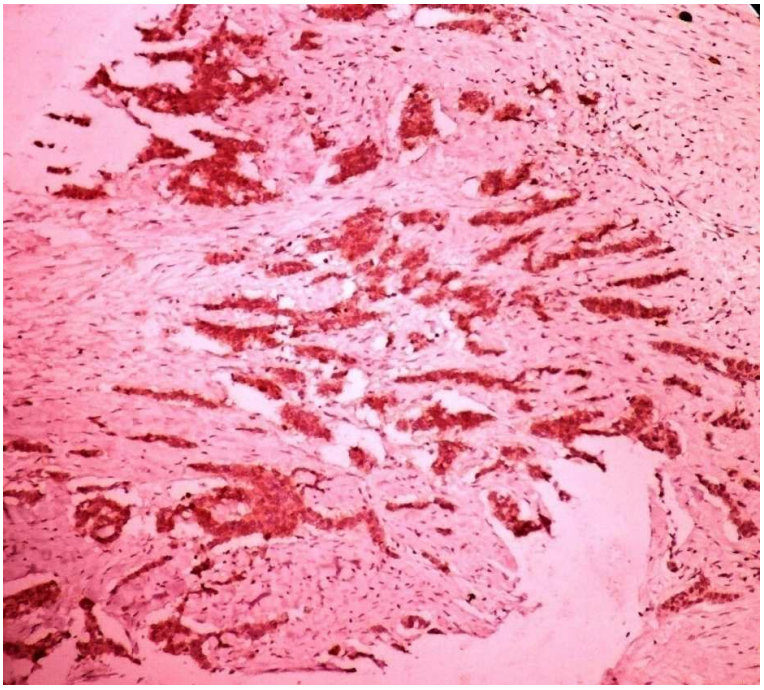


FIGURE 25. Positive staining (2+) for CD 117

INVASIVE DUCTAL CARCINOMA NOS.

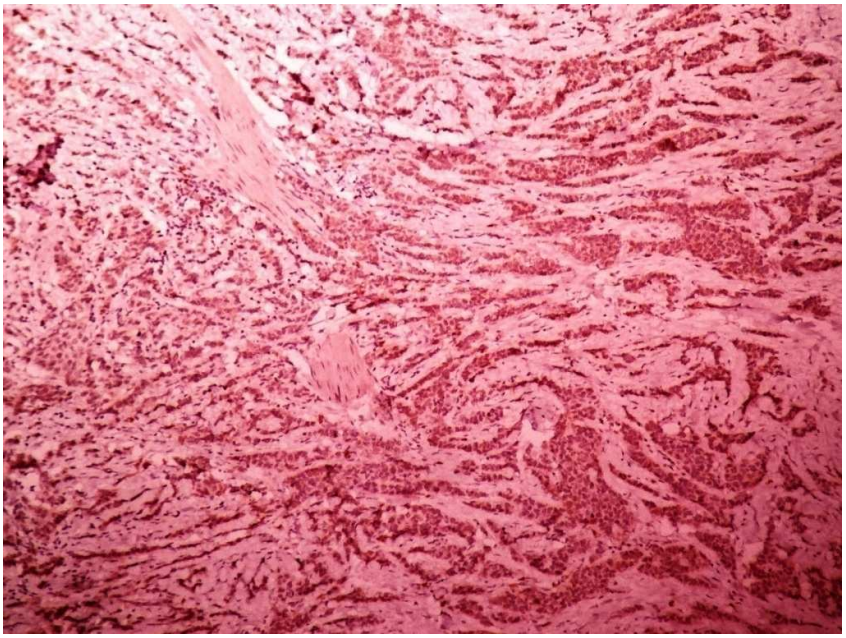


FIGURE 26. Positive staining (1+) for CD 117

MUCINOUS CARCINOMA OF BREAST.

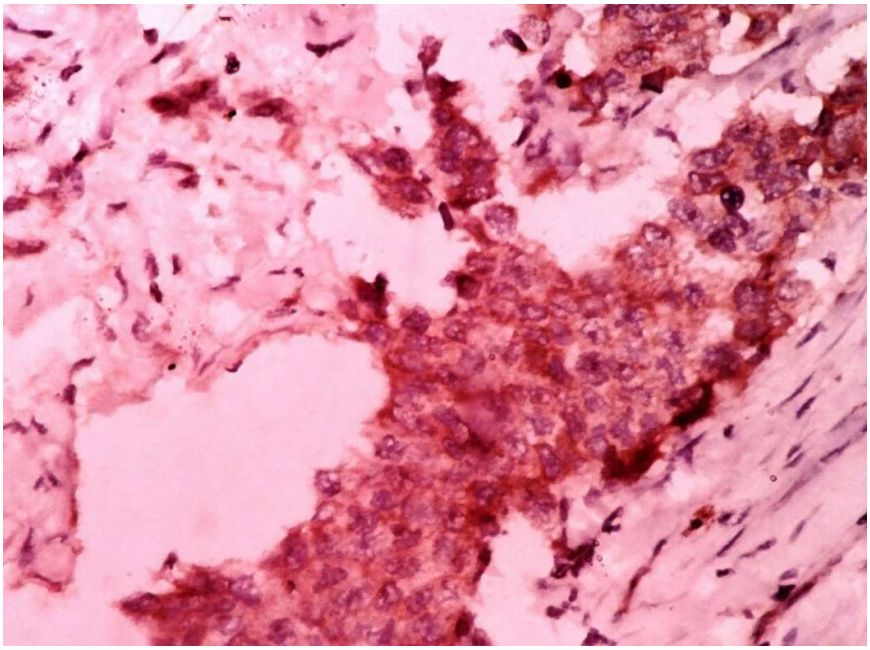


FIGURE 27. Positive staining (2+) for CD 117

APOCRINE CARCINOMA OF BREAST.

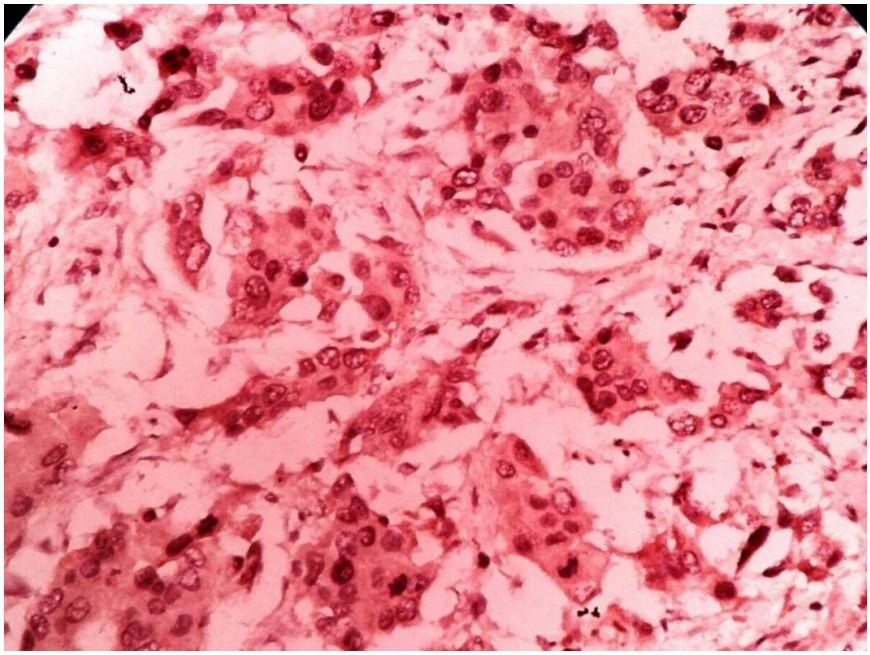


FIGURE 28. Positive staining(2+) for CD 117

INTRACYSTIC PAPILLARY CARCINOMA OF BREAST

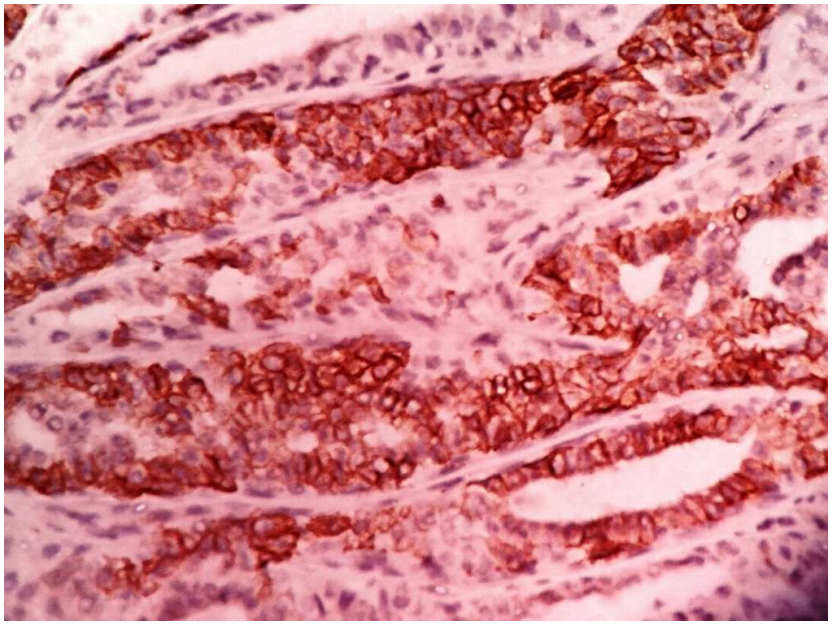


Figure 29. Positive staining (2+) for CD 117

METAPLASTIC CARCINOMA OF BREAST

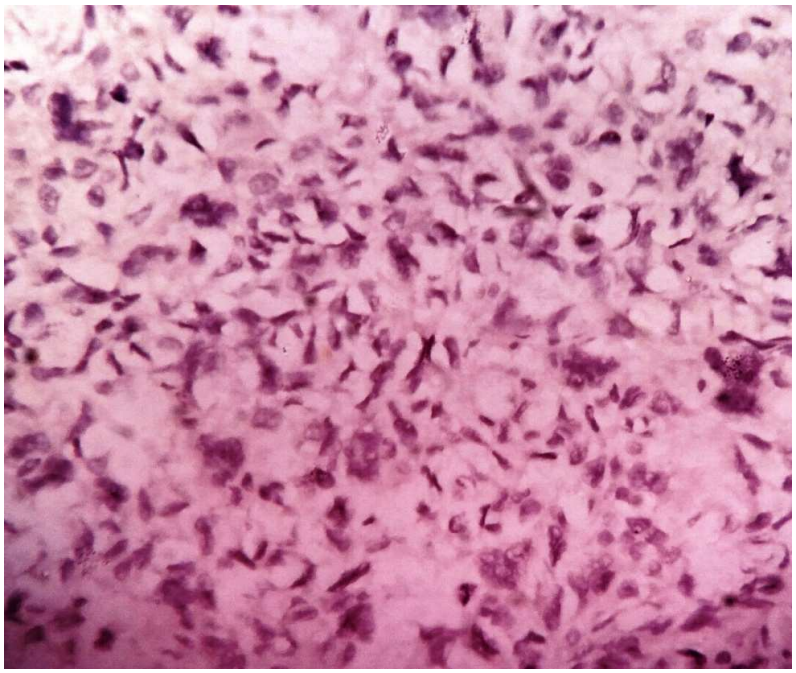


FIGURE 30. Negative for CD 117

DUCTAL CARCINOMA INSITU

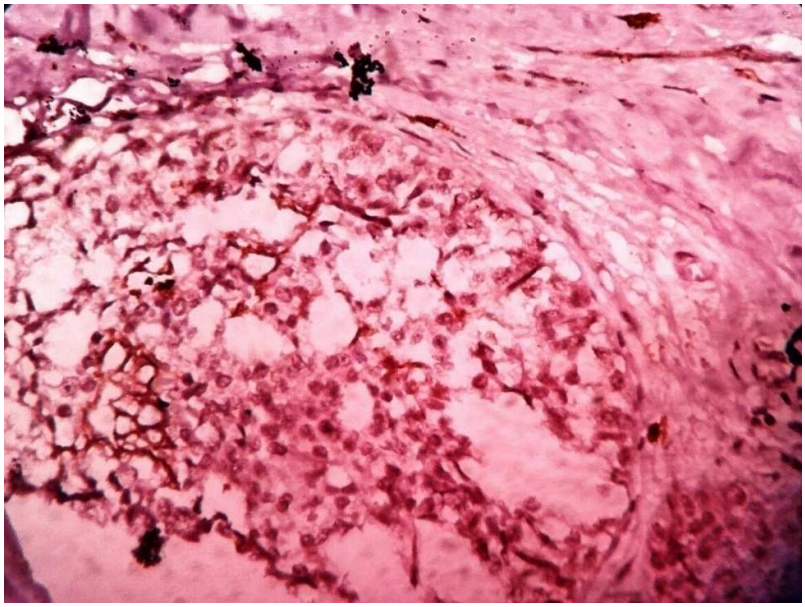


Figure 31 . Positive staining for CD 117

DISCUSSION

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

We can reduce the mortality of breast cancer by early detection, appropriate management and targeted therapies. Many theories underlie the pathogenesis of breast cancer and there are many prognostic factors. Apart from the prognostic factors like stage, grade, Lymph node status, ER, PR and HER2neu, many newer markers are under study. One such attempt was made to detect expression of C-KIT (CD117).

In this present study, Immunohistochemistry was done in 50 cases and the expression was correlated with the clinicopathological factors.

Madras Medical College being a tertiary care center, among the surgical specimens received breast specimens include 6.33 % of all cases. Malignant breast tumors constituted 40.5% of all the breast specimens received.

The youngest age presented with invasive ductal carcinoma was 30 years and oldest age group reported was 75 years with 49 as median age of presentation. This was compared with study by Micello et al,[124]Carreno et al,[125] Hu et al, [126]Honma et al [127]showed that in India there is a change in trend towards younger age group in the recent years. (TABLE 37) The highest

incidence of breast cancer was reported in 41 to 50 years age group. This is in concurrence with the study done by Rajesh Singh Laishram et al. [128]

Table-37: Comparison of Median age

	Median age of presentation
Micello et al.2010 [124]	58.7
Carreno et al.2007 [125]	61
Hu et al.2011 [126]	61
Honma et al.2012 [127]	56
Current study	49

Among the histological types, Invasive ductal carcinoma NOS type comprised the most common with 93.3%.This correlated with the study done by Albrektsen et al, [129] Shirley SE et al [130]and AM Dauda et al. [131]The incidence of IDC NOS type is higher in Indian women (89.62%) compared to that of western women accounting for the poor prognosis (Table38)

Majority of the breast carcinomas were T2 stage which is similar to the study done by Lakmini et al. But comparing with the study by Christine L [132] and Carter et al the proportion of T2 in Indian population (70.28%) is higher than in Western population (55.4%). (Table 39)

Table38: Comparison of distribution of histological subtypes of breast cancers

Histological subtypes	AM Dauda et al[131]	Shirley SE et al [130]	Albrektsen et al[129]	Current study
Invasive ductal carcinoma NOS	78.8%	69.3%	81.4%	93.9%
Mucinous carcinoma	2.4%	3.6%	2%	3.0%
Intracystic Papillary carcinoma	4.2%	3.5%	-	0.6%
Metaplastic carcinoma	2.4%	1.3	-	1.80 %
Apocrine carcinoma	-	-	-	1.89%

Table-39: Comparison of size of tumors (%)

Size	Christine L. Carter et al [132]	E F S Al-Joudi et al [133]	Lakmini et al [134]	Current study
T1	33.6	3.14	14.5	23.0%
T2	55.4	19.37	74	60.0%
T3	11	77.49	11.5	17.0%

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 [135] ,Carey et al [136]and G G Vanden Eynden et al [137](Table40).

Table 40: Comparison of grade of tumor (%)

Grade	Qiu J et al [135]	Carey et al [136]	G G Vanden Eyndenetal [137]	Current study
Grade I	33.3	25	32.63	27.3%
Grade II	54	26	36.84	56.4%
Grade III	12.7	49	30.53	12.1%

Fifty eight percent of the cases showed lymph node metastasis and 37.6% cases with 1-3 nodes positive. These data coincides with the study done by Jun Qiu et al [135] and S E Shirley et al [130] who reported nodal metastasis in 60.32% and 75.7% of their cases.

In our study there was lymphocytic infiltration in 74.5%, skin infiltration in 6.10% and necrosis in 47.3% of the cases, but there was 33% skin infiltration reported in the study conducted by Chanda Bewtra et al [138] and 38.1% necrosis in the study conducted by Gloria Perio et al. [139]

Table-41: Comparison of molecular classification

Molecular classification	Adedayo et al [140]	Current study
Lumina A	68.9	33.9%
Lumina B	10.2	17.0%
HER2neu positive	7.5	20.0%
Triple negative	13.4	29.1%

Our study showed maximum number of cases in Luminal A category constituting 33.9% of cases which is in concurrence with the study by Adedayo et al (140) who showed maximum (68.9%) cases in Lumina A. (Table 41)

COMPARISON OF C-KIT EXPRESSION WITH OTHER STUDIES

In our study, C-KIT was expressed in 40% of Invasive Breast carcinomas. The C-KIT expressions among various studies are given in the Table 42.

Table-42: Comparison of C-Kit Expression with other Studies

Studies	Percent
Abdallah et al(114)	90.5%
Susruthan et al(115)	52.0%
Maha M Amin et al(106)	29.0%
Palmu et al(116)	82.0%
Tahany M Shams et al (122)	75.0%
Hitoshi Tsuda et al(119)	34.0%
S Tsutsui et al (121)	27.0%
Hitoshi Tsuda et al (120)	10.0%
Present study	40.0%

The median age of our study was 49%. There was no significant correlation found between age of the patient and C D117 expression (P-

0.88). Susruthan et al [115] ,Tahany M Shams et al [122] and many other authors did not find any correlation with age and CD117 expression.

Tumor location and the side of the breast involved had no correlation with CD117 expression (p-0.201 and 0.907 respectively).

In our study tumor size did not correlate significantly with the CD117 expression (P-0.150) and it coincides with the study done by Tsutsui et al, Tahany M Shams et al and Sushruthan et al.

In our study Infiltrating ductal carcinoma showed more expression for c-kit receptors even though it was not statistically significant (P-0.08). The special subtypes like Mucinous Carcinoma, Apocrine Carcinoma, Intracystic papillary carcinoma showed 100% positivity. Abdhallah et al study showed 90.5% positive expression for c-kit in IDC NOS and 100% positivity in special subtypes. Sushruthan et al showed 49 % positivity in IDC NOS and 69% positivity in other subtypes. Hitoshi Tsuda et al study showed CD117 expression in 10% IDC NOS type.

In this present study, Grade I, II and III tumors showed 18.8%, 52.2% and 16.7% C-kit expression respectively which is statistically significant (P=0.028). Ronald Simon et al study showed significant relationship between c-kit positivity and Grade of the tumor. Hitoshi Tsuda et al showed 0%, 6%, 20% CD117 expression in Grade I, II , III tumors respectively. Sushruthan et al study showed 36%, 57% and 46%

CD117 expression in Grade I, II, III tumors respectively. Tahany M shams et al showed a significant correlation of Ckit Expression with the Grade and he showed 88%, and 45.5% positivity in Garde II and III tumors (p=0.0001). Abdallah et al also showed a statistically significant correlation between tumor grade and CD117 expression (P=0.015).

In this study we found more CD117 expression in lesser nodal involvement group. We found lack of CD117 expression in more lymph nodal metastasis, but it was not statistically significant. Maha Shomaf et al [(141)] also showed a lack of C-KIT expression in association with more lymph nodal involvement. Similarly Maha N Amin et al [(106)]concluded, a lack of CD 117 expression is found in higher nodal stage groups.

Tsutsui et al showed a significant relation between lymph nodal metastasis and negative expression of CD 117 expression (P=<0.0001).

Among Lymphovascular invasion in the tumors, present study found that, 43.8% of LVI positive group expressed CD 117 expression. This statistically insignificant results were coincided with studies by Maha Shomaf et al and Maha M Amin et al study which also showed an insignificant correlation between CD 117 expression and the presence of LVI. In contrast, Tahany M shams showed a significant positive expression of CD 117 expression in LVI positive tumors (P=<0.0001).

In this study, 44% of ER positive cases expressed C-kit immunoreactivity which is not statistically significant. This is in similar

with the study done by Sushruthan et al and Tsutsui et al . This is in contrast with Hitoshi Tsuda et al who found an inverse relation and Maha N Amin et al found a positive relation with expression of CD117 in ER positive cases.

In this present study, 44.4% of PR positive expressed CD117 positivity. This is in concordance with the study done by Sushruthan et al. Hitoshi Tsuda et al study, showed that CKIT overexpression was inversely correlated with PR expression. Maha M Amin et al study showed that there was statistically significant difference between expression of CD117 in tumor epithelial cells and expression of PR.

In this study, we found 35% positive expression of CD 117 in Her-2 neu positive tumors. This is similar to the study done by Sushruthan et al. Maha N Amin et al found that CD 117 expression was strongly associated with Her-2 positivity.

In our study, Luminal A group showed 35.5 % expression (n=6/17) and Luminal B showed 60% expression. Her-2 Enriched subtype showed 10% expression of CD117, and Basal- like breast carcinoma showed 53.8% CD 117 expression (n=7/13). Tahany M Shams et al showed 75% immune reactivity for CD117 in triple negative breast cancers. Nielsen et al study showed more expression of C-KIT in basal- like subtype invasive breast carcinomas. Susruthan et al showed

that, in Triple negative breast cancer patients the C-kit expression is high, suggesting a definitive role in targeted therapy.

Out of 18 DCIS associated breast carcinomas, 50% of cases with DCIS component expressed C-Kit (n=9). This study correlates well with the results given by Tsutsui et al [(121)], Yared et al (10%) [(142)] and Ulivi et al (44%). [(118)]

The present study revealed 100% positivity for CD 117 expression in the normal breast tissue which is in concurrence with the studies of Natali et al (100%) [(143)],Tsuura et al (100%)[(144)] and Ulivi et al (100%).

LIMITATIONS OF THE STUDY

1) The cases were selected on the basis of histopathological classification in the tertiary care Centre and not a population based study, which will not reflect the true prevalence of the general population

2) HER2neu expression has an intermediate stain scoring of 2+ which requires FISH for grading it as negative or positive.

3) Follow up details of the cases has not been available and a Targeted therapy has not been done which helps to assess the prognostic and theranostic significance of C-KIT receptor.

SUMMARY

This study is conducted in the Institute of Pathology, Madras Medical College, Chennai during the period between September 2014 to August 2016. It is a Prospective and retrospective study. Out of the 22562 specimens received during this period, 1430 (6.3%) cases were breast specimens. Of these 1430 specimens, breast malignancies constituted for 40.5% of all cases.

Detailed history regarding Patient's age, sex, side of the breast involved, Grade, Lymph node involvement, Lymphocytic infiltration, Necrosis, Skin infiltration by tumor and Hormonal status like status of ER, PR, HER2neu were assessed for 165 cases. For c-kit expression estimation 50 cases were randomly selected including 42 Infiltrating Ductal carcinoma-NOS cases and 08 cases of special types like Mucinous, Metaplastic, Apocrine, and Intracystic Papillary carcinoma.

Immunohistochemical analysis of c-kit was done in these cases. Slides were evaluated and scoring was done by Tsuada et al scoring system and results were compared with other Histopathological parameters and Hormonal receptors like ER, PR and HER2neu status.

- Highest incidence of breast carcinoma occur in the age group of 41-50 years.

- Infiltrating Ductal Carcinoma NOS is the most common primary malignant neoplasm of breast constituting 93.9% of cases.
- Most of the malignant tumors are Right sided.
- Sixty percent of cancers are presented in the size range of 2-5cms.
- Majority of the tumors are of Grade II accounting for 56.4% of all cases.
- Notably 58.2% cases presented with Lymph node metastasis and majority of them with N1 stage.
- Lymphovascular invasion was seen in 56.7% of cases and Lymphocytic infiltration seen in 74.5% cases.
- Necrosis was present in 47.3% of cases
- Skin infiltration was observed in 6.1% of cases
- Estrogen receptor expression was observed in 47.3% cases and Progesterone receptor was observed in 38.8% cases and 37% of cases were positive for HER2neu.
- As per Molecular classification, Lumina A constituted maximum number of cases with 33.9% and Lumina B constituted 17% cases. HER2neu alone positive in 20% cases and Triple negative in 29.1% cases.

- C-KIT expression was seen in 40% of cases.
- More number of C-KIT positive cases are in the age group 41-50 years though it was not statistically significant
- C-kit is expressed in 33.3% of Invasive Ductal Carcinomas NOS type.
- C-KIT expression is seen in 100% cases of Apocrine carcinoma, Mucinous carcinoma and Intracystic papillary carcinoma. Although this result was limited by small sample size, many studies show similar results. C-KIT expression was negative in Metaplastic carcinoma.
- C-KIT expression was decreased in higher T stage group patients but not statistically significant and this result is in concordance with many studies.
- There is a loss of C-KIT expression in high grade tumors and it is statistically significant and more expression seen in lower grade tumors.
- Many lymph node positive tumors are C-kit positive but not significantly associated.
- C-kit expression is not significantly associated with Lymphovascular invasion and Lymphocytic infiltration.

- There is loss of C-KIT expression in tumors with necrosis (65.5%) and 60.0% positivity in tumors with skin infiltration.

- ER positive tumors are not significantly associated with C-KIT expression. Similarly c-kit is not significantly expressed in PR positive tumors also.

- A total of 43.3% of HER2neu negative tumors are c-kit positive and 35% of HER2neu positive tumors are C-KIT positive.

- C-kit expressed in 43.8% both ER and PR Positive and 34.8% in ER/PR negative tumors.

- C-kit expression was seen maximum in basal like tumors explaining the implication of C-KIT related targeted therapy in this tumor.

- Interestingly, 53.8% of Triple negative tumors (ER-/PR-/HER2neu-) were C-KIT positive but it is not statiscally significant.

- The present study revealed 100% positivity for CD 117 expression in the normal breast tissue.This result is in concurrence with many studies.

- Fifty percent of DCIS component expressed CD 117 positivity and this correlated with many studies

CONCLUSION

In our study, among the surgical specimens received, breast specimens included 6.33% of all cases. Malignant breast tumors constituted 40.5% of all the breast specimens received.

The incidence of IDC NOS type comprised the highest among the primary breast cancers. Luminal A constituted the majority of cases and followed by Basal like subtype.

C-KIT is expressed in 40% of Primary breast carcinomas.

C-kit is expressed in 33.3% of Invasive Ductal Carcinomas NOS type

C-KIT expression is seen in 100% cases of Apocrine carcinoma, Mucinous carcinoma and Intracystic papillary carcinoma. Although this result was limited by small sample size, many studies showed similar results. C-KIT expression was negative in Metaplastic carcinoma.

There is a loss C-KIT expression in high grade tumors and it is statistically significant and in tumors with necrosis. More expression seen in lower grade tumors and this implicates C-KIT could be used as a prognostic marker.

C-kit expression is not related with Age of the patient, T size, Lymph nodal status, LVI and skin involvement and these results are in concordance with many studies.

ER and PR positivity did not correlate with C-KIT expression and it is expressed in 35% of HER2neu positive tumors and in 43.3% of HER2-neu negative tumors.

The normal breast tissue expressed 100% positivity for CD 117. Fifty percent DCIS component expressed CD 117 positivity. C-kit expression decreases on tumor progression from normal epithelium. C-kit has a definitive role in the pathogenesis of breast cancer.

C-kit expression was seen in 35.3 % of Luminal A subtypes of tumors. C-kit was expressed in 53.8% of triple negative breast carcinomas suggesting a definite role for targeted therapy; however a larger study is required for the evaluation of the same.

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REFERENCES

1) Parkin DM, Bray F, Ferlay J, Pisani P: Estimating the world cancer burden. Globocan 2000. Int J Cancer 2001; 94:153-156.

2) Sondik EJ: Breast cancer trends. Incidence, mortality, and survival. Cancer 1994; 74:995-999.

3) Estimated Cancer Incidence, Mortality and Prevalence worldwide in 2012, globacan.iarc.fr/Pages/fact_sheets_cancer.aspx.

4) Nadhakumar et al., three year report of Population based cancer registry 2009-2011., NCDIR – NCRP, Bangalore. Feb 2013; 1 – 11.

5) Balkrishna B Yeole, A P Kurkure.An Epidemiological Assessment of Increasing Incidence and Trends in Breast Cancer in Mumbai and Other Sites in India, during the Last Two Decades. Asian Pacific J Cancer Prev 2003; 4: 51-56.

6) Moore DH, Moore II DH, Moore CT: “Breast carcinoma etiological factors”. Adv Cancer Res 1983; 40:189-253.

7) Dr.R.Swaminatha, Dr.R.Rama, Dr.V.Shanta, An aside study on trends in cancer incidence in Chennai city 1982-2006 and statewide predictions of future burden in Tamil Nadu 2007-2016. Madras Metropolitan Tumor registry.

8) Skolnick MH, Cannon-Albright LA: Genetic predisposition to breast cancer. Cancer 1992;70: 1747-1754.

9) Kelsey JL, Gammon MD, John EM: Reproductive factors and breast cancer. Epidemiol Rev 1993; 15:36-47.

10) Pathak DR, Osuch JR, He J: Breast carcinoma etiology: current knowledge and new insight into the effects of reproductive and hormonal risk factors in black and white populations. Cancer 2000; 88:1230-1238.

11) Wang DY, Rubens RD, Allen DS, Millis RR, Bulbrook RD, Chau dary MA, Hayward JL: Influence of reproductive history of age at diagnosis of breast cancer and prognosis. Int J Cancer 1985; 36:427-432

12) Newcomb PA, Storer BE, Longnecker MP,Mittendorf R, Greenberg ER, Clapp RW, Burke KP, Willett WC, MacMahon B: Lactation and a reduced risk of premenopausal breast cancer. N Engl J Med 1994; 330:81-87.

13) IARC : Hormonal contraception and post-menopausal hormonal therapy. IARC monographs on the evaluation of carcinogenic risks to humans, vol. 72. Lyon, IARC Press, 1998.

14) Kelsey JL, Gammon MD: The epidemiology of breast cancer. CA Cancer J Clin 1991; 41:146-165.

15) Hoover R, Gray Sr LA, Cole P, MacMahon B: Menopausal estrogens and breast cancer. N Engl J Med 1976; 295:401-405.

16) Ross RK, Paganini-Hill A, Wan PC, Pike MC: Effect of hormone replacement therapy on breast cancer risk estrogen versus estrogen plus progestin. J Natl Cancer Inst 2000; 92:328-332.

17) Chen CL, Weiss NS, Newcomb P, Barlow W, White E: Hormone replacement therapy in relation to breast cancer. JAMA 2002; 287:734-741.

18) Romieu I, Berlin JA, Colditz G: Oral contraceptives and breast cancer. Review and meta-analysis. Cancer 1990; 66:2253-2263

19) Goss PE, Sierra S: Current perspectives on radiation-induced breast cancer. J Clin Oncol 1998; 16:338-347.

20) Clark CP, Peters GN, O'Brien KM: Cancer in the augmented breast. Diagnosis and prognosis. Cancer 1993; 72:2170-2174.

21) Berkel H, Birdsell DC, Jenkins H: Breast augmentation. A risk factor for breast cancer?. N Engl J Med 1992; 326:1649-1653.

22) Bonito D, Giarelli L, Falconieri G, BonifacioGori D, Tomasic G, Vielh P: Association of breast cancer and meningioma. Report of 12 new cases and review of the literature. Pathol Res Pract 1993; 189:399-404.

23) Schrager CA, Schneider D, Gruener AC, Tsou HC, Peacocke M: Clinical and pathological features of breast disease in Cowden's syndrome: an underrecognized syndrome with an increased risk of breast cancer. Hum Pathol 1998; 29:47-53.

24) Tan DS, Marchio C, Reis-Filho JS: Hereditary breast cancer: from molecular pathology to tailored therapies. J Clin Pathol 2008; 61:1073-1082.

25) Wooster R, Neuhausen SL, Mangion J, Quirk Y, Ford D,CollinsN, Nguyen K, Seal S, Tran T, Averill D, Fields P, Marshall G, Narod S, Lenoir GM, Lynch H, Feunteun J, Devilee P, Cornelisse CJ, Me nko FH, Daly PA, Ormiston W, McManus R, Pye C, Lewis CM, CannonAlbright LA,Peto J,Ponder BAJ, Skolnick MH, Easton, Goldgar DE, Stratton MR: Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12–13. Science 1994; 265:2088-2090.

26) Struewing JP, Hartge P, Wacholder S, Baker SM, Berlin M, Mc Adams M, Timmerman MM, Brody LC, Tucker MA: The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. N Engl J Med 1997; 336:1401-1408.

27) Tan DS, Marchio C, Reis Filho JS: Hereditary breast cancer: from molecular pathology to tailored therapies. J Clin Pathol 2008 ; 61:1073 -1082.

28) Palacios J, Robles-Frias MJ, Castilla MA, Lopez-Garcia MA, Benitez J: The molecular pathology of hereditary breast cancer. Pathobiology 2008; 75:85-94.

29) Bradbury AR, Olopade OI: Genetic susceptibility to breast cancer. Rev Endocr Metab Disord 2007; 8:225.

30) Garcia-Closas M, et al: Heterogeneity of breast cancer associations with five susceptibility loci by clinical and pathological characteristics. PLOS Genetics 2008; 4:e10000054.

31) Wiseman BS, Werb Z: Stromal effects on mammary gland development and breast cancer. Science 2002; 296:1046.

32) Schedin P: Pregnancy-associated breast cancer and metastasis. Nat Rev Cancer 2006; 4:281.

33) Joy Winter, "Morphological and immunophenotypic analysis of basal-like carcinoma of thebreast.Bioscience Horizons 2008; 1(1): 19-27.

34) Yoder BJ, et al: Molecular and morphologic distinctions between infiltrating ductal and lobular carcinoma of the breast. Breast J 2007; 13:172

35) AcsG, Lawton TJ, RebbeckTR et al, "Differential expression of E-cadherin in lobular and ductal neoplasms of the breast and its biologic and diagnostic implications". Am J Clin Pathol 2001; 115: 85-98

36) GoldsteinNS, BassiD, WattsJCetal,“E cadherin reactivity of 95 non invasive ductal and lobular lesions of the breast: implications for the interpretation of problematic lesions” . Am J Clin Pathol 2001; 115:534-542.

37) Lehr H-A, FolpeA, YazijiH, KommossF et al, “Cytokeratin 8 immunostaining pattern and E-cadherin expression distinguish lobular from ductal breast carcinoma”. Am J Clin Pathol 2000; 114:190-196.

38) Dabbs DJ,BhargavaR,ChivukulaM,Lobular versus ductal breast neoplasms:the diagnostic utility of p120 catenin.Am J Surg Pathol 2007;31:427-437.

39) EllisIO, ElstonCW: Histologic Grade. In: O'Malley FP, Pinder SE, ed. Breast Pathology, Elsevier; 2006:225-233.

40) Bertucci F, et al: Gene expression profiling shows medullary breast cancer is a subgroup of basal breast cancers. Cancer Res 2006; 66:4636.

41) EichhornJH, “Medullary carcinoma, provocative now as then”. Semin Diagn Pathol 2004;21:65-73.

42) ShoushaS, “Medullary carcinoma of the breast and BRCA1 mutation”. Histopathology 2000; 37:182-185

43) Kuroda H, Tamaru J, Sakamoto G et al, “Immunophenotype of lymphocytic infiltration in medullary carcinoma of the breast”. Virchows Arch 2005; 446:10-14.

44) EllisIO, ElstonCW, O'Malley FP, Pinder SE,. Histologic grade In Breast Pathology, Elsevier; 2006:225-233.

45) Lacroix Triki M, Suarez PH, et al:“Mucinous carcinoma of the breast is genomically distinct from invasive ductal carcinomas of no special type”.JPathol2010;222:282-298.

46) Luck AA, et al: The influence of basal phenotype on the metastatic pattern of breast cancer. ClinOncol 2008; 20:40.

47) DelaCruz C, MoriyaT, EndohM, WatanabeM, Takeyama J,YangM,Oguma M, SakamotoK, SuzukiT, HirakawaH, OritaY, OhuchiN, SasanoH: “Invasive micropapillary carcinoma of the breast: clinicopathological and immunohistochemical study”. Pathol Int 2004; 54:90-96.

48) Peppercorn J et al, molecular subtypes in breast cancer evaluation and management: divide and conquer cancer invest 26:1, 2008.

49) Constantinidou A, Jones RL, Reis-Filho JS: Beyond triple - negative breast cancer: the need to define new subtypes. Expert Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O,

PergamenschikovA, Williams C, Zhu SX, Lonning PE, Borresen -Dale AL, Brown PO, Botstein D: Molecular portraits of human breast tumours. Nature 2000; 406:747-752.

50) Schnitt SJ: Will molecular classification replace traditional breast pathology?. Int J SurgPathol 2010; 18:162S-166S.

51) Kang Sp et al: Triple negative breast cancer : current understanding of biology and treatment options, Curr Opin Obstet Gynecol , 2008 20:40.

52) Reis-Filho JS, TuttANJ : Triple negative tumors, a critical review histopathology. 52:108, 2008.

53) ClaireVerschraegen, VincentVinh-Hung, “Modeling the Effect of Tumor Size in Early Breast Cancer”. Ann Surg. 2005; 241(2):309-318.

54) FitzgibbonsPL, PageDL, WeaverDetal, “Prognostic factors in breast cancer”. College of American Pathologists Consensus Statement 1999.Arch Pathol Lab Med. 2000;124:966-978.

55) Lohrisch C, Jackson J, Jones A, Mates D, Olivotto IA: Relationshi p between tumor location and relapse in 6781 women with early invasive breast cancer. J ClinOncol 2000; 18:2828-2835.

56) Santiago R J, Harris E E, Qin L et al, “Similar long-term results of breast- conservation treatment for Stage I and II invasive lobular carcinoma compared with invasive ductal carcinoma of the

breast": The University of Pennsylvania experience. Cancer 2005; 103: 2447-2454.

57) PageDL, Special types of invasive breast cancer, with clinical implications. Am J Surg Pathol 2003;27:832-835.

58) Miremadi A, Pinder S E, Lee AHS et al, "Neuroendocrine differentiation and prognosis in breast adenocarcinoma". Histopathology 2002;40: 215-222.

59) Lash RH, Bauer TW, Medendorp SV: Prognostic significance of the proportion of intraductal and infiltrating ductal carcinoma in women treated by partial mastectomy. Surg Pathol 1990; 3:47-58.

60) Bauer TW, O'Ceallaigh D, Eggleston JC, Moore GW, Baker RR: Prognostic factors in patients with stage I, estrogen receptor-negative carcinoma of the breast. A clinicopathologic study. Cancer 1983; 52:1423-1431.

61) Yu L, Yang W, Cai X, Shi D, Fan Y, Lu H: Centrally necrotizing carcinoma of the breast: clinicopathological analysis of 33 cases indicating its basal-like phenotype and poor prognosis. Histopathology 2010; 57:193-201.

62) Carter D, Pipkin RD, Shepard RH, Elkins RC, Abbey H: Relations hip of necrosis and tumor border to lymph node metastases and

10-year survival in carcinoma of the breast. Am J SurgPathol 1978; 2:39-46.

63) Hultborn KA, Tornberg B: Mammary carcinoma. The biologic character of mammary carcinoma studied in 517 cases by a new form of malignancy grading. ActaRadiol (Stockh) 1960; 196:1-143.

64) Sears HF, Janus C, Levy W, Hopson R, Creech R, Grotzinger P: B reast cancer without axillary metastases. Are there high-risk biologic subpopulations?. Cancer 1982; 50:1820-1827.

65) Wertheim U, Ozzello L: Neoplastic involvement of nipple and skin flap in carcinoma of the breast. Am J Surg Pathol 1980; 4: 543-549.

66) Breast Cancer Study Group: Identification of breast cancer patients with high risk of early recurrence after radical mastectomy. Clinical and pathological correlations. Cancer 1978; 42: 2809-2826.

67) Davis BW, Gelber R, Goldhirsch A, Hartmann WH, Hollaway L, Russell I, Rudensta CM: Prognostic significance of peritumoral vessel invasion in clinical trials of adjuvant therapy for breast cancer with axillary lymph node metastasis. Hum Pathol 1985; 16:1212-1218.

68) Nime FA, Rosen PP, Thaler HT, Ashikari R, Urban JA : Prognostic significance of tumor emboli in intramammary lymphatics in patients with mammary carcinoma. Am J SurgPathol 1977; 1:25-30.

69) TanLK, GiriD, Panageas K et al, “Occult/micro metastases in axillary lymph nodes of breast cancer patients are significant: a retrospective study with long term follow-up”.Proc Am Soc Clin Oncol 2002;21:146.

70) Kang Y: New tricks against an old foe: molecular dissection of metastasis tissue tropism in breast cancer. Breast Dis 2006; 26:129.

71) Luck AA, et al: The influence of basal phenotype on the metastatic pattern of breast cancer. ClinOncol 2008; 20:40.

72) Quiet CA, Ferguson DJ, Weichselbaum RR, Hellman S: Natural history of node-negative breast cancer. A study of 826 patients with long-term follow-up. J ClinOncol 1995; 13:1144-1151.

73) Rakha EA, El-Sheikh SE, Kandil MA, El-Sayed ME, Green AR, Ellis IO: Expression of BRCA1 protein in breast cancer and its prognostic significance. Hum Pathol 2008; 39:857-865.

74) ReedW, SandstadB, HolmR, NeslandJM: “The prognostic impact of hormone receptors and c-erb B-2 in pregnancy-associated

breast cancer and their correlation with BRCA1 and cell cycle modulators". Int J Surg Pathol 2003 ;11:485-488.

75) Robson M: "Are BRCA-1 and BRCA-2 associated breast cancers different? Prognosis of BRCA-1 associated breast cancer". J Clin Oncol 2000; 18:113 S-118S.

76) VandeRijnM, Perou CM, et al: "Expression of cytokeratins17 and 5 identifies a group of breast carcinomas with poor clinical outcome". Am J Pathol 2002; 161:1991-1996.

77) Barnes DM, Hanby AM: Oestrogen and progesterone receptors in breast cancer: past, present and future. Histopathology 2001; 38: 271-274.

78) Hawkins RA, Roberts MM, Forrest APM: Oestrogen receptors and breast cancer. Current status. Br J Surg 1980; 67:162-165.

79) Battifora H, Mehta P, Ahn C, Esteban J: Estrogen receptor immunohistochemical assay in paraffin-embedded tissue. A better gold standard? Appl Immunohistochem 1993; 1:39-45.

80) Harvey JM, Clark GM, Osborne CK, Allred DC: Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. J ClinOncol 1999; 17:1474-1481.

81) MacGrogan F, Soubeyran I, DeMascarel I, Wafflart J, Bonichon F, Durand M, Avril A, Mauriac L, Trojani M, Coindre JM: Immunohistochemical detection of progesterone receptors in breast invasive ductal carcinomas: a correlative study of 942 cases. Appl Immunohistochem 1996; 4:219-227.

82) Taylor CR: Paraffin section immunocytochemistry for estrogen receptor: the time has come. Cancer 1996; 77:2419-2422.

83) Mohsin SK, Weiss H, Havighurst T, Clark GM, Berardo M, Roanh le D, To TV, Qian Z, Love RR, Allred DC: Progesterone receptor by immunohistochemistry and clinical outcome in breast cancer: a validation study. Mod Pathol 2004; 17:1545-1554.

84) Chang,H.R., Glaspy J., AllisonM.A., Kass,F.C., Elashoff, R.,Chung, D.U.,& Gornbein,J .(2010). “Differential response of triple-negative breast cancer to a docetaxel and carboplatin-based neo-adjuvant treatment”.Cancer,Vol.116, pp.4227-32.

85) Gupta D, Middleton LP, Whitaker MJ, Abrams J: Comparison of fluorescence and chromogenic in situ hybridisation for detection of HER-2/neu oncogene in breast cancer. Am J ClinPathol 2003; 119:381-387.

86) Papouchado BG, Myles J, Lloyd RV, Stoler M, Oliveira AM, Dow nsKelly E,Morey A, Bilous M, Nagle R, Prescott N, Wang L,

Dragovich L, McElhinny A, Garcia CF,Ranger,Moore J, Free H, Powell W, Loftus M, Pettay J, Gaire F, Roberts C, Dietel M, R oche P, Grogan T, Tubbs R: Silver in situ hybridization (SISH) for determination of HER2 gene status in breast carcinoma: comparison with FISH and assessment of inter observer reproducibility. Am J Surg Pathol 2010; 34:767-776.

87) Fisher ER, Redmond CK, Liu H, Rockette H, Fisher B collaborating NSABP investigators: Correlation of estrogen receptor and pathologic characteristics of invasive breast cancer. Cancer 1980; 45:349-353.

88) PerouCM, SorlieT, Eisen MB, et al. “Molecular portraits of human breast tumours”. Nature. 2000; 406:747-752.

89) Il Soo Moon, Hyun Sook Lee, Sung Dong Park, Immunonucleochemistry: a new method for insitu detection of antigens in the nucleus of cells in culture, Cytotechnology 2010; 62(2): 83-93.

90) Fred T. Bosman, Some recent developments in immunocytochemistry, The Histochemical Journal 1983; 15(3):189-200.

91) JacquesChevalier, JingYi, OdileMichel, Biotin and Digoxigenin as Labels for Light and Electron Microscopy in-Situ Hybridization

Probes: Where Do We Stand? J Histochem Cytochem1997; 45(4):481-491.

92) KrenacsL, Krenacs T, Stelkovics E, Heat-induced antigen retrieval for immune histochemical reactions in routinely processed paraffin sections. Mol Biol.2010; 588: 103-119.

93) FabioD' Amico, Evangelia S karmoutsou, Franca S tivala, State of the art in antigen retrieval for immunohistochemistry. Journal of Immunological Methods 2009; 341(1-2):1-18.

94) Bancroft JD, Marilyn Gamble (Ed), Theory and practice of histological techniques, Churchill Livingstone 2002

95) Yarden Y, Kuang WS, Yang-Feng T, Coussens L, Munemitsu S, Dull TJ, et al. Human protooncogene c-kit: A new cell surface receptor tyrosine kinase for an unidentified ligand. EMBO J 1987; 6: 3341-51.

96) Kitamura Y, Hirotab S. Kit as a human oncogenic tyrosine kinase. Cell Mol Life Sci 2004; 61: 2924-31.

97) Chui X, Egami H, Yamashita J, Kurizaki T, Ohmachi H, Yamamoto S, et al. Immunohistochemical expression of the c-kit proto-oncogene product in human malignant and non-malignant breast tissues. Br J Cancer1996; 73:1233-6.

98) Tsuura Y, Hiraki H, Watanabe K, Igarashi S, Shimamura K, Fukuda T, et al. Preferential localization of c-kit product in tissue mast cells, basal cells of skin, epithelial cells of breast, small cell lung carcinoma and seminoma/dysgerminoma in human: Immunohistochemical study on formalin-fixed, paraffin-embedded tissues. Virchows Arch 1994;424:135-41.

99) Ko CD, Kim JS, Ko BG, Son BH, Kang HJ, Yoon HS, et al. The meaning of the c-kit protooncogene product in malignant transformation in human mammary epithelium. Clin Exp Metastasis 2003;20:593-7.

100) Tsuura Y, Suzuki T, Honma K, Sano M. Expression of c-KIT protein in proliferative lesions of human breast. Sexual difference and close association with phosphotyrosine status. J Cancer Res Clin Oncol 2002;128:239-46.

101) Roussidis AE, Theocharis AD, Tzanakakis GN, Karamanos NK. The importance of c-KIT and PDGF receptors as potential targets for molecular therapy in breast cancer. Curr Med Chem 2007;14:735-43.

102) Tsuda H, Morita D, Kimura M, Shinto E, Ohtsuka Y, Mat.subara O, et al. Correlation of KIT and EGFR overexpression with invasive ductal breast carcinoma of the solid-tubular subtype,

nuclear grade 3 and mesenchymal of myoepithelial differentiation. Cancer Sci2005;96:48-53.

103) Diallo R, Rody A, Jackish C, Ting E, Schaefer KL, Kissler S, et al. C-kit expression in ductal carcinoma in situ of the breast: Co-expression with HER-2/neu. Hum Pathol 2006;37:205-11.

104) Crisi GM, Marconi SA, Makari-Judson G, Goulart RA. Expression of c-kit in adenoid cystic carcinoma of the breast. Am J Clin Pathol 2005;124:733-9.

105) Maha M. Amin, Amira K. El-Hawary, Omar Farouk, Relation of CD117 immunoreactivity and microvascular density in invasive breast carcinoma. Indian Journal of Pathology and Microbiology 2012; 55(4); 456-460.

106) Kondi-Pafiti A, Arkadopoulos N, Gennatas C, Michalaki V, Frangou-Plegmenou M, Chatzipantelis P. Expression of c-kit in common benign and malignant breast lesions. Tumori 2010; 96: 978-84.

107) Lennartsson J, Voytyuk O, Heiss E, Sundberg C, Sun J, Rönnstrand L. C-KIT signal transduction and involvement in cancer. Cancer Ther 2005;3:5-28.

108) Mundim FG, Logullo AF. Role of the expression of the tyrosine kinase receptor KIT in invasive ductal carcinomas of the breast: A review. Appl Cancer Res 2009;29:50-7.

109) Ko CD, Son BH, Ahn SH, Ka IW, Gong GY, Kim JC. Significance of mismatch repair protein expression in the chemotherapeutic response of sporadic invasive ductal carcinoma of the breast. Breast J 2004;10:20-6.

110) Simon R, Panussis S, Maurer R, Spichtin H, Glatz K, Tapia C. KIT (CD117)-positive breast cancers are infrequent and lack KIT gene mutations. Clin Cancer Res 2004;10:178-83.

111) Eroglu A, Sari A. Expression of c-kit proto-oncogene product in breast cancer tissues. Med Oncol 2007;24:169-74.

112) X Chui, H Egami, J Yamashita, T Kurizaki, H Ohmachi, S Yamamoto and M Ogawa" Immunohistochemical expression of the c-kit proto-oncogene product in human malignant and non-malignant breast tissues. British Journal of Cancer (1996) 73, 1233-1236

113) Abdallah M Khalil, M.D, Essam E Ayad, Samar A El-Sheikh MD " Immunohistochemical Expression Of c-Kit in Invasive breast carcinoma of Different Nuclear Grades" Med. J. Cairo Univ 2012, Vol.80(1);345-351.

114) Susruthan M, Rajendiran S, Jayanth V, Archana K, Viswanath Pai. C-kit expression in breast carcinoma – A study of 62 cases of breast carcinoma International journal of Recent Trends in Science and Technology. July 2015; 15(3): 597-602.

115) Palmu S, Soderstrom KO, Quazi K, Isola J, Salminen E . "Expression of c-kit and HER-2 tyrosine kinase receptors in poor prognosis breast cancer" Anticancer Research 2002;22(1A):411-414.

116) Ronald Simon, Soti Panussis, Robert Maurer, Hanspeter Spichtin, Kathrin Glatz, Coya Tapia, Martina Mirlacher, Alex Rufle, Joachim Torhorst, and Guido Sauter. KIT (CD117)-Positive Breast Cancers Are Infrequent and Lack KIT Gene Mutations, Clinical Cancer Research, 2004;178(10): 178–183.

117) Paola Ulivi, Wainer Zoli, Laura Medri, Dino Amadori, Luca Sarag oni, Franco Barbanti, Daniele Calistri, "c-kit and SCF Expression in Normal and Tumor Breast Tissue" Breast Cancer Research and Treatment 2004, Vol 83(1), pp 33–42.

118) Hitoshi Tsuda, Yoichi Tani, Joel Weisenberger, Shigehiro Kitada,Tadashi Hasegawa,Tetsuya Murata, Seiichi Tamai Frequent KIT and epidermal growth factor receptor overexpressions in undifferentiated-type breast carcinomas with 'stem-cell-like' features. Cancer Sci ,June 2005; vol. 96 (6): 333– 339.

119) Hitoshi Tsuda, Daisaku Morita, Mikihiko Kimura, Eiji Shinto, Yukiko Ohtsuka, Osamu Matsubara, Johji Inazawa Correlation of KIT and EGFR overexpression with invasive ductal breast carcinoma of the solid-tubular subtype, nuclear grade 3, and mesenchymal or myoepithelial differentiation Cancer Sci ,January 2005 ; vol. 96 (1): 48–53.

120) Tsutsui, K Yasuda, K Suzuki, H Takeuchi, T Nishizaki, H Higashi and S Era, A loss of c-kit expression is associated with an advanced stage and poor prognosis in breast cancer. British Journal of Cancer (2006) 94, 1874 – 1878.

121) Tahany M. Shams and Mohamed E. Shams Overexpression of c-KIT (CD117) in triple-negative breast cancer Egypt J Pathol 2011 ; 31:113–117.

122) Aydan Eroglu, Aliye Sari “Expression of c-kit proto-oncogene product in breast cancer tissues, Medical Oncology June 2007, Volume 24(2), pp 169–174.

123) Micello D, Marando A, Sahnane N et al. Androgen receptor is frequently expressed in HER2 positive, ER/PR negative breast cancers. Virchows Arch. 2010; 457 (4):467-476.

124) Carreno G, Del Caser JM et al. Local recurrence after mastectomy for breast cancer: analysis of clinicopathological, biological and

prognostic characteristics. Breast Cancer Res Treat. 2007;102 (1):61-73.

125) Hu R, Dawood S, Holmes MD et al. Androgen receptor expression and breast cancer survival in postmenopausal women. Clin Cancer Res.2011;17(7):1867-1874.

126) Honma N, Horii R, Iwase T et al. Clinical importance of androgen receptor in breast cancer patients treated with adjuvant tamoxifen monotherapy. Breast cancer.2012 Feb.

127) Rajesh Singh Laishram, Gegong Jongkey, Sharmila Laishram, Clinico-Morphological Patterns of Breast Cancer in Manipur, India. International Journal of Pathology 2011; 9(1): 40-43.

128) Albrektsen et al, Histological type and grade of breast cancer tumors by parity, age at birth, and time since birth: a register-based study in Norway. BMC Cancer 2010; 10: 226.

129) Shirley S E, Sinclair P A, Stennett M A et al, The pathology of breast cancer in Jamaica: the National Public Health Laboratory study. West Indian Med J. 2010;59 (2):177-81.

130) AM Dauda, M A Misauno and E OOjo, Histopathological Types of Breast Cancer in Gombe, North Eastern Nigeria: A Seven-Year Review. Afr J Reprod Health 2011; 15(1):107-109

131) Christine L.Carter, Carol Allen, Donald E. Henson, Relation of Tumor Size, Lymph Node Status, and Survivalin 24,740 Breast Cancer Cases.Cancer1989;63:181-187.

132) FSAl-Joudi, Z AIskandar,J Rusli,The Expression of p53 in Invasive Ductal Carcinoma of the Breast: A Study in the North-East States of Malaysia. Med J Malaysia 2008; 63(2): 96-99.

133) Lakmini K B Mudduwa et al, Quick score of hormone receptor status of breast carcinoma: Correlation with the other clinicopathological prognostic parameters, Indian Journal of pathology and microbiology 2009; 52(2): 159-162.

134) QiuJ, Yang R, RaoY, DuY, KalemboFW, Risk Factors for Breast Cancer and Expression of Insulin-Like Growth Factor-2 (IGF-2) in Women with Breast Cancer in Wuhan City, China. PLoSONE2012; 7(5):e36497.

135) Carey LA, Perou C M, Livasy C A et al, Race, Breast cancer Subtypes, and survival in the Carolina breast cancer study, JAMA 2006; 295(21): 2492–2502.

136) GG Van den Eynden, I Van der Auwera, SJ Van Laere, Distinguishing blood and lymph vessel invasion in breast cancer: prospective immunohistochemical study. Br J Cancer 200694 (11):1643-1649.

137) Chanda Bewtra et al, Clinicopathologic features of female breast cancer in Kumasi, Ghana, International Journal of Cancer Research. 2010; 6(3): 154-160

138) Glorio perio et al, Prognostic Implications of HER-2 Status in Steroid Receptor–Positive, Lymph Node–Negative Breast Carcinoma, Am J Clin Pathol 2007; 127: 780-786.

139) Adedayo A. Onitilo, Jessica M. Engel et al. Breast cancer subtypes based on ER/PR & HER2 expression: comparison of clinicopathologic features and survival. Clin Med Res.2009 Jun; 7(1-2):4-13.

140) Maha Shomaf, Al-Motassem Yousef, Jamal Masad, Murad Sahawneh, Ahmad Halawa C-kit (CD117) expression is not valuable to predict prognosis in invasive ductal carcinoma of breast. Journal of Interdisciplinary Histopathology www.scopmed.org

141) Yared MA, Middleton LP, Bernstam FM, Cristofanilli M, Sahin AA (2004) Expression of c-kit proto-oncogene product in breast tissue. Breast J 10: 323–327.

142) Natali PG, Nicorta MR, Sures I, Mottolese M, Botti C, Ullrich A (1992a) Breast cancer is associated with loss of the c-kit oncogene product. Int J Cancer 52: 713– 717.

143) Tsuura Y, Suzuki T, Honma K, Sano M (2002) Expression of c-kit protein in proliferative lesions of human breast: sexual difference and close association with phosphotyrosine status. J Cancer Res Clin Oncol 128: 239–246.

ANNEXURE I

PROFORMA

"A study on Immunohistochemical expression of C-kit in invasive breast carcinoma and its clinicopathological correlation"

Case number : Name :

HPE number : Age :

IP number: Sex :

Clinical diagnosis:

Side of Breast : Right/Left

Specimen : Simple Mastectomy / Modified radical mastectomy / Radical Mastectomy / Toilet mastectomy / Others

GROSS

Specimen size:

Nipple areola and Skin

Tumor size

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 : P resent / Absent

Necrosis : Present / Absent

Associated breast lesions :

Total number of nodes dissected :

Number of nodes involved :

ER STATUS,

PR STATUS,

HER-2 NEU STATUS.

MOLECULAR CLASSIFICATION

IHC – C-KIT MEMBRANOUS& CYTOPLASMIC STAINING

IHC SCORING

ANNEXURE II

WHO HISTOLOGICAL CLASSIFICATION OF EPITHELIAL BREAST TUMORS

INVASIVE BREAST CANCERS

Invasive ducta lcarcinoma not otherwise specified
Mixed type carcinoma
Pleomorphic carcinoma
Carcinoma with osteoclastic type of giant cells
Carcinoma with choriocarcinomatous features
Carcinoma with melanotic features
Invasive lobular carcinoma
Tubular carcinoma
Invasive cribriform carcinoma
Medullary carcinoma
Mucinous carcinoma
- Cystadenocarcinoma
- Signet ring carcinoma

Neuroendocrine tumors
- Solid neuroendocrine carcinoma
- Atypical carcinoid tumor
- Small cell/oat cell carcinoma
- Large cell neuroendocrine carcinoma

Invasive papillary carcinoma
Invasive micropapillary carcinoma
Apocrinecarcinoma
Metaplastic carcinoma
- Pure epithelial metaplastic carcinoma
  - Squamous cellcarcinoma
  - Adenocarcinoma with spindle cell metaplasia
  - Adenosquamous carcinoma
  - Mucoepidermoid carcinoma
- Mixed epithelial/ mesenchymal metaplastic carcinoma

Lipid rich carcinoma
Secretory carcinoma
Oncocytic carcinoma
Adenoid cystic carcinoma
Acinic cell carcinoma
Glycogen rich carcinoma
Sebaceous carcinoma
Inflammatory carcinoma
Intraductal papillary carcinoma
Intracystic papillary carcinoma
Microinvasive carcinoma

NON INVASIVE BREAST CANCERS

Ductal carcinoma insitu
Lobularcarcinomain situ
Atypical papilloma

BENIGN EPITHELIAL TUMORS

Tubular adenoma
Lactating adenoma
Apocrine adenoma
Pleomorphic adenoma
Ductal adenoma
Papilloma

FIBROEPITHELIAL TUMORS

Fibroadenoma
Phyllodes tumor
- Benign
- Borderline
- Malignant

Periductal stromal sarcoma
Mammary hamartoma

INTRADUCTALPROLIFERATIVE LESIONS

Atypical ductal hyperplasia
Flat epithelial atypia
Usual epithelial hyperplasia

METASTATIC TUMORS

ANNEXURE III

NOTTINGHAM MODIFICATION OF SCARF BLOOM

RICHARDSON GRADING SYSTEM

TUBULE FORMATION	**SCORE**
Tubule formation in >75% of the tumor	1
Tubule formation in 10 to75 % of the tumor	2
Tubule formation in <10% of the tumor	3

NUCLEAR PLEOMORPHISM	**SCORE**
Minimal variation in size and shape of nuclei	1
Moderate variation in size and shape of nuclei	2
Markedvariationinsize andshape ofthenuclei	3

MITOTIC RATE	**SCORE**
<10 Mitosis per10 high power field	1
10 to 20 mitosis per10 high power field	2
>20 mitosis per10 high power field	3

GRADE	***SCORE***
Grade 1:	3,4, 5
Grade 2:	6,7
Grade 3:	8,9

ANNEXURE-IV

IMMUNOHISTOCHEMISTRY PROCEDURE

1. Four microns thick sections were cut from formalin fixed paraffin embedded tissue samples and transferred to gelatin-chromealum coated slides.
2. The slides were incubated at 58ºC for overnight.
3. The sections were de-paraffinized in xylene for15 minutes x 2 changes.
4. The sections were dehydrated with absolute alcohol for 5 minutes x 2 changes.
5. The sections were washed in tap water for 10 minutes.
6. The slides were then immersed in distilled water for 5 minutes.
7. Heat induced antigen retrieval was done with microwave oven in appropriate temperature with appropriate buffer for 20 to 25 minutes.
8. The slides were then cooled to room temperature and washed in running tap water for 5 minutes.
9. The slides were then rinsed in distilled water for 5 minutes.
10. Wash with appropriate wash buffer (phosphate buffer) for 5 minutes x 2 changes.
11. Apply peroxidase block over the sections for 10 minutes.
12. Wash the slides in phosphate buffer for 5 minutes x 2 changes.
13. Cover the sections with power block for15 minutes.
14. The sections were drained (without washing) and appropriate primary antibody was applied over the sections and incubated for 45 minutes.
15. The slides were washed in phosphate buffer for 5 minutes x 2 changes.
16. The slides were covered with Super Enhancer for 30 minutes.
17. The slides were washed in phosphate buffer for 5 minutes x 2 changes.
18. The slides were covered with SS Label for 30 minutes.
19. Wash in phosphate buffer for 5 minutes x 2 changes.

20. DAB substrate was prepared by diluting 1 drop of DAB chromogen to1ml of DAB buffer.
21. DAB substrate solution was applied on the sections for 8 minutes.
22. Wash with phosphate buffer solution for 5 minutes x 2 changes.
23. The slides are washed well in running tap water for 5 minutes.
24. The sections were counter stained with Hematoxylin stain for 2 seconds (1dip).
25. The slides are washed in running tap water for 3 minutes.
26. The slides are air dried, cleared with xylene and mounted with DPX.

ANNEXURE-V

QUICK SCORE SYSTEM- ER, PR	
Score for Proportion	**Score for Intensity**
0 = no staining 1 = <1 percent nuclei staining 2 = 1-10 percent nuclei staining 3 = 11-33 percent nuclei staining 4 = 34-66 percent nuclei staining 5 = 67-100 percent nuclei staining	0 = no staining 1 = weak staining 2 = moderate staining 3 = strong staining

The scores are added together to obtain a total score that can range from 0 to 8. Tumors scoring 2 or lesser or negative and have a negligible chance of response.

STAINING PATTERN AND HER2-NEU SCORING

STAINING PATTERN	SCORE	HER 2/neu ASSESSMENT
No staining or membrane staining Observed in <10% of tumor cells	0	Negative
A faint /barely perceptible membrane staining observed in >10% of the tumor cells	1+	Negative
A weak to moderate complete membrane staining observed in >10% of the tumor cells.	2+	Positive
A strong complete membrane Staining observed in >30% of the tumor cells.	3+	Positive

C-KIT EXPRESSION SCORING (TSUDA ET AL)[103]

SCORE	STAINING PATTERN
Score 0	No staining was observed or staining was observed in less than 10% of epithelial cells
Score 1+	The cytoplasm was discretely and weakly to moderately stained in 10% or more of the epithelial cells
Score 2+	The cytoplasm was strongly stained with or without membrane staining in 10% or more of the epithelial cells
SCORE 0	NEGATIVE
SCORE 1+ and 2+	POSITIVE

ANNEXURE-VI

TNM Classification of carcinomas of the breast:

T	-	Primary Tumor
T_X	-	Primary tumor cannot be assessed
TO	-	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 : Paget disease associated with a tumor is classified according to the size of the tumor

T1	-	Tumor 2 cm or less in greatest dimension
TMIC	-	Micro invasion 0.1 cm or less in greatest dimension
T1a	-	more than 0.1 cm but not more than 0.5cm in greatest dimension
T1b	-	more than 0.5 cm but not more than 1 cm in greatest dimension.
T1c	-	more than 1 cm but not more than 2 cm in greatest dimension
T2	-	Tumour more than 2 cm but not more than 5 cm in greatest dimension.
T3	-	Tumour more than 5 cm in greatest dimension
T4	-	Tumour of any size with direct extension to chest wall or skin only as described in T4a to T4d.

Note: Chest wall includes ribs, intercostals muscles, and seratus anterior muscle but not pectoral muscle.

T4a	-	Extension to chest wall
T4b	-	Oedema (including peau'd orange) ulceration of the skin of the breast (or) satellite skin nodules confined to the same breast.
T4c	-	Both 4a and 4b, above
T4d	-	Inflammatory Carcinoma.

Notes:Microinvasion is the extension of cancer cells beyond the basement membrane into the adjacent tissues with no focus more than 0.1 cm in greatest dimension. When there are multiple foci of microvasion, the size of only the largest focus is used to classify the microinvasion.

Inflammatory carcinoma of the breast is characterized by diffuse brawny induration of the skin with an erysipeloid edge, usually with no underlying mass. If the skin biopsy is negative

and there is no localized measurable primary cancer, the T category is PTX when pathologically staging a clinical inflammatory carcinoma (T4d). Dimpling of the skin, nipple retraction, or other skin changes, except those in T4p and T4d may occur in T1, T2, or T3 without affecting the classification.

N - Regional Lymph Nodes

Nx - Regional lymph nodes cannot be assessed previously removed

N0 - No regional lymph node metastasis.

N1 - Metastasis in movable ipsilateral axillary lymph node (s)

N2 - Metastasis in fixed ipsilateral axillary lymph node (8) or in Clinically apparent ipsilateral internal mammary lymph node(s) in the absence of clinically evident axillary lymph node metastasis.

N2a - Metastasis in axillary lymph node(s) fixed to one another or to other structures.

N2b - Metastasis only in clinically apparent internal mammary lymph node (s) and in the absence of clinically evident axillary lymph node metastasis.

N3 - Metastasis in ipsilateralinfraclavicular lymph node (s) with or without axillary lymph node involvement, or in clinically apparent ipsilateral internal mammary lymph node (s) in the presence of clinically evident axillary lymph node metastasis or metastasis in ipsilateral supraclavicular lymph node (s) with or without axillary or internal mammary lymph node involvement.

N3a - Metastasis in infraclavicularlymphnode(s)

N3b - Metastasis in supraclavicular lymph nodes

M - Distant metastasis

Mx - Distant metastasis cannot be assessed

M0 - No distant metastasis

M1 - Distant metastasis

S.N	HPE NO	Age	Side	TL	TS	HT	G	LN	M	LVI	LYI	N	SK	AL	ER	PR	HER-2	CD117
1	3433/16	52	R	UOQ	2-5	MUC CA	I	<= 3	A	P	P	A	A	DCIS	POS	NEG	NEG	1+
2	2499/16	49	L	UIQ	< 2	IDC NOS	II	>9	A	P	P	A	A	FCD	POS	POS	POS	2+
3	1586/15	60	L	UOQ	> 5	METAP CA	NIL	Nil	A	A	A	P	A	FCD	NEG	NEG	NEG	NEG
4	3207/16	48	L	UIQ	2-5	IDC NOS	I	Nil	P	P	P	P	P	DCIS	POS	NEG	NEG	NEG
5	1334/15	37	R	UOQ	< 2	IDC NOS	II	Nil	A	A	A	A	A	FCD	POS	NEG	POS	1+
6	358/16	37	R	LIQ	2-5	IDC NOS	II	<= 3	A	P	P	P	A	Nil	NEG	NEG	NEG	1+
7	1689/15	50	L	UOQ	2-5	IDC NOS	II	Nil	P	P	A	A	A	FCD	POS	NEG	NEG	NEG
8	10285/15	55	R	LOQ	< 2	IDC NOS	II	<= 3	A	P	P	P	P	DCIS	POS	POS	NEG	1+
9	301/16	40	R	UOQ	< 2	IDC NOS	I	<= 3	A	A	P	A	A	Nil	POS	POS	NEG	NEG
10	6029/15	75	R	LOQ	< 2	IDC NOS	II	4-9	A	P	P	A	P	DCIS	NEG	NEG	POS	NEG
11	4007/15	52	L	CQ	< 2	IDC NOS	I	<= 3	A	A	P	A	A	Nil	POS	NEG	NEG	NEG
12	1198/15	30	L	LOQ	< 2	IDC NOS	I	4-9	A	A	P	A	A	FCD	POS	POS	NEG	NEG
13	2444/16	65	L	UOQ	< 2	ICPC	NIL	Nil	A	P	P	P	A	EH	POS	POS	NEG	2+
14	8572/14	40	L	UOQ	< 2	APO CA	NIL	Nil	A	A	A	A	A	DCIS	NEG	NEG	NEG	1+
15	6979/15	65	R	UOQ	< 2	MUC CA	NIL	<= 3	A	A	A	A	A	FCD	POS	POS	NEG	1+
16	2812/16	60	L	UOQ	2-5	IDC NOS	II	Nil	A	P	P	P	P	DCIS	POS	NEG	POS	1+
17	6365/15	48	L	LOQ	2-5	IDC NOS	II	Nil	A	A	P	P	P	DCIS	NEG	NEG	NEG	1+
18	69/16	38	L	LIQ	2-5	IDC NOS	II	Nil	A	A	P	P	A	DCIS	NEG	NEG	NEG	1+
19	7004/15	45	L	LIQ	2-5	IDC NOS	II	Nil	A	A	P	P	A	Nil	NEG	POS	POS	1+
20	6675/15	43	R	UIQ	< 2	METAP CA	NIL	Nil	A	P	P	A	P	DCIS	POS	POS	POS	1+
21	10469/14	45	R	UOQ	< 2	IDC NOS	II	<= 3	P	P	A	A	A	DCIS	NEG	POS	NEG	NEG
22	8092/15	65	L	LIQ	2-5	IDC NOS	II	>9	A	P	P	A	A	FCD	NEG	NEG	NEG	1+
23	3678/15	45	L	CQ	> 5	IDC NOS	I	4-9	A	P	P	P	A	DCIS	POS	POS	POS	NEG
24	9437/14	46	L	UIQ	2-5	IDC NOS	III	Nil	A	A	A	P	A	FCD	NEG	NEG	NEG	NEG
25	62/16	65	R	UOQ	2-5	IDC NOS	II	Nil	A	A	P	P	A	FCD	NEG	NEG	POS	NEG
26	1287/16	50	R	LOQ	> 5	IDC NOS	I	<= 3	P	P	P	A	A	FCD	POS	POS	POS	NEG
27	2710/16	60	R	UIQ	2-5	IDC NOS	I	<= 3	A	P	P	A	A	AD	POS	POS	NEG	NEG
28	3116/15	40	L	LIQ	< 2	IDC NOS	I	<= 3	A	P	P	P	A	DCIS	POS	POS	NEG	NEG
29	10225/15	55	L	UOQ	2-5	IDC NOS	III	<= 3	A	P	P	P	A	FCD	NEG	NEG	NEG	NEG
30	10072/15	35	R	LOQ	2-5	IDC NOS	III	Nil	A	A	P	P	A	FCD	NEG	NEG	POS	NEG
31	11719/14	42	L	UOQ	2-5	IDC NOS	I	<= 3	A	P	P	P	A	FCD	NEG	NEG	POS	NEG
32	7885/14	40	R	UOQ	< 2	IDC NOS	III	<= 3	A	P	P	P	P	DCIS	NEG	NEG	POS	NEG
33	7446/15	42	L	UOQ	2-5	IDC NOS	II	>9	A	P	P	P	A	FCD	NEG	NEG	POS	NEG
34	3439/16	40	R	LOQ	> 5	IDC NOS	II	<= 3	A	P	P	A	A	FCD	NEG	NEG	NEG	NEG
35	2327/15	33	R	LOQ	2-5	IDC NOS	I	Nil	A	P	P	A	P	DCIS	POS	POS	POS	1+
36	703/15	55	L	UOQ	< 2	IDC NOS	II	<= 3	A	P	A	P	P	FCD	NEG	NEG	NEG	2+
37	11184/14	67	L	UOQ	2-5	IDC NOS	III	<= 3	A	A	P	A	A	DCIS	NEG	NEG	NEG	NEG
38	5844/15	68	L	UOQ	2-5	IDC NOS	I	<= 3	A	P	P	A	A	FCD	POS	NEG	NEG	NEG
39	8690/15	61	L	UOQ	2-5	IDC NOS	I	<= 3	A	A	P	A	A	FCD	POS	POS	NEG	NEG
40	3198/16	52	R	UOQ	2-5	IDC NOS	I	<= 3	A	P	P	P	A	Nil	POS	POS	NEG	NEG
41	2840/15	43	L	CQ	2-5	IDC NOS	II	<= 3	A	A	P	P	P	DCIS	POS	POS	POS	NEG
42	3491/16	46	R	UOQ	< 2	IDC NOS	II	<= 3	A	P	P	A	A	FCD	NEG	NEG	POS	1+
43	6788/15	60	R	LIQ	> 5	METAP CA	II	<= 3	A	P	P	P	A	FCD	NEG	NEG	NEG	NEG
44	6189/14	65	R	UOQ	> 5	MUC CA	II	<= 3	A	P	A	A	A	FCD	POS	POS	NEG	1+
45	4709/15	45	R	UOQ	2-5	IDC NOS	II	4-9	A	P	P	A	A	DCIS	NEG	NEG	POS	NEG
46	4339/15	42	L	LIQ	2-5	IDC NOS	I	4-9	A	P	P	A	A	FCD	NEG	NEG	NEG	1+
47	46/15	57	L	CQ	2-5	IDC NOS	II	<= 3	A	A	A	A	A	FCD	NEG	NEG	POS	NEG
48	1600/15	35	L	UIQ	> 5	IDC NOS	II	>9	A	P	P	P	A	FCD	POS	NEG	POS	NEG
49	8287/14	55	L	UOQ	2-5	IDC NOS	III	<= 3	A	P	A	A	A	DCIS	POS	NEG	NEG	1+
50	7381/15	45	R	UIQ	2-5	IDC NOS	I	<= 3	A	A	P	A	A	FCD	NEG	NEG	POS	NEG
51	6194/14	45	L	UOQ	> 5	IDC NOS	I	<= 3	A	P	P	A	P	FCD	NEG	NEG	NEG	.
52	6219/14	65	L	UOQ	2-5	IDC NOS	III	Nil	A	P	A	P	A	FCD	NEG	NEG	NEG	.
53	6254/14	52	L	UOQ	> 5	IDC NOS	II	Nil	A	A	A	P	A	FCD	POS	POS	NEG	.
54	6266/14	70	L	UOQ	> 5	IDC NOS	II	Nil	A	A	A	A	A	FCD	POS	POS	NEG	.
55	6309/14	60	R	CQ	2-5	IDC NOS	I	>9	A	A	P	A	A	EH	POS	POS	NEG	.
56	6376/14	37	L	LOQ	2-5	IDC NOS	II	4-9	A	A	A	P	A	AD	NEG	NEG	NEG	.

57	6655/14	50	L	UOQ	< 2	IDC NOS	II	4-9	A	P	A	A	A	FCD	POS	POS	POS	.
58	6670/14	40	L	UOQ	< 2	IDC NOS	I	<= 3	A	P	A	P	A	Nil	POS	POS	NEG	.
59	6906/14	41	R	UOQ	> 5	IDC NOS	II	4-9	A	P	P	P	A	FCD	POS	POS	NEG	.
60	6929/14	55	L	UIQ	< 2	IDC NOS	II	Nil	A	P	A	P	A	Nil	POS	POS	POS	.
61	7004/14	35	R	CQ	2-5	IDC NOS	II	Nil	A	P	P	P	A	DCIS	NEG	POS	POS	.
62	7125/14	67	R	UOQ	< 2	IDC NOS	II	Nil	A	A	P	P	A	FCD	NEG	NEG	NEG	.
63	7209/14	55	L	LOQ	< 2	IDC NOS	II	Nil	A	P	P	A	A	FCD	POS	POS	NEG	.
64	7302/14	48	L	UOQ	< 2	IDC NOS	II	Nil	A	P	A	A	A	Nil	NEG	NEG	NEG	.
65	7421/14	38	L	LIQ	< 2	IDC NOS	I	Nil	A	A	A	A	A	DCIS	POS	POS	POS	.
66	7435/14	46	R	UIQ	2-5	IDC NOS	II	4-9	A	A	P	P	A	DCIS	NEG	NEG	NEG	.
67	7516/14	55	R	CQ	2-5	IDC NOS	II	<= 3	A	A	A	A	A	Nil	POS	POS	POS	.
68	7721/14	33	R	UIQ	2-5	IDC NOS	II	4-9	A	A	P	P	A	FA	POS	POS	NEG	.
69	7839/14	57	L	CQ	2-5	IDC NOS	II	4-9	A	P	P	P	A	Nil	NEG	NEG	NEG	.
71	8076/14	56	R	UOQ	< 2	IDC NOS	II	4-9	A	P	A	P	P	DCIS	POS	NEG	POS	.
72	8280/14	60	R	CQ	< 2	IDC NOS	II	Nil	A	A	P	A	A	FCD	NEG	NEG	NEG	.
73	8326/14	55	L	UOQ	2-5	IDC NOS	II	<= 3	A	P	P	P	A	FCD	NEG	NEG	NEG	.
74	8383/14	53	R	UOQ	2-5	IDC NOS	II	<= 3	A	P	P	P	A	Nil	NEG	NEG	NEG	.
75	8459/14	46	L	UOQ	> 5	IDC NOS	II	<= 3	A	P	P	P	P	DCIS	POS	POS	NEG	.
76	8589/14	37	R	LIQ	2-5	IDC NOS	III	Nil	A	P	P	A	A	Nil	POS	POS	NEG	.
77	8672/14	50	R	CQ	2-5	IDC NOS	I	<= 3	A	P	P	A	A	FA	NEG	NEG	POS	.
78	8932/14	40	R	CQ	2-5	IDC NOS	I	<= 3	A	P	P	A	A	FA	POS	POS	NEG	.
79	9065/14	30	L	UOQ	2-5	IDC NOS	III	<= 3	P	P	A	A	P	FCD	POS	POS	POS	.
80	9083/14	45	L	UOQ	2-5	IDC NOS	II	Nil	P	P	P	P	A	Nil	NEG	NEG	NEG	.
81	9229/14	57	L	UOQ	2-5	IDC NOS	II	4-9	A	P	A	A	A	FCD	NEG	NEG	POS	.
82	9247/14	65	L	UOQ	2-5	IDC NOS	II	<= 3	A	A	P	A	A	DCIS	POS	POS	NEG	.
83	9275/14	60	L	CQ	> 5	IDC NOS	III	Nil	P	P	P	P	P	FCD	POS	POS	NEG	.
84	9438/14	56	R	CQ	2-5	IDC NOS	II	4-9	A	A	A	A	A	Nil	POS	POS	NEG	.
85	9444/14	30	R	CQ	2-5	METAP CA	NIL	Nil	A	A	A	A	A	Nil	NEG	NEG	NEG	.
86	9667/14	40	L	CQ	2-5	IDC NOS	II	<= 3	A	A	P	P	A	FCD	POS	POS	NEG	.
87	9762/14	44	R	UOQ	2-5	IDC NOS	II	Nil	A	P	P	P	A	FA	POS	POS	POS	.
88	9956/14	40	L	UIQ	> 5	IDC NOS	I	Nil	A	A	P	A	A	FA	POS	POS	POS	.
89	10005/14	52	L	CQ	2-5	IDC NOS	II	<= 3	A	A	P	A	A	FCD	NEG	NEG	POS	.
90	10119/14	31	R	UOQ	> 5	IDC NOS	II	Nil	A	A	A	A	A	FCD	POS	POS	NEG	.
91	10371/14	40	R	CQ	2-5	IDC NOS	II	4-9	A	P	P	A	A	ADH	POS	POS	NEG	.
92	10407/14	43	R	CQ	2-5	IDC NOS	I	<= 3	P	P	A	A	A	FCD	NEG	NEG	POS	.
93	10449/14	45	L	CQ	> 5	IDC NOS	III	<= 3	P	A	A	A	A	FCD	NEG	NEG	NEG	.
95	10540/14	53	L	UOQ	2-5	IDC NOS	II	4-9	A	P	P	A	A	FCD	POS	POS	POS	.
96	10598/14	67	L	CQ	2-5	IDC NOS	II	Nil	A	P	A	A	A	FCD	POS	NEG	POS	.
97	10850/14	55	R	CQ	> 5	IDC NOS	II	<= 3	A	P	A	A	A	FA	NEG	NEG	POS	.
98	10939/14	50	R	CQ	> 5	IDC NOS	III	>9	P	P	P	A	A	ADH	POS	POS	NEG	.
99	11008/14	63	R	UOQ	2-5	IDC NOS	II	<= 3	A	P	P	A	A	FCD	POS	POS	NEG	.
100	11141/14	50	R	UOQ	> 5	IDC NOS	II	Nil	A	A	P	P	P	FCD	POS	POS	POS	.
106	11994/14	42	R	CQ	> 5	IDC NOS	II	<= 3	A	A	P	A	A	Nil	NEG	NEG	POS	.
107	122/15	45	L	CQ	2-5	IDC NOS	II	Nil	A	A	A	P	A	FCD	NEG	NEG	POS	.
108	339/15	40	R	LOQ	2-5	IDC NOS	II	Nil	A	A	A	A	A	FCD	NEG	NEG	NEG	.
109	352/15	46	R	CQ	2-5	IDC NOS	II	Nil	A	P	A	A	A	FCD	NEG	NEG	POS	.
110	412/15	55	L	LIQ	2-5	IDC NOS	III	<= 3	A	P	P	A	A	FCD	NEG	NEG	POS	.
111	11314/14	45	L	CQ	2-5	IDC NOS	II	Nil	A	A	A	P	A	FCD	NEG	POS	POS	.
111	617/15	45	R	CQ	2-5	IDC NOS	I	4-9	A	A	P	P	A	Nil	NEG	NEG	POS	.
112	11413/14	50	R	CQ	2-5	IDC NOS	II	Nil	A	A	P	P	A	Nil	NEG	NEG	POS	.
112	1097/15	40	R	CQ	> 5	IDC NOS	III	Nil	A	P	A	A	P	ADH	NEG	NEG	POS	.
113	11474/14	50	L	UOQ	2-5	IDC NOS	II	Nil	A	P	P	P	A	FCD	NEG	NEG	NEG	.
113	1157/15	50	R	LIQ	> 5	IDC NOS	II	Nil	A	A	A	A	A	FCD	NEG	NEG	NEG	.
114	11537/14	65	L	UOQ	2-5	IDC NOS	III	Nil	A	A	A	A	A	FCD	POS	POS	NEG	.
114	1240/15	55	R	UIQ	2-5	IDC NOS	I	Nil	A	A	P	A	A	Nil	NEG	NEG	NEG	.
115	11889/14	37	R	UOQ	2-5	IDC NOS	II	<= 3	A	A	A	A	A	FCD	POS	NEG	NEG	.
115	11928/14	60	R	CQ	> 5	IDC NOS	II	Nil	A	P	P	P	A	Nil	NEG	NEG	POS	.

115	1424/15	50	R	UOQ	2-5	IDC NOS	I	4-9	A	A	A	A	A	DCIS	POS	POS	POS	.
116	1590/15	60	R	CQ	2-5	IDC NOS	II	Nil	A	A	P	A	A	FCD	NEG	NEG	POS	.
117	1968/15	55	R	CQ	> 5	IDC NOS	III	Nil	A	A	P	P	A	DCIS	NEG	NEG	NEG	.
118	2044/15	45	R	LOQ	2-5	IDC NOS	II	4-9	A	A	P	P	A	FCD	NEG	NEG	POS	.
119	2741/15	48	R	CQ	2-5	IDC NOS	I	Nil	A	A	A	P	A	FCD	NEG	NEG	POS	.
120	2849/15	46	R	LOQ	2-5	IDC NOS	I	Nil	A	A	A	P	A	FCD	POS	POS	NEG	.
121	2858/15	55	L	LIQ	2-5	IDC NOS	II	<= 3	A	A	P	A	A	DCIS	NEG	NEG	NEG	.
122	2863/15	50	L	UOQ	< 2	IDC NOS	III	<= 3	A	P	P	P	A	FCD	NEG	NEG	NEG	.
123	2995/15	40	L	UIQ	2-5	IDC NOS	II	Nil	A	A	P	P	A	Nil	NEG	NEG	POS	.
124	3115/15	54	R	UOQ	< 2	IDC NOS	II	Nil	A	A	P	P	A	FCD	POS	POS	POS	.
125	3405/15	49	R	UOQ	2-5	IDC NOS	I	<= 3	A	P	P	A	A	DCIS	POS	POS	NEG	.
126	3525/15	50	L	UIQ	2-5	IDC NOS	I	<= 3	A	P	P	P	A	FCD	NEG	NEG	NEG	.
127	3854/15	40	L	UOQ	< 2	IDC NOS	II	<= 3	A	P	P	P	A	FCD	NEG	NEG	POS	.
128	3893/15	60	R	UIQ	< 2	IDC NOS	I	Nil	A	A	P	A	A	FCD	NEG	NEG	NEG	.
129	4431/15	65	L	UOQ	2-5	IDC NOS	I	Nil	A	A	P	A	A	FCD	POS	NEG	NEG	.
130	4556/15	65	R	LOQ	2-5	IDC NOS	I	>9	A	P	P	A	A	AD	POS	NEG	NEG	.
131	5470/15	49	R	LOQ	< 2	IDC NOS	II	<= 3	A	P	P	A	A	FCD	POS	POS	NEG	.
132	5550/15	34	R	UIQ	2-5	IDC NOS	II	Nil	A	P	P	P	A	FCD	NEG	NEG	NEG	.
133	5603/15	69	R	UIQ	2-5	IDC NOS	II	Nil	A	P	P	A	A	FCD	NEG	NEG	NEG	.
134	5756/15	50	L	UOQ	2-5	IDC NOS	II	Nil	A	P	P	P	A	FCD	NEG	NEG	POS	.
135	6009/15	45	R	LIQ	2-5	IDC NOS	I	4-9	A	P	P	A	A	FCD	POS	NEG	NEG	.
136	6259/15	65	R	LIQ	< 2	MUC CA	II	Nil	A	A	P	A	A	DCIS	POS	POS	NEG	.
137	6432/15	47	R	UIQ	2-5	IDC NOS	II	<= 3	P	P	P	A	P	FCD	NEG	NEG	POS	.
138	6487/15	35	R	UIQ	> 5	IDC NOS	I	4-9	A	P	P	A	A	FCD	NEG	NEG	NEG	.
139	7197/15	57	R	UOQ	> 5	IDC NOS	II	Nil	A	A	P	P	A	DCIS	NEG	NEG	POS	.
140	7299/15	32	R	UOQ	2-5	IDC NOS	II	Nil	A	A	P	P	A	FCD	NEG	NEG	NEG	.
141	7454/15	45	L	LOQ	2-5	IDC NOS	II	4-9	A	P	P	P	A	AD	NEG	NEG	POS	.
142	7537/15	60	L	UOQ	2-5	IDC NOS	I	<= 3	A	P	P	P	A	FCD	NEG	NEG	NEG	.
143	7685/15	58	R	CQ	2-5	IDC NOS	I	<= 3	A	P	P	P	A	FCD	POS	POS	POS	.
144	8185/15	45	R	UIQ	2-5	IDC NOS	I	Nil	A	P	P	A	A	FCD	NEG	NEG	NEG	.
145	8425/15	40	L	LOQ	2-5	IDC NOS	I	Nil	A	A	P	A	A	AD	NEG	POS	NEG	.
146	8433/15	54	L	LOQ	< 2	IDC NOS	II	>9	A	P	P	A	A	FCD	POS	POS	NEG	.
147	8459/15	37	L	LIQ	2-5	IDC NOS	II	Nil	A	A	P	P	A	FCD	NEG	NEG	NEG	.
148	8783/15	49	L	UIQ	2-5	IDC NOS	II	4-9	A	P	P	P	A	FCD	POS	POS	POS	.
149	9051/15	40	R	UIQ	< 2	IDC NOS	II	Nil	A	A	P	P	A	Nil	POS	POS	NEG	.
150	9413/15	30	L	UOQ	2-5	IDC NOS	II	<= 3	A	P	P	A	A	FCD	POS	NEG	NEG	.
151	9636/15	50	R	UOQ	< 2	IDC NOS	I	>9	A	P	P	P	A	FCD	NEG	NEG	NEG	.
152	9667/15	40	L	UIQ	< 2	IDC NOS	II	<= 3	A	P	P	P	A	FCD	POS	POS	POS	.
153	10105/15	51	R	UOQ	2-5	IDC NOS	II	Nil	A	P	P	A	A	FCD	POS	POS	NEG	.
154	10175/15	39	L	UOQ	2-5	IDC NOS	I	Nil	A	P	P	A	A	FCD	NEG	NEG	POS	.
155	10560/15	37	L	UIQ	> 5	IDC NOS	III	4-9	A	P	P	A	A	FCD	POS	POS	NEG	.
156	10881/15	40	R	LOQ	> 5	IDC NOS	II	<= 3	A	P	P	P	P	FCD	NEG	POS	NEG	.
157	549/16	47	R	LOQ	2-5	IDC NOS	II	4-9	A	P	P	P	A	FCD	POS	POS	NEG	.
158	980/16	39	R	LOQ	< 2	IDC NOS	II	Nil	P	A	P	P	A	FCD	NEG	NEG	NEG	.
159	1189/16	48	R	LOQ	2-5	MUC CA	NIL	<= 3	A	P	P	A	A	Nil	POS	NEG	NEG	.
160	1716/16	56	R	LIQ	2-5	IDC NOS	I	<= 3	A	P	P	A	A	FCD	NEG	NEG	NEG	.
161	1879/16	66	R	UOQ	2-5	IDC NOS	I	<= 3	A	A	P	P	A	FCD	POS	NEG	NEG	.
162	2338/16	50	R	UIQ	< 2	IDC NOS	II	<= 3	A	A	P	P	A	FCD	POS	NEG	NEG	.
163	2172/16	62	R	LIQ	2-5	IDC NOS	I	Nil	A	A	P	P	A	FCD	POS	NEG	NEG	.
164	2660/16	60	L	CQ	< 2	IDC NOS	II	<= 3	A	P	P	P	A	FCD	NEG	NEG	NEG	.
165	3452/16	45	R	UOQ	2-5	IDC NOS	III	Nil	A	A	P	P	A	Nil	NEG	NEG	NEG	.
165	3626/16	45	L	UOQ	2-5	IDC NOS	II	Nil	A	A	P	A	A	FCD	NEG	NEG	NEG	.

KEY TO MASTER CHART

R	:	Right
L	:	Left
TL	:	Tumor Location
UOQ	:	Upper Outer Quadrant
UIQ	:	Upper Inner Quadrant
CQ	:	Central Quadrant
LIQ	:	Lower Inner Quadrant
LOQ	:	Lower Outer Quadrant
HT	:	Histological Type
IDC NOS	:	Infiltrating Ductal Carcinoma- Not Otherwise Specified
ICPC	:	Intra Cystic Papillary Carcinoma
METAP CA	:	Metaplastic Carcinoma
MUC CA	:	Mucinous Carcinoma
APO CA	:	Apocrine Carcinoma
M	:	Margin
G	:	Grade
AL	:	Associated Lesion
DCIS	:	Ductal Carcinoma InSitu
FCD	:	Fibrocystic Disease
SA	:	SelerosingAdenosis
UDH	:	Usual Ductal Hyperplasia
ADH	:	Atypical Ductal Hyperplasia
FA	:	Fibroadenoma
LVI	:	LymphoVascular Invasion

LYI	:	Lymphocytic Infiltration
N	:	Necrosis
SK	:	Skin Involvement
LN	:	Lymph Node Status
ER	:	Estrogen Receptor
PR	:	Progestrone Receptor
CD117	:	C-KIT
H2N	:	HER2neu
A	:	Absent
P	:	Present
POS	:	Positive
NOS	:	Negative

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 diagnose certain cases of Breast cancer easily with the help of certain special tests.
- 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 IMMUNOHISTO-CHEMICAL EXPRESSION OF C-KIT IN INVASIVE BREAST CARCINOMA AND CLINICOPATHOLOGICAL CORRELATION"**

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 IMMUNOHISTO-CHEMICAL EXPRESSION OF C-KIT IN INVASIVE BREAST CARCINOMA AND CLINICOPATHOLOGICAL CORRELATION".**

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 tumors 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 understand 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_____

ஆராய்ச்சி தகவல் தாள்

ஆராய்ச்சி தலைப்பு : **மார்பக புற்றுநோய்களில் c-kit எனும் நோய்எதிர்ப்பு திசுவேதியியல் குறியீட்டின் வெளிப்பாடு மற்றும் மருத்துவ நோய்குறியியல் தொடர்புகளை பற்றி ஆராய்தல்.**

ஆய்வாளர் : மரு. **N.** லாவண்யா
நோய்குறியியல் துறை,
சென்னை மருத்துவக் கல்லூரி,
சென்னை - 600003.

தங்களது மார்பக புற்றுநோய் கட்டி (அறுவை சிகிச்சை செய்யப்பட்ட கட்டி) இங்கு பெற்றுக் கொள்ளப்பட்டது.

இராஜீவ் காந்தி அரசு பொது மருத்துவமனைக்கு வரும் நோயாளிகளிடம் இருக்கும் மார்பக புற்றுநோய் கட்டிகளைப் பற்றிய ஒரு ஆராய்ச்சி இங்கு நடைபெற்று வருகின்றது.

இந்த மார்பக புற்றுநோய் கட்டிகளில் c-kit எனும் நோய்எதிர்ப்பு திசு வேதியியல் குறியீடு வெளிப்பாடு மற்றும் மருத்துவ நோய்குறியியல் தொடர்புகளை பற்றி ஆராய்வரே எனது ஆய்வின் நோக்கமாகும்.

நீங்களும் இந்த ஆராய்ச்சியில் பங்கேற்க நாங்கள் விரும்புகிறோம். இந்த ஆராய்ச்சியில் உங்களுடைய திசுக்களை எடுத்து சில சிறப்புப் பரிசோதனைக்கு உட்படுத்தி அதன் தகவல்களை ஆராய்வோம். அதனால் தங்களது நோயின் ஆய்வறிக்கையோ அல்லது சிகிச்சையோ பாதிப்புக்குள்ளாகாது என்பதையும் தெரிவித்துக் கொள்கிறோம்.

முடிவுகளை அல்லது கருத்துகளை வெளியிடும் போதோ அல்லது ஆராய்ச்சியின் போதோ தங்களது பெயரையோ அல்லது அடையாளங்களையோ வெளியிட மாட்டோம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த ஆராய்ச்சியில் பங்கேற்பது தங்களுடைய விருப்பத்தின் பேரில் தான் இருக்கிறது. மேலும் நீங்கள் எந்நேரமும் இந்த ஆராய்ச்சியில் இருந்து பின்வாங்கலாம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த சிறப்புப் பரிசோதனைகளின் முடிவுகளை ஆராய்ச்சியின் போது அல்லது ஆராய்ச்சியின் முடிவின் போது தங்களுக்கு அறிவிப்போம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த ஆய்வை பற்றிய சந்தேகங்களுக்கு தொடர்பு கொள்ள வேண்டியவர் : மரு. **N.**லாவண்யா. செல் : 9444894598

பங்கேற்பாளர் கையொப்பம்..... இடம் :..... தேதி :.....

பங்கேற்பாளர் பெயர் மற்றும் விலாசம் .....

ஆராய்ச்சியாளர் கையொப்பம்..... இடம் :..... தேதி :.....

ஆராய்ச்சி ஒப்புதல் கடிதம்

ஆராய்ச்சி தலைப்பு : **மார்பக புற்றுநோய்களில் c-kit எனும் நோய்எதிர்ப்பு திசுவேதியியல் குறியீட்டின் வெளிப்பாடு மற்றும் மருத்துவ நோய்குறியியல் தொடர்புகளை பற்றி ஆராய்தல்.**

சென்னை மருத்துவக் கல்லூரி நோய்குறியியல் துறையில் பயிலும் முதுகலை மருத்துவர் N. லாவண்யா, அவர்கள் மேற்கொள்ளும் இந்த ஆய்வில் பங்குகொள்ள ..... ஆகிய நான் முழு மனதுடன் சம்மதிக்கிறேன்.

எனக்கு விளக்கப்பட்ட விஷயங்களை நான் புரிந்து கொண்டு நான் எனது சம்மதத்தைத் தெரிவிக்கிறேன்.

இந்த ஆராய்ச்சியில் பிறரின் நிர்ப்பந்தமின்றி என் சொந்த விருப்பத்தின் பேரில் தான் பங்கு பெறுகிறேன் மற்றும் நான் இந்த ஆராய்ச்சியிலிருந்து எந்நேரமும் பின்வாங்கலாம் என்பதையும் அதனால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் நான் புரிந்து கொண்டேன்.

நான் மார்பக புற்றுநோய் கட்டி நோய்கள் குறித்த இந்த ஆராய்ச்சியின் விவரங்களைக் கொண்ட தகவல் தாளைப் பெற்றுக் கொண்டேன்.

நான் என்னுடைய சுயநினைவுடன் மற்றும் முழு சுதந்திரத்துடன் இந்த மருத்துவ ஆராய்ச்சியில் என்னை சேர்த்துக் கொள்ள சம்மதிக்கிறேன்.

எனக்கு அறுவை சிகிச்சை செய்யப்பட்டு நோய்க்குறியியல் துறையில் சதைப் பரிசோதனைக்கு பயன்பட்ட மெழுகுக்கட்டிகளை வைத்து ஆராய்ச்சி மற்றும் சிறப்புப் பரிசோதனை செய்யது கொள்ள சம்மதம் தெரிவிக்கிறேன்.

பங்கேற்பாளர் கையொப்பம்..... இடம் :..... தேதி :.....

பங்கேற்பாளர் பெயர் மற்றும் விலாசம் .....

ஆராய்ச்சியாளர் கையொப்பம்..... இடம் :..... தேதி :.....