A COMPARATIVE STUDY OF THE EXPRESSION OF HER2/neu IN INVASIVE DUCTAL CARCINOMA OF BREAST ASSOCIATED WITH AND WITHOUT DUCTAL CARCINOMA IN SITU

DISSERTATION SUBMITTED TO

THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY

CHENNAI

in partial fulfillment of

the requirements for the degree of

M.D. (PATHOLOGY)

BRANCH - III

TIRUNELVELI MEDICAL COLLEGE HOSPITAL

TIRUNELVELI

APRIL-2015

CERTIFICATE

This is to certify that this Dissertation entitled "**A COMPARATIVE STUDY OF THE EXPRESSION OF HER2/neu IN INVASIVE DUCTAL CARCINOMA OF BREAST ASSOCIATED WITH AND WITHOUT DUCTAL CARCINOMA IN SITU** " is the bonafide original work of **Dr.B.DHIVYA**, during the period of her Post graduate study from 2012 – 2015, under my guidance and supervision, in the Department of Pathology Tirunelveli Medical College & Hospital, Tirunelveli, in partial fulfillment of the requirement for M.D., (Branch III) in Pathology examination of the Tamilnadu Dr.M.G.R Medical University will be held in April 2015.

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DECLARATION

I solemnly declare that this dissertation titled **"A COMPARATIVE STUDY OF THE EXPRESSION OF HER2/neu IN INVASIVE DUCTAL CARCINOMA OF BREAST ASSOCIATED WITH AND WITHOUT DUCTAL CARCINOMA IN SITU "** submitted by me for the degree of M.D, is the record work carried out by me during the period of 2012-2015 under the guidance of **Prof. Dr.Sithy Athiya Munavarah,** Professor of Pathology, Department of Pathology, Tirunelveli Medical College, Tirunelveli. The dissertation is submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, towards the partial fulfilment of requirements for the award of M.D. Degree (Branch III) Pathology examination to be held in April 2015.

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ACKNOWLEDGEMENT

I take immense pleasure at this opportunity to acknowledge all those who have helped me to make this dissertation possible.

I express my heartfelt thanks to the **Dean, Tirunelveli Medical College,** for permitting me to undertake this study. I express my profound sense of gratitude to **Dr.K.Shantaraman ,MD.,** my respected Professor and Head of Department of Pathology, Tirunelveli Medical College, Tirunelveli, for his valuable advice, constant guidance and motivation in the preparation of this work.

I consider it my privilege and honour to have worked under the unstinted guidance, encouragement, and supervision of my respected guide **Dr.Sithy Athiya Munavarah MD.,** Professor of Pathology.

I thank **Dr.S.Vallimanalan MD, Dr.K.Swaminathan M.D., Dr. J.Suresh Durai, MD., Dr.Arasi Rajesh, MD.,** Professors of Pathology, for their constant support and inspiration. I also thank the Assistant Professors, for their encouragement and support. I sincerely thank the Professors and faculties of the Department of General Surgery for providing me the patients for my study. I take this opportunity to thank all my postgraduate colleagues for their valuable support. My thanks to all the technicians and other members of the Department of Pathology for their kind help at different stages of this study.

I thank all my family members for their encouragement and support during this study.

Dr. **B.Dhivya**

ABBREVATIONS

ASCO	-	American Society Of Oncologists
ER	-	Estrogen Receptor.
HER2/neu	-	Human Epidermal Growth Factor/neuroblastoma
IDC/DCIS	-	Invasive Ductal Carcinoma with Ductal Carcinoma In Situ.
IDC	-	Invasive Ductal Carcinoma without ductal carcinoma in situ.
IDC, NOS	-	Invasive ductal carcinoma, Not otherwise specified.
IHC	-	Immunohistochemistry.
LVI	-	Lymphovascular invasion.
PR	-	Progesterone Receptor.
TDLU	-	Terminal Duct Lobular Unit.
WHO	-	World Health Organization

CONTENTS

ABSTRACT

AIM:

The clinical outcome of breast carcinoma varies in every individual due to its molecular heterogeneity. Nowadays there is a rising interest whether the associated DCIS in invasive ductal carcinoma of breast affects the prognosis and overall survival of the patient. Only a very few studies has assessed the expression of molecular markers between IDC-DCIS and pure IDC(IDC without DCIS). So the present study was undertaken to study the expression of HER2/neu in invasive ductal carcinoma of breast associated with and without DCIS and also to find the correlation with other clinico-pathological variables with such expression.

MATERIALS AND METHODS:

Two groups were categorized based on the presence or absence of in situ component in invasive ductal carcinoma of breast. A total of 50 mastectomy specimens were studied for the expression of HER2/neu by IHC , which includes 25 cases of invasive ductal carcinoma associated with DCIS (IDC-DCIS) and 25 cases of invasive ductal carcinoma without DCIS (IDC).

RESULTS:

There is no statistical difference in HER2/neu expression between IDC-DCIS and pure IDC (IDC without DCIS) .The expression of Ki 67 was

significantly higher (p value 0.022) in IDC without DCIS (60%) than IDC-DCIS (28%).The expression of HER2/neu is associated with large tumor size, positive lymph node status and ER status in IDC without DCIS. Ki 67 which is considered a predictor of chemotherapy is correlated with HER2/neu expression in both the groups but significantly higher in pure IDC (without DCIS).

CONCLUSION:

IDC associated with DCIS (IDC-DCIS) shows a less malignant behavior compared to IDC without DCIS. Since molecular markers play an important role in tumor carcinogenesis and progression further studies to be done in large scale which might help in identifying the subgroup for targeted therapy.

Key words: Breast carcinoma, heterogeneity, carcinogenesis, HER2/neu, Ki 67.

INTRODUCTION

Breast carcinoma is the most common malignant tumor among women .It constitutes 25% of all the cancers in women worldwide. [1]The incidence of breast carcinoma has been rising due to increased mammographic screening and change in lifestyle .[2]

The clinical outcome of breast carcinoma varies with every individual due to the heterogeneous nature of the tumor .Increased use of mammography has led to the early detection of in situ carcinoma which represents 20-45% of all newly detected mammographic breast lesions.[3]Many studies showed about 50% of untreated DCIS progresses to invasive breast cancer. The duration required for the progression of in situ components to invasive cancer and the factors predicting such progression is largely unknown.[4]

In tumors with both invasive and in situ components (IDC-DCIS), it is believed that the invasive cancer develops from the in situ component. In tumors without DCIS component, the invasive ductal carcinoma (IDC) is presumed to be arising directly from atypical ductal hyperplasia (ADH). It is also debated whether the pure IDC (IDC without DCIS) is distinct type from IDC associated with DCIS (IDC-DCIS). [(5-7)]

Recent studies have tried assessing the effect of presence of in situ carcinoma in invasive ductal carcinoma (IDC-DCIS) on the prognosis and overall survival of the patients. Studies done by Wong et al[6] showed

that the presence of in situ component is associated with a low aggressive potential. Chagpar et al[8] concluded that the presence of in situ component is associated with increased disease free survival and overall survival. However presence of in situ component is yet to be proven as independent predictor of the outcome.

Several molecular markers have been implicated in the development and prognosis of the breast cancer including hormone receptors (ER/ PR), HER2/neu, p53,Ki 67 etc. In addition to predict the prognosis, these markers are also used for target directed therapy and to assess the response to treatment. The effect of these markers in the IDC-DCIS is yet to be evaluated, which might have an effect on the therapeutic approach to breast carcinoma.

Only a very few studied the expression of HER2/neu and the hormone receptors (ER/PR) between the two groups (IDC and IDC-DCIS), however the results are not consistent.

Hence the present study was undertaken to evaluate the expression of HER2/neu in Invasive ductal carcinoma associated with DCIS (IDC-DCIS) and invasive ductal carcinoma without DCIS (IDC) and to assess the correlation of other clinical and prognostic factors associated with such expression.

AIMS AND OBJECTIVES

1. To evaluate the expression of HER2/neu in Invasive ductal carcinoma associated with and without DCIS.
2. To correlate the expression of HER2/neu with other prognostic factors
   a. Clinical variables (age, menopausal status)
   b. Pathological variables (tumor size, tumor grade, tumor necrosis, nipple invasion, lymphovascular invasion, lymph node status, ER , PR status and proliferation index)
3. To determine whether the association of HER2/neu with other prognostic factors differ between IDC with and without DCIS.

REVIEW OF LITERATURE

History:

Breast cancer was first noted by ancient Egyptians more than 3500 years ago. They described it as bulging tumors in breast which has no cure .The descriptions of Edwin Smith and George Ebers Papyrus about breast tumors were consistent with the present descriptions of breast cancer. In 460 BC Hippocrates described breast cancer as a humoral disease. He also named cancer as karkinos which is a Greek word for crab. It was named so because the tumor seemed to have tentacles like legs of crab.[9]

In 1757 Henri Le Dran suggested along with surgical removal of the tumor, removal of infected lymph nodes in arm pits will help to cure breast cancer. Claude Nicolas Le Cat also argued that breast cancer was only cured by surgical therapy.[10]

By 19th century William Halstead introduced radical breast surgery for breast cancer. In the year of 1976 Fisher noticed that breast conserving surgery followed by chemotherapy or radiotherapy is as effective as radical mastectomy. By 1995 there was a development in the hormonal therapies for breast cancer.[9]

ANATOMY OF BREAST

The breast is a modified apocrine sweat gland. It forms an important accessory organ of female reproductive system.

Embryology

The mammary glands develops from the ectodermal mammary ridges at the fifth week of fetal development. They extend on the ventral surface of the fetus from the axillary to inguinal region bilaterally. Around seventh week in utero major part of mammary ridge disappear .

A small portion of mammary ridge persist in the fourth or fifth intercostals space called the primary mammary buds . Primary buds of ectoderm starts penetrating downward into the underlying mesoderm .By 12th week of gestation ,the primary mammary buds develop into secondary buds which will eventually form the mammary lobules.

In fifth month in utero, the ectodermal penetration produce 15 –20 radial branching ingrowths into the developing breast. Small lumina will develop within the mammary buds which forms the lactiferous ducts and their branches . The lactiferous ducts converge to open into a shallow mammary pit , which then transforms into nipple during infancy.[11]

Anatomy

The breast is covered by skin and subcutaneous tissue and it lies on the pectoral muscle separated by a fascia. It extends from the 2nd to 6th rib vertically and from the lateral border of sternum to the mid axillary line horizontally. A small extension called axillary tail of spence extends laterally towards the axilla.

The nipple lies at the level of 4th intercostal space. Nipple is pierced by 15-20 lactiferous duct .Surrounding the nipple is a circular pigmented area called areola. Areola is rich in modified sebaceous glands. Fibrous strands extend from the dermis into the breast, which attach the skin and nipple to the breast called the suspensory ligaments of Cooper.

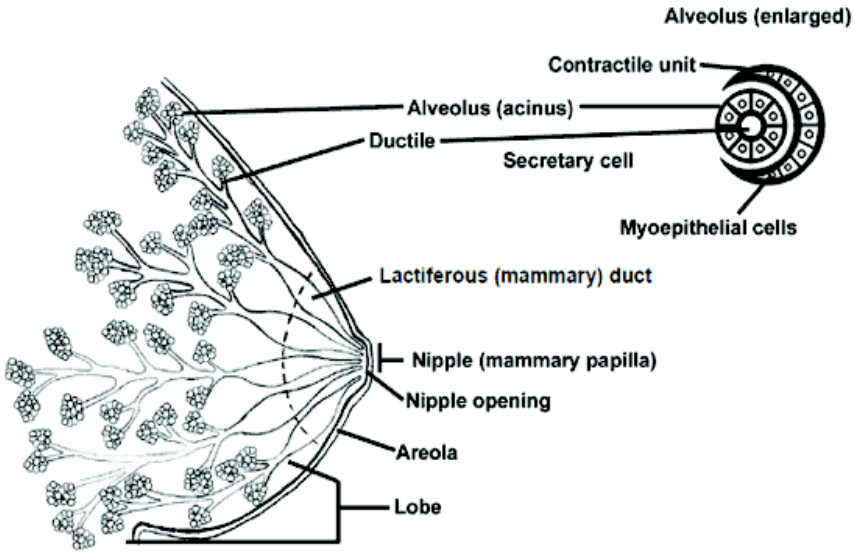


Fig 1: Anatomy of breast

The breast parenchyma is composed of glandular tissue which is arranged topographically into lobes. The lobes is made up of terminal duct lobular unit [TDLU] and the large duct system. The TDLU is the

secretory portion of breast. It consists of lobule and terminal ductule. Each lobule in turn is a cluster of acini. The TDLU connects with the lactiferous (collecting) duct by means of sub segmental and segmental duct. The lactiferous duct opens in to the nipple .A fusiform dilatation called the lactiferous sinus is present between the lactiferous and segmental duct. [12,11]

The intralobular stroma envelopes the acini of the lobules. It consists of breast-specific hormonally responsive fibroblast-like cells admixed with scattered lymphocytes. The stroma appears myxoid .The interlobular stroma is made of dense fibrous connective tissue admixed with adipose tissue. [12]

Blood supply

Arterial supply of breast is by,

i. Internal thoracic artery.
ii. Branches of the lateral thoracic, superior thoracic and acromiothoracic arteries.
iii. Lateral branches of posterior intercostal arteries.

Venous drainage of the breast follows the course of arteries forming an anastomotic circle in the subcutaneous tissue beneath the nipple-areola complex.

From this the veins run as,

1. Superficial veins draining into internal thoracic vein.
2. Deep vein draining into internal thoracic, axillary and posterior intercostal veins.[13]

Nerve supply:

Nerve supply is by anterior and lateral cutaneous branches of 4th and 6th intercostal nerves.[13]

Lymphatic drainage

1. Axillary lymph nodes: Lymphatic drainage is mainly into the anterior group of axillary nodes. Posterior, lateral, central and apical groups of nodes also receive lymphatic drainage either directly or indirectly.
2. The internal mammary nodes which lies along internal thoracic vessels.
3. Supraclavicular node, cephalic node, posterior intercostal, subdiaphragmatic and subperitoneal lymph plexus[13]

Lymphatic vessels of breast:

1. The superficial lymphatics drains overlying skin of breast except nipple and areola .They pass radially to the surrounding lymph nodes (axillary, internal mammary, supraclavicular and cephalic node)

2. The deep lymphatics drain the parenchyma ,nipple and areola of breast. About 75% of lymph drains into axillary nodes, 20% into internal mammary nodes and 5 % into posterior intercostal nodes.[11]

Histology

The keratinizing squamous epithelium of the overlying skin dips into the orifices of the nipple. It then changes in to a double-layered cuboidal epithelium .The entire ductal-lobular unit is lined by two cell layers: luminal epithelial cells and basally located myoepithelial cells. Luminal cells are either columnar or cuboidal depending on their function.[12] It has been proposed that a committed stem cell in the terminal duct give rise to both luminal and myoepithelial cells.[(2,12)] The entire glandular epithelial system lies on a continuous basement membrane. Occasional scattered endocrine cells are also found in normal breast.[12]

The nipple was formed by the lactiferous duct along with the sebaceous unit. The epidermis of nipple and areola is similar to that of normal skin but shows increased melanin content in basal layer. It also shows occasional clear cells called Toker cells in the basal layer.

The luminal cells in the lobules are capable of producing milk .The contractile myoepithelial cells assist in milk ejection during lactation and also produces structural support to lobules.[12]

Immunohistochemically the luminal epithelial cells were positive for keratin, EMA, lactalbumin, GCDFP-15. Myoepithelial cells were positive for S-100, Smooth Musle Actin, calponin, caldesmon(duct portion) and also shows nuclear reactivity for p63.[12]

Physiology of breast

Estrogen and progesterone plays a major role in the development of breast. During the first half of menstrual cycle the lobules are relatively inactive. After ovulation, under the influence of estrogen and increasing progesterone levels, cell proliferation increases and the number of acini increases per lobule. The intralobular stroma will become edematous. During menstruation, as the estrogen and progesterone levels begins to fall there will be regression of the lobules with disappearance of the stromal edema.

During pregnancy the breast become completely mature and functional .There will be a progressive increase in number and size of the lobules which are separated by relatively scant stroma. After delivery the luminal cells start producing colostrum (high in protein) .As the progesterone level drops in the next 10 days there will be milk secretion(higher in fat and calories).On cessation of lactation the epithelial cells undergo apoptosis ,the lobules regress and become atrophic. But however full regression will not occur. During premenopause there will

be involution of the lobules. In elderly females the lobules may become completely atrophic.[12]

Intraductal proliferative lesions

Intraductal proliferative lesions originate from terminal duct lobular unit. They are confined within mammary duct lobular system. They are associated with an increased risk for the subsequent development of invasive carcinoma.

The WHO working group proposed a modified ductal intraepithelial neeoplasia(DIN) which is as follows[14]

Table 1: Classification of Intraductal proliferative lesion

Traditional terminology	**DIN terminology**
Usual ductal hyperplasia	(No DIN equivalent)
Flat epithelial atypia	DIN1A
Atypical ductal hyperplasia	DIN1B
DCIS grade I	DIN1C
DCIS grade II	DIN2
DCIS grade III	DIN3

UDH has a risk of 1.5 times to develop into invasive carcinoma.ADH has a risk of 4-5 fold to develop into invasive carcinoma and DCIS have a risk of 8-10 fold.[15]

Usual Ductal hyperplasia

UDH is a benign ductal proliferative lesion. Though it is not a precursor lesion, long term follow up of the patients with UDH shows a slightly elevated risk of invasive carcinoma.[16]

Microscopy

It shows secondary lumens of varying size and shape. The lumens are often peripherally distributed and in the center the proliferating cells are arranged in a streaming pattern.UDH have a admixture of any of the two or more cell types (epithelial, myoepithelial or metaplastic apocrine cells).

Flat epithelial atypia

It is also termed as low grade clinging carcinoma, columnar cell hyperplasia and columnar cell lesion. Only a very few cases have progressed to invasive breast cancer .Several studies showed that flat epithelial atypia is frequently associated with DCIS, lobular carcinoma in situ and tubular carcinoma.[17]

Microscopy

The mature epithelial cells lining the ducts are replaced by a single or stratified layers of mild atypical cells .Apical snouts are usually seen.[18]The affected TDLU are usually distended. Proliferating atypical cells are monotonous with uniform cuboidal to columnar cells forming 3-5 cell layers

Atypical ductal hyperplasia

ADH is a neoplastic intraductal lesion with a moderately elevated risk of developing into invasive breast cancer.ADH was first proposed by Page-Dupont team [19] for some of the proliferative lesions which did not show all the features of in situ carcinoma.

Based on this, a group organised by college of American Pathologists [20] categorised fibrocystic disease into three groups ,

1. No or mild hyperplasia : There is no risk for subsequent invasive breast carcinoma.
2. Moderate or florid hyperplasia : There is risk of 1.5-2 times that of general population to develop into invasive carcinoma.
3. Atypical ductal or lobular hyperplasia:It has a risk of 5 times that of general population to develop into invasive carcinoma.

Though ADH shows cytological and architectural features similar to that of low grade DCIS, it also shows one or more of the following features,

a) Features of Usual ductal hyperplasia
b) Only partial involvement of TDLU.

Architectural patterns seen in ADH will be micropapillae, fronds, tufts, bridges, cribriform and solid patterns. The individual cells are monomorphic with oval to rounded nuclei.

DUCTAL CARCINOMA IN SITU

Ductal carcinoma in situ is a neoplastic intraductal lesion with increased epithelial proliferation with mild to marked nuclear atypia with a tendency to progress towards invasive ductal carcinoma.[14] DCIS is believed to originate from TDLU.[12] DCIS has a relative risk of 8-11 times to develop into invasive ductal carcinoma .

Extensive intraductal carcinoma:

The tumors shows intraductal component comprising about 25 % of the tumor area with invasion in the surrounding breast parenchyma.[21]

Several morphological types of DCIS exist which includes the following,

1. Comedocarcinoma

Comedocarcinoma may attain a large size and become palpable. About half of the cases are located in the central quadrant .Multicentricity has been reported in about 33 % of the cases.[22]

Gross:

It presents as cluster of thick walled ducts with intervening breast parenchyma .The ducts extrudes plugs of necrotic material when they are compressed. The necrotic material grossly resemble that of comedones and so it is named as comedocarcinoma.

Microscopy:

The duct shows solid growth of large pleomorphic tumor cells with increased mitotic activity. The diameter of the duct is larger and they usually have a central necrosis .The ducts in turn are surrounded by concentric fibrosis with mild mononuclear cell infiltration. Coarse calcifications are predominant in the necrotic areas which can be identified by mammography. The ducts involved by comedocarcinoma are usually surrounded by myoepithelial cells. [23]

When a diagnosis of comedocarcinoma is made, two factors have to be determined .It includes degree of intraductal spread which can result in pagets disease and areas of stromal invasion.

IHC:

They are negative for hormone receptors. HER2/neu overexpression and high TP53 mutations are usually seen.[22]

2. Solid type

In this type the duct lumen shows proliferation of medium sized cells which are smaller and uniform than the cells of comedocarcinoma but larger than the cells of lobular carcinoma.[24]

3. Cribriform type

On microscopy it shows proliferation of uniform epithelial cells forming microlumens bridging most of the duct lumen. It can be seen in all levels of main duct system. The microlumina may contain secretion,

punctate calcifications and small numbers of degenerated cells. Azzopardi designated that cribriform type is often associated with trabecular bars and roman bridges.[25]

Trabecular bars: They are rigid row of cells with the long axis of the nucleus are perpendicular to the long axis of the bar.

Roman bridges: They are curvilinear trabecular bars which connects the two portions of epithelial lining.

4. Papillary type

It comprises only a small percentage of breast carcinoma. Within the ducts the neoplastic epithelium shows papillary projections supported by fibrovascular stroma.[12]

Micropapillary Variant

The ducts are lined by neoplastic cells giving rise to papillary fronds that protrudes into the duct lumen. The papillae lack a fibrovascular core .The lining cells may vary from cytologically homogenous to highly pleomorphic .

5. Clinging type

This lesion shows duct lined by one or two layers of malignant cells with a large empty lumen. It may present as high grade or low grade lesions. [12,26]

6. Cystic hypersecretory type

This lesion shows cystic formations which are induced by the abundant secretory material inside the duct.[12]

OTHER VARIANTS:

- DCIS with signet ring cells
- DCIS with multinucleated Giant cells
- DCIS with apocrine cytology
- Clear cell DCIS
- DCIS with squamous features
- Neuroendocrine DCIS
- Spindle cell DCIS
- Small cell DCIS

Grading of DCIS:

Grade of intraductal carcinoma has been used to predict the risk of invasive carcinoma after breast conservation therapy.[26] There is no universally accepted grading system for DCIS.[14]Grading is based on architecture, nuclear grade, lesion size, cell polarity and presence or absence of necrosis. Atleast 6 classifications have been proposed and most classifications gave importance to the architecture, nuclear grade and necrosis.[27] Silverstein et al proposed a classification based on nuclear grade.[28] Presence or absence of necrosis is taken as a part of prognostic index.

There is a significant correlation between the grade of intraductal carcinoma and its corresponding invasive component. The risk of developing invasive carcinoma is higher for comedocarcinoma and lower for other insitu types.[29,30]

DCIS is generally graded as high, intermediate and low grade.[12,14,24]A case is classified on the basis of the highest grade present.[31]

The Van Nuys Prognostic Index (VNPI)

In addition to the following histological groups margin status and tumor size is also included in VNPI. A significant correlation was found between VNPI and risk of recurrence on follow up study.[32]

Group 1: Non-high nuclear grade without necrosis

Group 2: Non-high nuclear grade with necrosis

Group 3: High nuclear grade with or without necrosis

Low grade DCIS

Low grade DCIS is composed of monomorphic cells with uniform sized nucleus showing diffuse finely dispersed chromatin pattern, occasional nucleoli and mitosis. Necrosis will be absent in low grade DCIS.

Intermediate grade DCIS

Intermediate grade DCIS is composed of cells cytologically similar to that of low grade DCIS but some of the ducts may show intraluminal

necrosis. The nuclei may be of intermediate grade with coarse chromatin and occasional nucleoli. Intermediate grade can be used when the ducts show,

a) Cribriform, solid or papillary pattern with necrosis. But it should not show nuclear anaplasia like comedocarcinoma .
b) The above mentioned patterns shows high grade nucleus in the absence of necrosis.

High grade DCIS

High grade DCIS is composed of atypical cells usually with central necrosis. The nucleus will be poorly polarized and markedly pleomorphic with coarse clumped chromatin .The nucleoli will be prominent .Mitotic figures will be conspicuous. The presence of central comedonecrosis is not mandatory to be graded under high grade DCIS. Even a single layer of highly anaplastic cells lining the ducts can also be categorised as high grade DCIS.[14] High grade DCIS can be termed for

a) Ducts showing comedocarcinoma type DCIS.
b) Other types of DCIS showing high grade nuclear features with or without necrosis.[12,14,26]

Immunohistochemistry of ductal carcinoma in situ

DCIS in contrast to lobular carcinoma in situ shows E-Cadherin expression.[33]High grade DCIS also shows P-cadherin expression.

1. Hormone receptors

Studies done by Bur et al. in 1992 showed that 80% of the intraductal carcinomas are estrogen-receptor positive. Estrogen positivity is seen in 91 % of noncomedo lesions compared to that of 57% comedo lesions. [34]

2. HER2/neu overexpression

Immunoreactivity for the HER2-neu oncogene is seen in 42% to 61% of in situ carcinomas.[35] Studies done by Ho et al. found that HER2/neu amplification was higher in comedocarcinoma (69%) compared to that noncomedo type intraductal carcinomas(18%).[36] Studies done by Van de vijver et al[35] and Collins et al[37] showed that comedocarcinoma showed HER2/neu amplification in 85% to 100% of cases whereas micropapillary, cribriform patten of intraductal carcinomas does not show HER2/neu overexpression.

CARCINOMA BREAST

Breast cancer is the most common malignant cancer among women.[1] The incidence has increased nowadays .This is due to increasing awareness of the people and use of diagnostic modalities like mammography ,fine needle aspiration and core biopsy. But the mortality has decreased due to early screening, which detects the tumor at an early curable stage and also by means of better effective treatment modalities.[2]

Risk factors

Several risk factors have been established for the development of breast carcinoma. The common denominator for most of the factors will be strong and prolonged estrogen stimulation in a genetically susceptible background.[14]

1. Country of birth

The incidence is high in northern Europe and North America (91.4 new cases per 100 000 women/year), intermediate in southern European and low in most Asian and African countries. In the United States due to increased mammographic screening there has been a increase in the detection of breast carcinoma. Due to earlier diagnosis and improved therapy the mortality has begun to fall in some regions like North America, Western Europe, and Australia.[38] Nowadays the incidence of breast carcinoma has been increased in less developed countries owing to gradual change of lifestyle of women.[12]

2. Age

Majority of the breast cancers are detected during the reproductive age group.[12]

3. Family history

The risk is 2-3 times higher than general population if the first degree relative of the women had breast cancer .[39]

4. Menstrual history

Early menarche and late menopause is associated with increased risk of carcinoma.[40]

5. Reproductive history

There will be a increased risk in case of nulliparity and late age at first child birth. It has been documented that there is a reduced risk of breast carcinoma in premenopausal women who have lactated.[41]

6. Exogenous estrogens

Recent studies showed that there is an increased risk of breast carcinoma in women under hormone replacement therapy than women using estrogen alone.[42]

7. Ionising radiation

An increased risk of breast cancer has been documented on exposure to ionizing radiation particularly when the exposure is at the time of breast development.[43]

8. Precancerous lesion

Complex fibroadenoma, florid hyperplasia without atypia, solitary papilloma without atypical hyperplasia, sclerosing adenosis are associated with a risk of 1.5-2 times than that of general population . Atypical ductal hyperplasia and atypical lobular hyperplasia are associated with a risk of 4.0-5.0 than that of general population.[16]

9. Genetic predisposition

About 5-10 % of breast cancers are familial.[12] In various studies it has been reported that the risk of developing breast carcinoma due to BRCA1 mutation will be 56% to 90% .Women carrying BRCA2 mutation have a risk of 37% to 84% .BRCA1 mutation may account for about 45% of cases of hereditary breast carcinoma and they are usually of poorly differentiated type with high proliferative rate.[14]

10. **Diet**

Western diet (High caloric diet rich in proteins and animal fat), obesity, increased alcohol consumption ,sedentary lifestyle are associated with increased risk of breast carcinoma.[14]

Clinical presentation

The most common symptom will be breast lump (60-70%) followed by pain(14-18%) .Nipple discharge (7-9%) is the least common presenting symptom. With the introduction of mammographic screening there is an increased detection of asymptomatic cases. About 40-50% of cases present with breast lump in the upper outer quadrant .Following that they present in decreasing order of frequency from central, upper inner, lower outer to lower inner quadrant.[14]

Breast mass should be evaluated by triple assessment which includes clinical examination, imaging studies(mammography

,ultrasound) and tissue sampling either by fine needle aspiration cytology or needle core biopsy.[12,14]

INVASIVE DUCTAL CARCINOMA

Invasive ductal carcinoma are tumors in which stromal invasion is detectable .Regardless of the presence of in situ component and the relative proportion of in situ and invasive component they are included under invasive carcinoma. [12]

Invasive carcinomas can be classified into two major categories- ductal and lobular type. Invasive ductal carcinoma comprises 75-85% of mammary carcinoma .Invasive ductal carcinoma, not otherwise specified comprises majority of duct carcinoma .Other relatively infrequent forms of infiltrating ductal carcinoma include tubular, medullary, metaplastic, colloid carcinoma etc.,[26]

CYTOARCHITECTURAL TYPES

1. INVASIVE DUCTAL CARCINOMA,NOS TYPE

IDC, NOS type comprises 75 % of all the cases of breast carcinoma. It represents the prototype of all breast carcinomas.[44]

Gross:

The tumor is usually a ill circumscribed firm tumor.It shows a yellowish gray cut surface.The trabeculae radiates through the surrounding parenchyma in to the adjacent fat with a crab like or stellate configuration. In case of larger tumors areas of necrosis, hemorrhage and

cystic degeneration may be present. In older days the term scirrhous carcinoma has been used for tumors with hard consistency. The hard consistency is due to presence of large amounts of stroma .

Microscopy:

The tumor shows various growth pattern like diffuse sheets, well defined nests, cords, trabeculae and also as individual cells. Glandular differentiation may be well developed to barely detectable. The individual tumor cells are usually large and pleomorphic compared to that of classical invasive lobular carcinoma. The tumor shows prominent nuclei and nucleoli and increased mitotic figures. About 60% of the cases shows areas of necrosis. The amount of stroma varies from scant to abundant desmoplastic stroma. Elastic tissue are present in about 90% of cases. The presence of chalky streaks on gross examination is due to the presence of elastosis involving the vessel and duct walls .About 60% of the cases show calcification. The interphase between the tumor and stroma shows mononuclear cell inflammatory infiltrates.[12]

Studies done by Fisher et al. showed that lymphatic, blood vessel and perineural invasion was found in 33%,5% and 28% of the cases.[45]

IHC:

The tumor cells are positive for low molecular weight keratin(8,18 and 19) and EMA. Other sensitive breast related markers are mammoglobin and GCDFP 15.The basement membrane components

collagen 4 and laminin shows a discontinuous linear pattern or it may be totally absent.[12,14]

2. **INVASIVE CRIBRIFORM CARCINOMA**

Invasive cribriform carcinoma is a rare form of breast malignancy.

Microscopy:

The tumor shows a cribriform appearance similar to that of its intraductal counterpart but in addition it shows stromal invasion. Cribriform pattern is often seen in association with tubular formations. Page et al proposed that the relative proportion of the two elements determine the term to be used.[46]

Prognosis: The tumor has a excellent prognosis.

3. TUBULAR CARCINOMA

Pure tubular carcinoma comprises less than 2% of invasive breast cancer. But in mammographic screening 9-19 % of cases can be detected .It is easily detectable due to its spiculate nature and cellular stroma.

Gross:

The gross feature of tubular carcinoma is similar to that IDC, NOS type with poorly circumscribed margins and hard consistency. But the size of the tumor is usually small with a mean diameter of 1 cm.

Microscopy:

The tumor shows haphazard arrangement of glands without any organoid configuration. The characteristic feature of tubular carcinoma is

irregular and angulated contour of the glands .The lining cells show apocrine type snouts in the apical cytoplasm. They lack myoepithelial cells and basement membrane. The lumina of the glands are open and filled with basophilic secretion. It shows a cellular desmoplastic reaction. The tumor is cellular with fat invasion in the periphery. Because of the well differentiated nature of the glands, scant pleomorphism and absence of necrosis it simulates benign conditions like microglandular adenosis , sclerosing adenosis and radial scar .[12,24]

DCIS can be seen in majority of the cases. The in situ component is usually of low grade showing cribriform or papillary pattern.

Prognosis:

Tubular pattern can be seen associated with invasive ductal carcinoma, NOS type or sometimes with invasive lobular carcinoma. In this instances, the term tubular NOS and tubular mixed can be employed. When the tubular pattern is more than 75 % ,the tumor shows better prognosis than ductal carcinoma NOS type. The term tubular carcinoma can be best employed for tumors in which tubular pattern is present for atleast 90 % of the tumor. These tumors are associated with favorable prognosis

4. MUCINOUS CARCINOMA

Mucinous carcinoma was classified under mucin producing carcinoma .Other mucin producing carcinomas are mucinous

cystadenocarcinoma, columnar cell mucinous carcinoma and signet ring cell carcinoma.[14]

The tumor usually occurs in postmenopausal women. It is also called as mucoid, gelatinous or colloid carcinoma.

Gross:

The tumor is well circumscribed .Cut surface of the tumor shows a characteristic glistening and gelatinous appearance.

Microscopy:

The tumor cells are arranged usually in small clusters floating in a mucinous pool which are surrounded by bands of fibrous septa. The tumor cells show little pleomorphism. Mitotic rate is usually low. The mucin is usually extracellular .The mucin may be acid or neutral type.

The in situ component is usually difficult to recognize.

Histochemically the mucins are o-acylated forms of sialomucin. Immuohistochemically there is strong MUC2 positivity in cytoplasm. Estrogen and progesterone receptors are always positive whereas HER2/ neu will be negative.[47]

Few studies suggest that mucinous carcinoma can be classified as A and B based on the endocrine differentiation. Type A tumors shows tumor cells arranged as trabeculae with minimal intracytoplasmic mucin. The cells do not show argyrophilia. Type Btumors shows sheets of tumor cells with abundant intracytoplasmic mucin. Argyrophilia can be

demonstrated in the tumor cells. Nodal metastasis is very low which accounts for 2-4% of node metastasis.[12,26]

IHC:

They are positive for estrogen and progesterone receptors. They usually do not show HER2/neu overexpression or p53 accumulation.

5. MEDULLARY CARCINOMA

The tumor is most common in patients under 50 years of age. The tumor is common among carriers of BRCA1 mutation.

Gross:

The tumor is well circumscribed, solid and homogenous.

Microscopy:

The tumor grows in a diffuse pattern with minimal or absent glandular differentiation. The individual tumor cells are large ,pleomorphic with large nuclei and prominent nucleoli. The cell borders are indistinct which gives the tumor a syncytial arrangement. Spindle cell metaplasia, bizarre tumor giant cells and extensive necrosis may occur. The tumor shows prominent lymphoplasmacytic infiltrate at the periphery of the tumor which is an characteristic feature of medullary carcinoma. The infiltrate was thought due to the reaction of host tissues to the neoplasm. They are usually peripheral T cell type.

IHC:

They are positive for CK7, p53.They are negative for hormone receptors (ER,PR),HER2/neu and comes under Triple negative phenotype. The tumor expresses HLA-DR antigen which could be the possible reason for the prominent lymphoplasmacytic infiltrate. Though axillary lymph node involvement are common, only low axillary group of lymph nodes will be usually involved. The prognosis will be better than IDC,NOS type.[12,26]

***Atypical medullary carcinoma**:*

The tumor shows same growth pattern features as that of typical medullay carcinoma but has no more than two classic microscopic features.

The tumor shows

a. Syncytial growth comprising > 75% of the tumor
b. Atypical features
c. Focal tumor infiltration at the margins
d. Uniform nuclei and rare mitosis
e. Mild to absent lymphoplasmocytic infiltration at the margins and focal tubule formation seen. [48]

6. INVASIVE PAPILLARY CARCINOMA

The tumor is rare and occurs more frequently in the postmenopausal women. Most commonly papillary carcinomas present as

in situ lesions. The invasive component can be papillary or it may show features of IDC, NOS type. As the presence of invasion in these tumors are not clearcut, it should be applied for cases with well differentiated true papillary structures only. When a tumor with papillary pattern is seen, metastatic papillary carcinoma from other sites should also be excluded.[12,26]

The tumor may have axillary lymph node metastasis particularly in solid variant of papillary carcinoma. Prognosis of the tumor is better compared to that of invasive ductal carcinoma, NOS type.[49]

7. INVASIVE MICROPAPILLARY CARCINOMA

Invasive miropapillary carcinoma is a distinct rare variant of invasive ductal carcinoma. When the micropapillary pattern is found throughout the tumor it is referred as pure invasive micropapillary carcinoma. When it is present as a part of conventional IDC it is called as mixed invasive micropapillary carcinoma .But the criteria to distinguish these two is not clear cut. Some authors suggest atleast 50 % of the tumor should be micropapillary to call it as pure invasive micropapillary carcinoma

Microscopy:

The tumor is composed of clusters of cells arranged in micropapillary or tubular pattern. The tumor cells are found free floating in clear spaces. Fibrovascular core will be absent in the micropapillary

clusters. The clusters exhibit a “inside out” arrangement. The apical cells are polarized outside and this can be evidenced by MUC 1 staining.

The nuclear grade of this tumor cells will be high. About half of the cases may show psammoma bodies. In situ component seen in these cases is usually micropapillary and sometimes show cribriform pattern**.**

Lymphatic invasion was reported in more than 50 % of the cases. Lymph node metastasis usually occur. The tumor have a bad prognosis.

IHC:

Estrogen receptor were positive in 72-75 % of cases and 45 % cases were positive for progesterone receptor.36 % of the cases show Her 2-neu overexpression. [50]

8. APOCRINE CARCINOMA

Apocrine carcinoma is very rare comprising 0.5 % of all breast carcinoma. The tumor is composed entirely or predominantly of apocrine type cells. The tumor cells are large with abundant eosinophilic cytoplasm with vesicular nucleus and prominent nucleoli. Glandular differentiation can be seen with apocrine snouts. Diagnosis of apocrine carcinoma should only be made when the architectural features are those of a malignant tumor. Immunohistochemically they are positive for GCDFP-15.Estrogen and progesterone receptors will be negative.[51]

9. SECRETORY CARCINOMA

Secretory carcinoma are rare tumors and seen in children. It can also occurs in adults. It has a excellent Prognosis.

Gross:

The tumors are usually small and well circumscribed.

Microscopy:

The tumor is composed of tubuloalveolar and papillary structures. The lumina contain eosinophilic PAS positive, diastase resistant material. The malignant cells have a pale staining vacuolated cytoplasm. Nucleoli may be prominent. Mitosis is scanty.

IHC:

There is a strong immunoreactivity for S-100 and a-lactalbumin.

10. METAPLASTIC CARCINOMA

Metaplastic carcinoma represents tumor predominantly with cell type other than epithelial and glandular component. It includes many categories but which overlap with each other. Metaplastic carcinoma is more aggressive than invasive ductal carcinoma. Metastasis is usually hematogenous rather than lymph node metastasis.

Gross:

The tumors are circumscribed, firm to hard in consistency. Degenerated cystic areas can be seen in cases with squamous metaplasia. Some of the tumors may have infiltrative borders.

Classification of metaplastic carcinoma

Table 2:Classification of metaplastic carcinoma.

Purely epithelial	**Mixed epithelial and mesenchymal**
Squamous • Large cell keratinizing, spindle cell,acantholytic • Adenocarcinoma with spindle cell differentiation • Adenosquamous, including mucoepidermoid	• Carcinoma with chondroid metaplasia • Carcinoma with osseous metaplasia • Carcinosarcoma(specify components).

A.Squamous cell carcinoma

Gross:

The tumors are large with cystic spaces filled with keratin.

Microscopy:

In pure squamous cell carcinoma the central cystic cavity is lined by malignant squamous cells. Most cases represent squamous metaphase.

Other two variants which can be seen will be acantholytic squamous cell carcinoma and adenosquamous carcinoma. Low grade adenosquamous carcinoma is said to have a favourable prognosis whereas acantholytic squamous cell carcinoma have a aggressive behavior.[52]

B. Carcinosarcoma

Microscopically the sarcoma like component can be malignant fibrous histiocytoma, osteosarcoma, chondrosarcoma, angiosarcoma or a

combination of various components. When the transition between sarcomatous and carcinomatous component is gradual and sharp, it is termed carcinosarcoma.[53]

When the transition to cartilaginous or osseous elements is direct without an intervening spindle cell component or osteoclastic giant cells, it is called matrix producing carcinoma.

Molecular studies suggest that the epithelial and sarcoma like components originate from same stem cell.

IHC:

The sarcoma like elements acquire vimentin and other mesenchymal features .It is referred to as the phenotypic switch. The cells are occasionally positive for epithelial markers.

SPREAD RELATED VARIANTS

1. Inflammatory carcinoma

The diagnosis of inflammatory carcinoma is essentially based on clinical criteria. Clinically the entire breast is red, warm. The skin shows widespread edema which resembles that of mastitis. It has been believed that clinical appearance is due to widespread carcinomatosis of dermal lymphatic vessels. Skin biopsy is usually performed to reveal dermal lymphatic permeation. Histopathological examination of some of the cases show a undifferentiated carcinoma.

The prognosis is usually bad. Studies done by Charafe -Jauffret et al.[54] found most inflammatory carcinoma are negative for estrogen and positive for MIB1,E-Cadherin and HER2/ neu.

PAGETS DISEASE

Pagets disease was originally described by Sir James Paget in 1874.It is a crusted lesion of nipple caused by underlying breast carcinoma. About 1-2% of patients with mammary carcinoma show pagets disease. The accompanying breast carcinoma is usually a intraductal carcinoma.[55]It may be associated with or without stromal invasion. The epidermis of the nipple shows characteristic Paget's cells in the the keratinizing epithelium. The cells may be singly scattered in the superficial epidermis. They may also form clusters in the basal portions of the epidermis. Individual cells have a pale or clear cytoplasm and their nuclei have a prominent nucleoli.

Intraductal carcinoma is usually of comedo or solid growth pattern.About 10 % of the cases show cribriform or papillary carcinoma and 40 % the cases show mixed type of in situ carcinoma. 79-100 % the cases are strongly positive for HER2/neu.The underlying insitu lesions are frequently HER2/neu positive .

HISTOLGICAL GRADING OF DUCTAL CARCINOMA

Grading of breast cancer was first attempted by Greenhough in 1925.[56]He used about 18 features and it is not popular. In 1993 Haagensen evaluated 15 histological features which mainly includes growth pattern, cell morphology and the stromal reaction.

The most popular grading system till date was proposed by Bloom in 1950.[57]His grading system was based on three main features which includes degree of tubule formation ,nuclear features and mitotic activity .He classified breast carcinoma into 2 categories –low grade and high grade tumors.

In 1957 this classification was upgraded by modifications of Bloom and Richardson .[58]It is also based on degree of tubule formation, nuclear pleomorphism and mitotic activity. But in this classification score of 1 to 3 was given to each criteria according to mild, moderate or marked degrees. A total score of 3 to 9 was given as follows,

Table 3 : Bloom And Richardson grading system 1957

Score 3-5	Grade 1	Well differentiated tumors
Score 6-7	Grade 2	Moderately differentiated tumors
Score 8-9	Grade 3	Poorly differentiated tumors

Elston highlighted the importance of proper tissue fixation, appropriate section thickness for histological grading .He used the grading system only for invasive ductal carcinoma. Special types like

mucinous, medullary carcinoma are excluded. Elston and Ellis modified Bloom and Richardson grading system by quantifying the mitotic activity.[59]This is also referred as the Nottingham modification of Bloom and Richardson system.

Table 4: Nottingham modification of Bloom-Richardson Histological grading system

Criteria	Score
Tubule and gland formation	
Majority of tumor (>75%)	1
Moderate degree (10-75%)	2
Little or none (< 10%)	3
Nuclear pleomorphism	
Small, regular, uniform	1
Moderate variation in size, shape	2
Marked variation in size, shape	3

Mitotic count

Mitotic count is also graded as 1-3.But it depends on the field diameter used. Mitotic figures are to be counted from the most mitotically active area.10 high power fields should be counted from the same area but need not to be contiguous. Poorly preserved area should be ignored.

Table 5: Scoring of mitotic count

Field diameter0.59mm	Field diameter 0.44mm	score
0-9	0-5	1
10-19	6-10	2
>20	>11	3

Final grading score

GRADE	SUM OF POINTS
I	3–5
II	6–7
III	8–9

PROGNOSTIC FACTORS

1. Age

Women < 50 years have the best prognosis. Older patients have a higher rate of recurrence and distant metastasis.

2. Pregnancy

Carcinoma breast manifesting during pregnancy are generally aggressive with overexpression of HER2/neu and low expression of hormone receptors .

3. BRCA1 status

Studies showed that BRCA1 mutation carriers have a low survival rate.

4. Skin invasion

Invasion of overlying skin is associated with decreased survival rate.

5. Nipple invasion

Nipple areola complex involvement vary from 0 to 58 %.[60-62]The nipples are usually involved by pagets disease, invasive ductal or lobular carcinoma and lymphovascular invasion. Nipple involvement by invasive carcinoma is mostly due to direct invasion of the tumor into the retroareolar region.[62] Nipple involvement is associated with high incidence of axillary node metastasis.

6**. Presence or absence of invasion**

The single most important prognostic determinant of breast carcinoma is the presence of invasive component. In case of tumor with both an invasive and in situ component ,the invasive component is proportional to the nodal metastasis. The in situ component is directly related to the incidence of multicentricity and indirectly with occult metastasis.[12]

7. Size of the tumor

Tumor diameter should be measured in three planes to the nearest millimeter. The greatest diameter is taken as the size of the tumor. When the tumor size is less than 1 cm or tumor with large in situ component measurements should be taken using stage micrometer in histological

sections. The invasive component is the better predictor of the total tumor size than the DCIS component.

Numerous studies showed that tumor size correlates with the prognosis. Multivariate analysis by Nottingham/Tenovas Primary Breast cancer study showed tumor size is an independent prognostic variable. In case of node metastasis tumor size is considered as a strongest predictor of tumor dissemination and relapse. [63]

8. Histological type

Variants of invasive ductal carcinoma like tubular carcinoma, cribriform carcinoma, medullary carcinoma, papillary carcinoma, pure mucinous carcinoma and secretoty carcinoma have a more favorable prognosis than Invasive ductal carcinoma, NOS type. Signet ring cell carcinoma has a bad prognosis.[63]

9. Histological grade

Nottingham modification of the Bloom–Richardson system is used to grade the tumor. It is mainly used in invasive ductal carcinoma, NOS type. But a few suggest it can also be employed for special types of invasive duct carcinoma and lobular carcinoma.[64]

10. Tumor necrosis

Spontaneous tumor necrosis is associated with tumors showing high histological grade and increased incidence of lymph node metastases.[65]

11. Lymphovascular emboli

Invasion of tumor cells into blood and lymphatic vessels is one of the critical steps for metastasis.[66] The presence of tumor emboli within peritumoral endothelial lined spaces was defined as lymphovascular invasion (LVI).The identification of LVI may permit the determination of patients at increased risk for axillary involvement and distant metastases.[67]

12. Lymph node status

Lymph node status is a powerful prognostic factor.[68] It should be based on histopathological confirmation rather than clinical examination. Many studies showed that patients with regional node involvement have a bad prognosis than those without node involvement.

Ten year survival rate for node negative patients will be 75 % while compared to that only 25-30% in node positive patients. Prognosis is also dependent on the number and the level of regional lymph nodes. The prognosis will be poor if greater number of nodes is involved .[69]

As per pathological lymph node staging in TNM staging, patients are categorized based on the number of nodes showing metastasis. (Annexure 2)

NSABR categorises patients under two divisions for therapeutic purpose. They categorized patients with 1-3 positive nodes and cases with 4 or more positive nodes.

13. Hormone receptors

Expression of ER and/or PR is generally associated with a better outcome. Survival and response to hormone therapy are most favorable among women with tumors positive for both ER and PR, intermediate for tumors discordant on receptor status, and least favorable for tumors negative for both.[70] The interrelationship of ER, PR, and HER2/neu has come to have an important role in the management of breast cancer. HER2/neu expression is generally inversely correlated with ER and PR expression.[71] It has been showed that breast carcinoma overexpressing HER2/neu do not respond to tamoxifen therapy.

14. HER2 /neu expression

HER2 /neu overexpression correlates with the higher tumor grade. It is a poor prognostic factor especially when associated with lymph node metastasis.[73] It is an excellent predictor of response to the drug trastuzumab but a weak predictor for chemotherapy.

15. Cell Proliferation

Cell proliferation has emerged as an important parameter especially in node positive patients. Tumors with increased proliferation rate behave aggressively. The simple method to assess proliferation will be the mitotic count .Nowadays Ki 67 has been used to determine cell proliferation by immunohistochemistry[74] .Other methods

include flow cytometry, S phase fraction are used to assess cell proliferation.

15. Microvessel density.

Invasive breast carcinoma with prominent vascular component in the surrounding stroma behaves aggressively than other tumors.[75]

IMMUNOHISTOCHEMISTRY

Immunohistochemistry (IHC) or immunocytochemistry is the application of immunologic principles and techniques to demonstrate specific antigens in cells and tissue based on the antigen antibody interaction and it exploit the specificity at light microscopic level.[12]

Various stages of development of Immunohistochemistry include peroxidase–antiperoxidase method (1970), alkaline phosphatase labeling method(1971), avidin biotin method (1977) and two layer dextrin polymer technique(1993).

Steps of immunohistochemistry:

ANTIGEN RETRIEVAL:

Antigen retrieval is done to unmask the antigen determinants of fixed tissue sections. This can be done by

1. Proteolytic enzyme digestion
2. Microwave antigen retrieval
3. Microwave and trypsin antigen retrieval
4. Pressure cooker antigen retrieval

Proteolytic enzyme digestion:

Enzymes like trypsin and proteinases are used to breakdown the formalin cross linkages and unmask the antigen determinants. But there is a disadvantage of antigen destruction and inadequate digestion

Microwave antigen retrieval:

In this formalin fixed paraffin sections are boiled in various buffers for rapid and uniform heating. Currently this is the most common method used.

Pressure cooker antigen retrieval:

In this method also the tissue sections are boiled in buffers to unmask the antigens. This method is used to retrieve large number of slides.

DETECTION SYSTEMS:

After adding specific antibodies to the antigens, the antigen antibody complex should be detected. This is done by direct and indirect methods.

Direct method:

The primary antibody is directly conjugated with flurochrome. Commonly used flurochromes are horse radish peroxidase and alkaline phosphatase.

Indirect method :

It is a two-step method .First the labeled secondary antibody reacts with primary antibody which is bound to specific antigen. The use of peroxidase enzyme complex or avidin biotin complex further increases the sensitivity of immunohistochemical stains.

HORMONE RECEPTORS

The presence of hormone receptors (estrogen, progesterone) correlates with the response of the tumor to endocrine therapy and chemotherapy. Though estrogen and progesterone are codependent variables, estrogen receptor status is the powerful predictive factor in breast cancer management.[76]

ER and PR are expressed in 80 % and 60 % of the breast cancer, respectively.[77] There is not much correlation between receptor positivity and cytoarchitectural type of breast. But however some studies suggest that mucinous , tubular, lobular carcinoma shows high estrogen positivity. Medullary, apocrine, metaplastic carcinoma are estrogen negative. [78] These hormone receptors are measured semiquantitatively. There are various scoring systems like Quick score, H score and Allred score. Nowadays Allred score is the most established score.It consists of a score for intensity (0 to 3) and a score for the proportion of nuclear staining(0 to 5).The final score is obtained by the sum of proportion score and intensity score which ranges from 0 to 8.[(77,79)] (Annexure 4)Invasive

tumor with an Allred score of more than 2 was considered to be positive for hormonal receptors.[79]

Ki 67

Ki 67 is a proliferation marker. It was first identified after immunization with hodgkin's lymphoma. The Ki 67 monoclonal antibody reacts with the nuclear non histone protein in all phases of cell cycle except G0.[80]Many studies concluded that there is significant correlation between high proliferative index and other prognostic factors like advanced clinical stage, high grade, lymph node status, estrogen receptor negativity and HER2/neu positivity.[81] High proliferative index has both predictive and prognostic significance in breast carcinoma. High Ki 67 index is correlated with decreased overall survival and relapse free survival irrespective of the nodal status.[82]

Conversely tumors with higher proliferation rate have a better response to chemotherapy.

Ki-67 is usually expressed as the percentage of positive cells among 500 tumor cells. Ki 67 shows nuclear positivity. The cells should be counted in 10 high power fields. Cut off value for proliferation rate is not clear cut. There are many inter laboratory variations. Some studies used 10% as cut off limit. Few studies followed mean, median or arbitrary values .Few authors suggest that cut off value depend on clinical objective. A cut-off value 0f 10 % can be used to exclude patients with

slow proliferating tumors from chemotherapy. So that overtreatment can be avoided. A cut off of 25 % can be used to identify patients sensitive to chemotherapy. Some studies concluded 20% cut off limit correlates well with prognosis. [81,83-85] In St Gallens Consensus held in 2013 most of the panel members suggested that threshold 0f 20% correlates well with the overall survival of the patients.[86]

HER2/neu

The HER2 gene was originally termed neu as it was first derived from rat neuro/glioblastoma cell lines.[87] Studies done by Semba & colleagues[88]and Di Fiore[89]& associates showed that the primary sequence of HER2 was related to ERBB2 family. Followed by that Akiyama and co workers demonstrated that HER2 is a 185 KD glycoprotein with tyrosine kinase activity. [90] HER2/neu (c-erbB-2) is proto oncogene which encodes a transmembrane glyocoprotein with tyrosine kinase activity belonging to the epidermal growth factor receptor family. HER2 /Neu is located on the chromosome 17q21 .It encodes a 185 kDa protein.

Several studies show that about 10-34% of breast carcinomas show over expression of HER2/neu protein.[91,92] HER2/neu acts as both prognostic and predictive factor. Patients with HER2/neu positivity show a worst prognosis by decreasing survival rate and increasing disease recurrence rate. Studies done by Alfred Carr et al[93] and Amanda McCann

et al[94] concluded that HER2/neu is an independent prognostic indicator for overall survival of patients with breast carcinoma.

Many studies show that HER2/neu over expression is associated with high proliferative index, high histological grade, positive axillary lymph node status, p53 accumulation and lack of estrogen and progesterone receptors.

Besides this HER2/neu acts as a therapeutic target as it is easily accessible as cell surface receptor. The drug which acts against this will be a human monoclonal antibody, trastuzumab which was approved in 1998 by FDA. This drug was effective in patients who are positive for HER2/neu and associated with node metastasis. It improves response rates, survival rate when used alone or with chemotherapy. Trastuzumab also reduces the disease recurrence rate by 33 %.[95]

HER2/neu also predicts the response to anthracycline and taxane based therapy whereas the effects of non-anthracyclines and non taxane are inferior.[96] It was found that HER2/neu positive tumors shows relative resistance to endocrine therapy.[91]

So accurate testing of HER2/neu is essential. Various methods like immunohistochemistry, FISH, gene based assays like southern blot, PCR methods and enzyme assays can be used to to HER2/neu amplification.The most frequent method used to assess HER2/neu is immunohistochemistry. The advantages of IHC over other methods are

simple and cost effectiveness and result can be interpreted by light microscopy.

IHC is performed in formalin fixed paraffin embedded tissue blocks. The results are interpreted based on the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines.[97]

Table 6: Scoring system for HER2/neu as per ASCO guidelines

0	No staining at all or very slight partial membrane staining in less than 10% of tumor cells.
1+	Faint barely perceptible membrane staining in more than 10% of tumor cells. Cells stained in only part of the membrane.
2+	Weak to moderate complete membrane staining observed in more than 10% of tumor cells.
3+	Strong complete membrane staining in more than 30% of tumor cells

Recommended guidelines by ASCO

1. The type of fixative that can be used is 10% neutral buffered formalin
2. The ideal time of fixation should be between 6-48 hrs
3. The most reliable antibody is rabbit monoclonal antibody,4B5

If the results are 2 + it is recommended to perform FISH. Recently ASCO/CAP [97] has published their recommendations for HER2/neu testing.

The result is taken as positive in case of ,

1. 3+ staining by IHC
2. More than 6 HER2/neu gene copies in FISH
3. FISH ratio >2.2

The result is taken as negative when there is

1. 0 or 1+ positivity by IHC
2. < 4 HER2/neu copies per nucleus by FISH
3. FISH ratio <1.8

MOLECULAR SUBTYPE OF BREAST CARCINOMA

In the year 2000 Perou and colleagues tried to segregate breast carcinoma based on their gene expression profiles into distinct subgroups. He used microarray to demonstrate gene expression. It was accepted with a hope that this will provide new approach to the biology of breast cancer. It was also believed that it may impact on the therapeutic approach of the patient.[98]

The subtypes recognized by the gene expression are luminal A, luminal B, HER2/neu type, basal like and normal breast like. It was suggested that normal breast like subtype is most propably an artifact rather than genuine subtype. It is due to lack or sparsity of tumor in the tissue samples used for microarray analysis.

As the different subtypes show specific characteristics, they may benefit from different therapeutic approach. Among the subtypes, basal like type shows the worst prognosis.[98]

Luminal A

It comprises 50% of the invasive breast cancer. This is seen in tubular carcinoma, cribriform carcinoma, low grade invasive ductal carcinoma NOS type and classic lobular carcinoma. It express luminal cytokeratins and high expression of hormone receptors. They respond well to chemotherapy. Response to chemotherapy is variable. The tumor will have good prognosis.

Luminal B

It comprises 20% of invasive breast carcinoma. It corresponds to invasive ductal carcinoma NOS type and micropapillary carcinoma. It express cytokeratins and moderate to weak of hormone receptors. Proliferation rate is higher compared to that of luminal A type. It shows variable expression for chemo and endocrine therapy.

HER 2/neu

It comprises about 15 % of invasive breast cancer. The tumors are usually high grade with lymph node metastasis. They are negative for hormone receptors .HER2/neu amplification and high proliferation is seen. Patients responds to trastuzumab and anthracycline based chemotherapy. The patients usually have a poor prognosis.

Basal like

It comprises about 15 % of invasive breast carcinoma. High grade IDC NOS, metaplastic and medullary carcinoma comes under this category. They are negative for hormone receptors and HER2/neu but shows a high proliferation rate.

The tumor respond to platinum based chemotherapy but do not show response to endocrine therapy and trastuzumab.

An effort is also made to classify the tumors based on the immunnohistochemical expression. The panel of markers include estrogen receptor(ER), progesterone receptor, HER2/neu, EGFR, cytokeratin 5/6 and Ki 67.[99]

Based on the reactivity they are classified as follows,

Table 7: Molecular typing of breast carcinoma according to expression of IHC markers

Immunoprofile	**Luminal A**	**Luminal B**	**HER2/*neu***	**Basal-like**
ER, PR	ER and/or PR+	ER and/or PR+	ER –, PR –	ER –, PR–
HER2 and others	HER2 – Low Ki-67 (<14%)	HER2+ or HER2 – Ki-67 =14%	HER2+	HER2– CK5/6 and/or EGFR+

Drawbacks

But the classification has some drawbacks .Relatively small number of cases are used do define molecular subtypes and few less common distinct subtypes are missed out. The basal like subtype also contains tumors with favorable prognosis like medullary carcinoma, secretory carcinoma which shows the need of a low grade basal like subtype.[90]Currently the clinical value of molecular classification is not well established.

MATERIALS AND METHODS

The present study is a two group comparative study. Cases of modified radical mastectomy specimens received for routine histopathological evaluation in the department of Pathology, Tirunelveli medical college from January 2013 to September 2014 were taken for this study .

Two group based on the presence or absence of in situ component associated with the invasive ductal carcinoma is studied for the expression of HER2/ neu and its clinical outcome. Approval of the Institute Ethical Committee was obtained to conduct this research study.

Inclusion criteria

Invasive breast carcinoma with

- Invasive ductal carcinoma with DCIS(IDC-DCIS)
- Invasive ductal carcinoma without DCIS.(IDC)

Exclusion criteria

- Invasive breast cancer with the histopathological diagnosis other than IDC (i.e. Invasive lobular carcinoma of breast etc.)
- Patients who had received neoadjuvant chemotherapy

Brief procedure:

Demographic profile, relevant clinical history like age, menopausal status, etc. were recorded for all patients from the clinical case records.

The received mastectomy specimens are subjected to fixation in 10% neutral buffered formalin .After adequate fixation(usually 24 hours) surgical grossing was done according to the standard protocol and a detailed gross description was made .Extensive sampling is done to search for DCIS and then tissue sections are taken and subjected to routine manual tissue processing and paraffin embedding. Sections of 4-5 μ thickness were taken and routinely stained with haematoxylin and eosin (H & E) and mounted with DPX mountant .The slides are examined and the tumor was graded according to Modified Bloom and Richardson grading system. Other prognostic variables like tumor size, presence /absence of DCIS component, nipple invasion, presence/absence of necrosis, lymphovascular invasion, number of lymph nodes showing metastasis were also assessed.

Invasive ductal carcinomas without any DCIS component (IDC) after extensive sampling were taken as one group. The cases with invasive ductal carcinoma associated with DCIS (IDC-DCIS) were taken as the comparative group.

Immunohistochemistry was performed on formalin-fixed and paraffin embedded tissue sections using standard protocol. (Annexure 3) IHC for HER2/neu was performed used rabbit polyclonal antibody (Thermo Scientific). HER2/Neu was interpreted as per ASCO guidelines (Table 6). Membrane staining with 3 + and 2+ immunoreactivity were

HER2/neu considered positive. In both the comparative groups HER2/neu amplification was assessed only in the invasive component.

Immunohistochemistry for ER and PR was performed using rabbit monoclonal antibody (Thermo Scientific) and Allred score was used to interpret ER and PR staining. A score of $\geq$ 3is considered positive. The scoring system is explained in Annexure 4.

IHC for Ki 67 was performed using mouse monoclonal antibody(Thermo Scientific). Expression of Ki 67 was reported as the percentage of positive tumor cells. A distinct nuclear immuno reactivity for Ki -67 was considered positive. The Ki-67 labeling index was determined by observing 500 tumor cell nuclei in areas of the section with highest labeling frequency. A cut off point of 20 % was taken to separate the cases into two groups: High proliferative rate(>20%) and Low proliferative rate ($\leq$ 20%)

STATISTICAL ANALYSIS :

Statistical analysis was performed by SPSS software Version 17(SPSS, Chicago, IL, USA). Pearson's chisquare and Fisher exact test were used to evaluate the statistical differences between the two groups and to find the relation between HER2/neu with other prognostic factors. P value <0.05 is considered significant.

OBSERVATION AND RESULTS

The study analyzed a total of 50 modified radical mastectomy specimens of patients with carcinoma breast in which 25 cases were of invasive ductal carcinoma associated with DCIS(IDC-DCIS) and another 25cases were of invasive ductal carcinoma without DCIS(IDC)

1. AGE:

Table 8 :Age distribution of patients between IDC with and without DCIS

	AGE IN YEARS				
	≤ 40	**41-50**	**51-60**	**61-70**	**71-80**
IDC-DCIS (25)	1	9	9	5	1
IDC (25)	-	11	7	6	1
Total cases (50)	1	20	16	11	2
Chi square test	p value - 0.819				

In the present study, age of the patients ranged from 34-80 years with a median age of 54 years. Majority of the cases belonged to 41-50 years (40%) .

IDC-DCIS , had equal number of patients in the age group of 41-50 years(36%) and 51-60 years(36%) with the median age of 54 years.

In IDC the most common age group was between 41-50 years (44 %) with the median age of 53 years. The difference between two groups in relation to age was not significant.(Table 8 & chart 1)

2. MENOPAUSAL STATUS:

Table 9: Distribution of menopausal status between IDC with and without DCIS

	MENOPAUSAL STATUS	
	Premenopausal	**Postmenopausal**
IDC-DCIS (25)	12	13
IDC (25)	8	17
Total cases (50)	20	30
Chi square test	p value - 0.248	

In our study majority of the cases belong to postmenopausal age group which constitutes 60 %.In IDC-DCIS 48 % of the cases are in premenopausal group and 52 % in postmenopausal group. In IDC without DCIS 32% of cases are in premenopausal group and 68% in postmenopausal group.

Among the premenopausal population 60 % of the cases belong to IDC-DCIS category. (Table 9 & chart 2)

3. TUMOR SIZE

Table 10: Distribution of tumor size between IDC with and without DCIS

	TUMOR SIZE		
	< 2 cm	2-5 cm	>5 cm
IDC-DCIS (25)	4	15	6
IDC (25)	2	14	9
Total cases (50)	6	29	15
Chi square test	P value - 0.521		

In both the groups majority of the patients had a tumor size between 2-5 cm comprising 58% followed by 30% showing >5cm .Only 6 cases (12%) show tumor size below 2 cm. In IDC-DCIS 15 cases(60%) are in the size of 2-5 cm. 16 % of the cases are in seen in <2 cm and 24% are in >5 cm of tumor size. In IDC 56% (14 cases) of the cases are in the size of 2-5 cm.8% of the cases showed <2 cm tumor size whereas 36% of the cases show > 5 cm tumor size.

IDC-DCIS shows a small tumor size in 16 % of cases compared to that of 8 % in IDC alone. > 5cm tumor size is seen higher in IDC without DCIS. (Table 10 & chart 3)

4. TUMOR TYPE

Table11 : Histological type of tumor in IDC-DCIS and IDC.

	HISTOLOGICAL TYPE				
	IDC, NOS	**Medullary carcinoma**	**Mucinous carcinoma**	**Invasive papillary carcinoma**	**others**
IDC-DCIS (25)	25	-	-	-	-
IDC (25)	23	1	1	1	-
Total cases (50)	47	1	1	1	-

Majority of the cases belong to invasive ductal carcinoma, NOS type. In IDC-DCIS all the cases belong to IDC/NOS type. In IDC without DCIS medullary, mucinous and invasive papillary carcinoma are present with one case each.(Table 11;Chart 4)

5. Distribution of DCIS in IDC with DCIS

5 A: DCIS types (In IDC-DCIS)

Table 12 : Distribution of various types of DCIS in IDC-DCIS

DCIS pattern	No of cases	Percentage
Comedocarcinoma	17	68%
Solid	3	12%
Cribriform	2	8%
Micropapillary	1	4%
Mixed	2	8%

The DCIS component associated with IDC was predominantly comedo type constituting 68 %.Among other patterns solid type comprised 12 %, cribriform type 8% ,mixed (all non comedo types) 8 % and micropapillary type 4 %.(Table 12 & Chart 5)

5 B.DCIS GRADE

Table 13 : Histological grade of DCIS in IDC-DCIS

DCIS GRADE	No of cases	Percentage
High Grade	20	80%
Intermediate Grade	-	-
Low Grade	5	20%
Total	25	100

80 % of the IDC-DCIS is associated with high grade DCIS and the rest 20 % of the tumors showed low grade DCIS.(Table 13 & chart 6)

6. GRADE OF INVASIVE DUCTAL CARCINOMA

Table 14 : Distribution of histological grade of IDC in IDC with and without DCIS

	Grade of IDC		
	Grade 1	Grade 2	Grade
IDC-DCIS (25)	6	16	3
IDC (25)	3	17	2
Total cases (50)	9	33	5
Chi square test	P value - 0.593		

Most of the cases i.e.66 % of the cases are showing histological grade 2.Grading is not done in case of medullary carcinoma,mucinous carcinoma and invasive papillary carcinoma.

In IDC-DCIS 24% of the cases show grade 1, 64 % of the cases showed grade 2 and 12% the cases showed grade 3. In IDC without DCIS 12 % of the cases showed grade 1, 68% of the cases showed grade 2 and 8 % of the cases show grade 3. (Table 14 & chart 7)

7. NECROSIS

Table 15:Distribution of tumor necrosis in IDC with and without DCIS

	Necrosis	
	Present	**Absent**
IDC-DCIS (25)	4	21
IDC (25)	4	21
Total cases (50)	8	42
Fisher exact test	P value - 1.000	

In our study necrosis was seen in only 16% of the cases in IDC-DCIS.

In both IDC with and without DCIS 16% of the cases showed tumor necrosis.

There is no statistical significance in relation to tumor necrosis between IDC-DCIS and IDC. (Table 15 & chart 8)

8. NIPPLE INVASION

Table 16: Nipple invasion in IDC with and without DCIS

	Nipple invasion	
	Present	**Absent**
IDC-DCIS (25)	1	24
IDC (25)	1	24
Total cases (50)	2	48
Fisher exact test	P value-1.000	

In our study only 2 cases (4%) showed nipple invasion. In both IDC with and without DCIS, each one case presented with nipple invasion .There is no statistical significance in relation to nipple invasion between IDC with and without DCIS. (Table 16 & chart 9)

9. LYMPHOVASCULAR INVASION

Table 17 : Lymphovascular invasion in IDC with and without DCIS.

	Lymphovascular invasion	
	Present	**Absent**
IDC-DCIS (25)	6	19
IDC (25)	8	17
Total cases (50)	14	36
Chi square test	P value -0.529	

In our sudy 50 cases 28% of the cases showed lymphovascular invasion

In IDC-DCIS 6 cases (24%) of the cases showed lymphovascular invasion. In IDC without DCIS 8 cases(32%) of the cases showed lymphovascular invasion. (Table 17 & chart 10)

There is no statistical significance in relation to lymphovascular invasion between IDC with and without DCIS

10. LYMPH NODE STATUS

Table 18 : Distribution of lymph node status between IDC-DCIS and IDC

	Node status			
	1-3 nodes	**4-9 nodes**	**>9 nodes**	**Negative**
IDC-DCIS (25)	6	6	2	11
IDC (25)	5	9	4	7
Total cases (50)	11	15	6	18
Chi square test	P value - 0.522			

In our study 64% of the total cases showed lymph node metastasis. 15 cases i.e. 30 % of the cases show nodal involvement in the range of 4-9 nodes.

In IDC-DCIS 44% of the cases did not show nodal involvement.24% of the cases showed nodal involvement with 1-3 and 4-9 nodes. Higher number of nodal involvement i.e. >9 nodes are seen in only 8 % of the cases.

In IDC alone only 28 % of the cases did not show node metastasis. Majority of the cases show 4-9 node involvement with 36 % .1-3 nodes are involved in 20 % of the cases.16% of the cases showed >9 nodes. (Table 18 & chart 11)

There is no statistical significance in relation to lymph node status between IDC with and without DCIS

11. Estrogen receptor status

Table 19: Estrogen receptor status in IDC with and without DCIS

	Estrogen receptor status	
	Positive	**Negative**
IDC-DCIS (25)	16	9
IDC (25)	13	12
Total cases (50)	29	21
Chi square test	P value - 0.390	

In the present study 58% of the cases showed estrogen positivity

In IDC-DCIS 16 cases (64 %) showed estrogen positivity.In IDC without DCIS 52 % of the cases showed estrogen receptor positivity.

Estrogen positivity is higher in IDC-DCIS compared to IDC without DCIS. There is no significant difference in relation to estrogen status between IDC with and without DCIS. (Table 19 & chart 12)

12. Progesterone receptor

Table 20: Progesterone receptor status in IDC with and without DCIS

	Progesterone receptor status	
	Positive	**Negative**
IDC:DCIS (25)	11	14
IDC (25)	9	16
Total cases (50)	20	30
Chi square test	P value-0.390	

In the total 50 cases 40% showed progesterone positivity.

In the present study in IDC:DCIS 44% of the cases showed progesterone positivity. In IDC without DCIS 36 % of the cases showed progesterone receptor positivity.

Progesterone positivity is higher in IDC:DCIS compared to IDC without DCIS. There is no significant difference in relation to progesterone status between IDC with and without DCIS.(Table 20 & chart 13)

13. HER2/neu

Table 21: HER2/neu expression between IDC with and without DCIS

	HER2/neu	
	Positive	**Negative**
IDC-DCIS (25)	6	19
IDC (25)	9	16
Total cases (50)	15	35
Chi Square Test	P value = 0.354	

In our study 15 cases i.e, 30% of the cases showed HER2/neu overexpression. In IDC-DCIS 6 cases (24%) showed HER2/ neu positivity.In IDC without DCIS 9 cases (36%) showed HER2/ neu positivity. Among the HER2/neu positive cases 60 % of the cases are seen in IDC without DCIS. Only 30% of the cases are seen in invasive ductal carcinoma associated with DCIS.

There is no significant difference in relation to HER2/neu status between IDC with and without DCIS. (Table 21 & chart 14)

14.Ki 67

Table 22 :Distribution of Ki 67 between IDC with and without DCIS

	Ki 67	
	Low (≤ 20)	**High(>20)**
IDC-DCIS (25)	18	7
IDC (25)	10	15
Total cases (50)	28	22
Chi Square Test	**P value = 0.022**	

In the total study group 56% of the cases showed low proliferative index and 44 % of the cases showed high proliferation index.

In IDC-DCIS only 28% of the cases show high proliferative index however in IDC without DCIS 60% showed a higher proliferative index which is statistically significant.(p value- 0.022) (Table 22 & chart 15)

CORRELATION OF HER2/ neu WITH OTHER PROGNOSTIC FACTORS BETWEEN IDC WITH AND WITHOUT DCIS

1.Age

Table 23: Association of HER2/neu in relation to age

Age in Years	Total Cases(50)	
	HER2/neu +	HER2/neu -
≤ 40	1	-
41-50	9	11
51-60	4	12
61-70	1	10
71-80	-	2
Pearson chi square test	P value=0.333	

In our study HER2/neu amplification was higher in the age group of 41-50 years with 45 %. In the age group of ≤ 40 years only one case is present which showed positivity for HER2/neu. In 51-60 years 25% of the cases showed HER2/neu positivity. In 61-70 years only 9% the cases showed HER2/neu and age(Table 23)

Table 24 : Association of HER2/neu in relation to age between IDC with and without DCIS .

AGE IN YEARS	IDC-DCIS(25)		IDC(25)	
	HER2/ neu +	HER2/ neu -	HER2/ neu+	HER2/ neu -
≤ 40	1	-	-	-
41-50	3	6	6	5
51-60	2	7	2	5
61-70	-	5	1	5
71-80	-	1	-	1
Pearson chi square test	P value=0.239		P value=0.341	

In both IDC with and without DCIS higher number of positivity is seen in the age group of 41-50 years. (Table 24 ; Chart 16 & Chart 17)

2. Menopausal Status

Table 25 : Association of menopausal status with HER2/ neu

Menopausal status	Total cases(50)	
	HER2/neu +	HER2/neu 2-
Pre menopausal	7	13
Post menopausal	8	22
Pearson chi square test	P value= 0.528	

HER2/ neu positivity was higher in the postmenopausal age group . Among the premenopausal group 35% of the cases showed HER2/neu

positivity. Among the postmenopausal group also 26.6% the cases showed HER2/neu positivity. There is no significant association between HER2/neu and menopausal status.(Table 25)

Table 26 :Association of HER2/neu in relation to menopausal status between IDC with and without DCIS

Menopausal status	IDC-DCIS(25)		IDC(25)	
	HER2/ neu+	HER2/ neu -	HER2/ neu+	HER2/ neu -
Pre menopausal	4	8	3	5
Post menopausal	2	11	6	11
Pearson chi square test	P value=0.293		P value=0.914	

In IDC-DCIS premenopausal group showed 33.3% positivity and 15.3% positivity in post menopausal group. In IDC without DCIS premenopausal group showed 37.5% positivity and 35.2% positivity in post menopausal group. (Table 26 ; Chart 18 & chart 19)

3.Tumor size

Table 27 : Association of HER2/ neu with tumor size

Tumor size	All cases(50)	
	HER2/neu+	HER2/neu -
<2 cm	1	5
2-5 cm	6	23
>5 cm	8	7
Pearson chi square test	P value=0.0609	

In our study HER2/neu positivity is higher in > 5 cm tumors with 16% positivity of the total cases followed by 12 % in 2-5 cm and 2 % with tumor size <2 cm.

Among the tumors with <2 cm size 16 % of the cases are HER2/neu positive,20% of the cases showed positivity among the 2-5cm tumor size and 53% of the cases showed positivity with tumor size > 5cm. (Table 27)

Table 28 : Association of HER2/neu with tumor size between IDC-DCIS and IDC

Tumor size	IDC-DCIS(25)		IDC(25)	
	HER2/ neu+	HER2/ neu -	HER2/ neu +	HER2/ neu -
<2 cm	-	4	1	1
2-5 cm	4	11	2	12
>5 cm	2	4	6	3
Pearson chisquare test	P value=0.447		**P value=0.034**	

In both IDC-DCIS and pure IDC higher rate of positivity was seen in > 5cm tumor size. In IDC-DCIS 33.3% of the cases show positivity for HER2/neu in tumor size > 5cm.In IDC without DCIS group among tumors with >5cm size, 66.6% of the cases show positivity .

IDC without DCIS showed a statistically significant relation between the size of the tumor and HER2/neu. (Table 28 ; Chart 20 & 21)

4. Tumor grade

Table 29 : Association of HER2/ neu with tumor grade

Tumor grade	All cases (50)	
	HER2/neu +	HER2/neu -
Grade 1	1	8
Grade 2	10	23
Grade 3	3	2
Pearson chisquare test	P value= 0.158	

HER2/neu positivity was higher in grade 3 tumors with 60% positivity followed by 30.3 % in grade 2 tumors and 11.1% in grade1 tumors.(Table 29)

Table 30 : Association of HER2/neu with tumor grade between IDC-DCIS and IDC

Tumor grade	**IDC-DCIS**		**IDC**	
	HER2/neu+	**HER2/neu-**	**HER2/neu+**	**HER2/neu-**
Grade 1	1	5	0	3
Grade 2	3	13	7	10
Grade 3	2	1	1	1
Pearson chi square test	P value=0.101		P value=0.359	

In IDC-DCIS one case showed positivity out of 3 cases with histological grade 3.Among grade 2 tumors 18.7 % of the cases showed HER2/neu positivity.

In IDC, 2 cases presented with histological grade 3 in which one case showed positivity for HER2/neu. Among grade 2 tumors 41.1% of the cases showed HER2/neu positivity.HER2/neu positivity is not seen among grade1 tumors. (Table 30 ;Chart 22 & 23)

5.NECROSIS

Table 31:Association of HER2/neu with tumor necrosis .

Necrosis	Total cases(50)	
	HER2/neu+	HER2/neu -
Present	2	6
Absent	13	29
Fisher exact test	P value= 1.000	

Among the 8 cases with tumor necrosis only 2 cases (25%) showed HER2/neu positivity. There is no significant correlation between necrosis and HER2/neu overexpression.(Table 31)

Table 32:Association of HER2/neu with tumor necrosis between IDC-DCIS and IDC without DCIS

Necrosis	IDC-DCIS(25)		IDC(25)	
	HER2/neu+	HER2/neu -	HER2/neu+	HER2/neu -
Present	-	4	2	2
Absent	6	15	7	14
Pearson chi square test	**P value=0.540**		**P value=0.602**	

In IDC-DCIS HER2/neu positivity is absent among tumors with necrosis. Among 4 cases with necrosis 2 cases showed positivity in IDC without DCIS. There is no significant association between tumor necrosis and HER2/neu overexpression in both IDC with and without DCIS. (Table 32; Chart 24 & 25)

6. Nipple invasion

Table 33:Association of HER2/neu with nipple invasion.

Nipple invasion	Total cases(50)	
	HER2/neu+	HER2/neu -
Present	1	1
Absent	14	9
Fisher exact test test	P value - 1.000	

Among the 2 cases with nipple invasion one case showed positivity for HER2/neu. There is no significant relation between nipple invasion and HER2/neu positivity.(Table 33)

Table 34:Association of HER2/neu with nipple invasion in IDC with and without DCIS

Nipple invasion	IDC-DCIS(25)		IDC(25)	
	HER2/neu+	HER2/neu -	HER2/neu+	HER2/neu -
Present	-	1	1	-
Absent	6	18	8	16
Pearson chi square test	P value – 1.000		P value-0.360	

In IDC-DCIS only one case presented with nipple invasion which did not show HER2/neu overexpression. In IDC without DCIS one case which presented with nipple invasion showed HER2/neu positivity.

There is no significant relation between nipple invasion and HER2/neu positivity in both IDC with and without DCIS.(Table 34 ; Chart 26 & 27)

7. Lymphovascular invasion

Table 35 : Association of HER2/neu with lymphovascular invasion

LVI	Total cases(50)	
	HER2/neu+	HER2/neu -
Present	6	8
Absent	9	27
Fisher exact test	P value= 0.304	

Among 14 cases with lymphovascular invasion 6 cases (42.8%) showed HER2/neu positivity. There is no significant association between lymphovascular invasion and HER2/neu positivity.(Table 35)

Table 36 :Association of HER2/neu with lymphovascular invasion in IDC with and without DCIS

LVI	IDC-DCIS(25)		IDC(25)	
	HER2/neu+	HER2/neu -	HER2/neu+	HER2/neu -
Present	2	4	4	4
Absent	4	15	5	12
Fisher exact test	P value= 0.606		P value= 0.394	

In IDC-DCIS 2 cases (33.3%) showed HER2/neu positivity among cases with lymphovascular invasion.In IDC without DCIS 50 % of the cases showed HER2/neu positivity in cases with lymphovascular invasion. Among cases without lymphovascular invasion 29.4 % of the cases showed HER2/neu positivity.

There is no significant association between lymphovascular invasion and HER2/neu positivity in both IDC with and without DCIS. (Table 36; Chart 28 & 29)

8.**Lymph Node Status**

Table 37 :Association of HER2/neu with lymph node status.

Lymph node	**Total cases**	
	HER2/neu+	**HER2/neu -**
Positive	13	19
Negative	2	16
Pearson chisquare test	**P value=0.028**	

Among the HER2/neu positive cases 13 cases i.e. 86.6 % show nodal metastasis. Among the node positive cases,40 % of the cases show HER2/neu positivity. Among the node negative cases, only 11 % of the cases show HER2/neu positivity.There is a significant association between HER2/ neu and lymph node metastasis.(Table 37)

Table 38 :Association of HER2/neu with lymph node status between IDC-DCIS and IDC

Lymph node	IDC-DCIS(25)		IDC(25)	
	HER2/neu+	**HER2/neu -**	**HER2/neu+**	**HER2/neu -**
Positive	5	9	9	10
Negative	1	10	0	6
Pearson chi square test	P value=0.180		**P value=0.0305**	

In IDC-DCIS 35.7 % of the node positive cases showed HER2/neu positivity.In IDC without DCIS 47.3% of the node positive cases show HER2/ neu positivity which was statistically significant. (Table 38; Chart 30 & 31)

9. Estrogen receptor

Table 39: Association of HER2/neu with Estrogen receptor

Estrogen receptor	Total cases(50)	
	HER2/neu+	**HER2/neu -**
Positive	3	26
Negative	12	9
Chi square test	**P value <0.0001**	

Among ER positive tumors only 10.3 % of cases showed HER2/neu positivity.Among ER negative tumors 63.1 % of cases showed HER2/neu positivity. HER2/neu positivity is higher in cases which are negative for estrogen receptor.(Table 39)

Correlation between ER and HER2/neu was done by Kendall tau b test.It showed a correlation co efficient of - 0.504 which is statistically significant.(P value : < 0.0001). This shows that there is a inverse relation between estrogen receptor status and HER2/neu expression which is statistically significant.

Table 40:Association of HER2/neu with Estrogen receptor in IDC with and without DCIS

Estrogen receptor	**IDC-DCIS(25)**		**IDC(25)**	
	HER2/neu+	**HER2/neu -**	**HER2/neu+**	**HER2/neu -**
Positive	2	14	1	12
Negative	4	5	8	4
	P value=0.142		**P value=0.003**	

In IDC-DCIS among ER positive tumors only 12.5 % of the cases showed HER2/neu positivity. Among ER negative tumors 44.4% of the cases showed HER2/neu positivity.

In IDC without DCIS, only 7.6 % of the cases showed HER2/neu positivity among ER positive tumors. Among ER negative tumors 66.6% of the cases showed HER2/neu positivity. (Table 40;Chart 32 & 33).Kendall tau b test showed correlation co-efficient of -0.614 with a significant p value(0.003).

10.Progesterone receptor

Table 41: Association of HER2/neu with progesterone receptor

Progesterone Receptor	Total cases(50)	
	HER2/neu+	HER2/neu -
Positive	2	18
Negative	13	17
Pearson chisquare test	**P value= 0.012**	

Among PR positive tumors 10 % of the cases showed HER2/neu overexpression. Among PR negative tumors 43.3% of the tumors showed HER2/neu overexpression. There is a signicant association between PR and HER2/neu overexpression. Kendall tau b test for correlation showed a correlation co efficient of -0.356 with a significant p value.

This shows there is a inverse relationship between PR and HER2/neu overexpression which is statistically significant.

Table 42 :Association of HER2/neu with progesterone receptor in IDC with and without DCIS

Progesterone receptor	IDC/DCIS(25)		IDC(25)	
	HER2/neu+	HER2/neu -	HER2/neu+	HER2/neu -
Positive	1	10	1	8
Negative	5	9	8	8
P value	0.180		0.088	

In IDC/DCIS among PR positive tumors only 9% of the cases are HER2/neu positive.Among PR negative tumors 35.7 % of the tumors are HER2/neu positive.

In IDC without DCIS , among PR positive tumors only 11% of the cases are HER2/neu positive.Among PR negative tumors 50% of the tumors are HER2/neu positive.(Table 42 ; Chart 34 & 35)

11.ki 67

Table 43 : Association of HER2/neu with Ki 67

Ki 67	**Total cases(50)**	
	HER2/neu +	**HER2/neu -**
Low Ki 67 (≤20)	2	26
High Ki 67 (>20)	13	9
Pearson chisquare test	**P value <0.0001**	

In our study among 28 cases with low proliferative index only 2 cases(7%) are positive for HER2/neu. Among 22 cases with higher proliferative index 59 % of the cases shows HER2/neu overexpression. There is a statistical significance between ki 67 and HER2/neu overexpression.(Table 43)

Table 44: Association of HER2/neu with Ki 67 between IDC-DCIS and IDC

Proliferation rate	IDC-DCIS(25)		IDC(25)	
	HER2/neu +	HER2/neu -	HER2/neu+	HER2/neu-
Low (≤20)	1	17	1	9
High (>20)	5	2	8	7
Pearson chi square test	**P value=0.0005**		**P value=0.027**	

In IDC-DCIS 5 % of the cases showed HER2/neu overexpression in low proliferative index group and 71.4 % of the cases showed HER2/neu overexpression among cases with higher proliferative index.

In IDC without DCIS 10 % of the cases showed overexpression in low proliferative index group and 53.3 % of the cases showed overexpression among cases with higher proliferative index. (Table 44; Chart 36 & 37)

There is a significant association between HER2/neu and Ki67 in both IDC-DCIS and IDC.

CHART 1: DISTRIBUTION OF AGE IN IDC-DCIS AND IDC

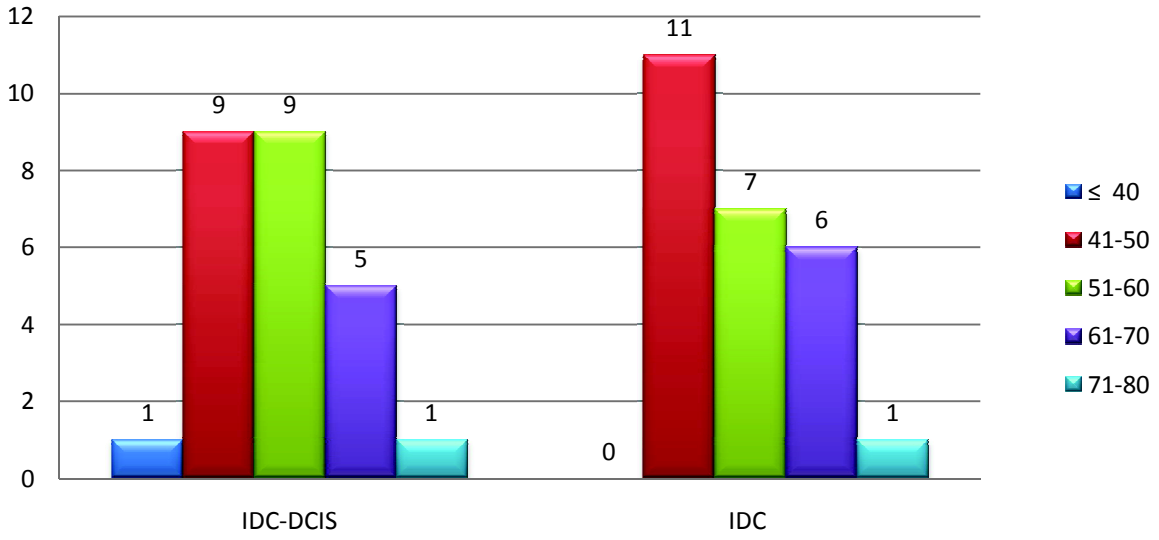
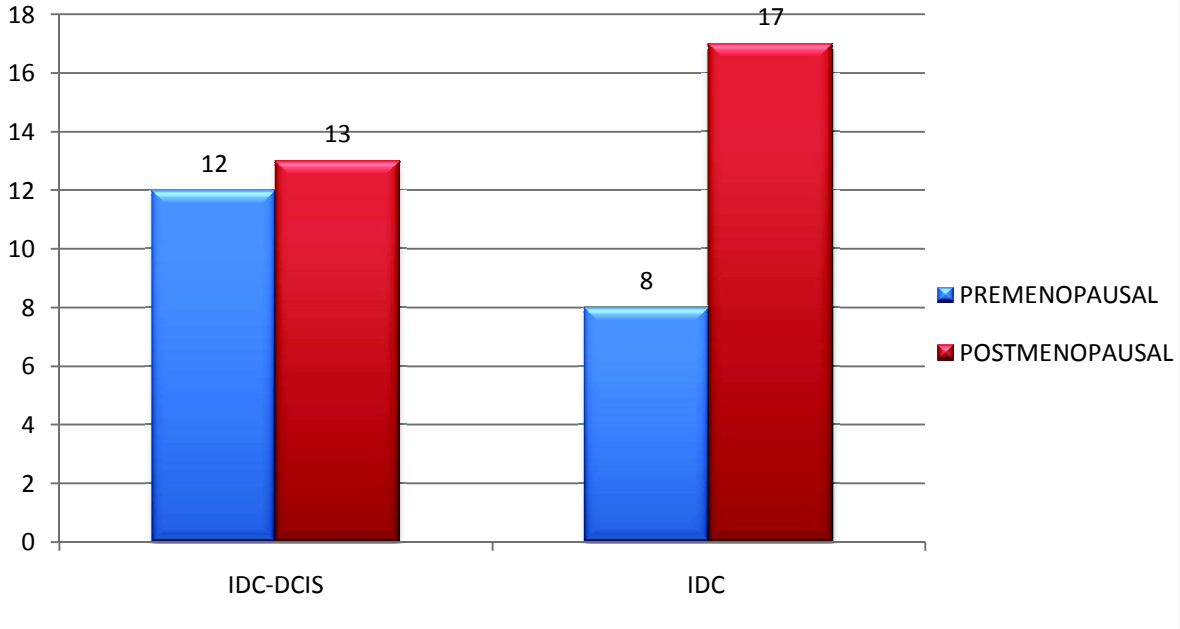


CHART 2: DISTRIBUTION OF MENOPAUSAL STATUS BETWEEN IDC-DCIS AND IDC



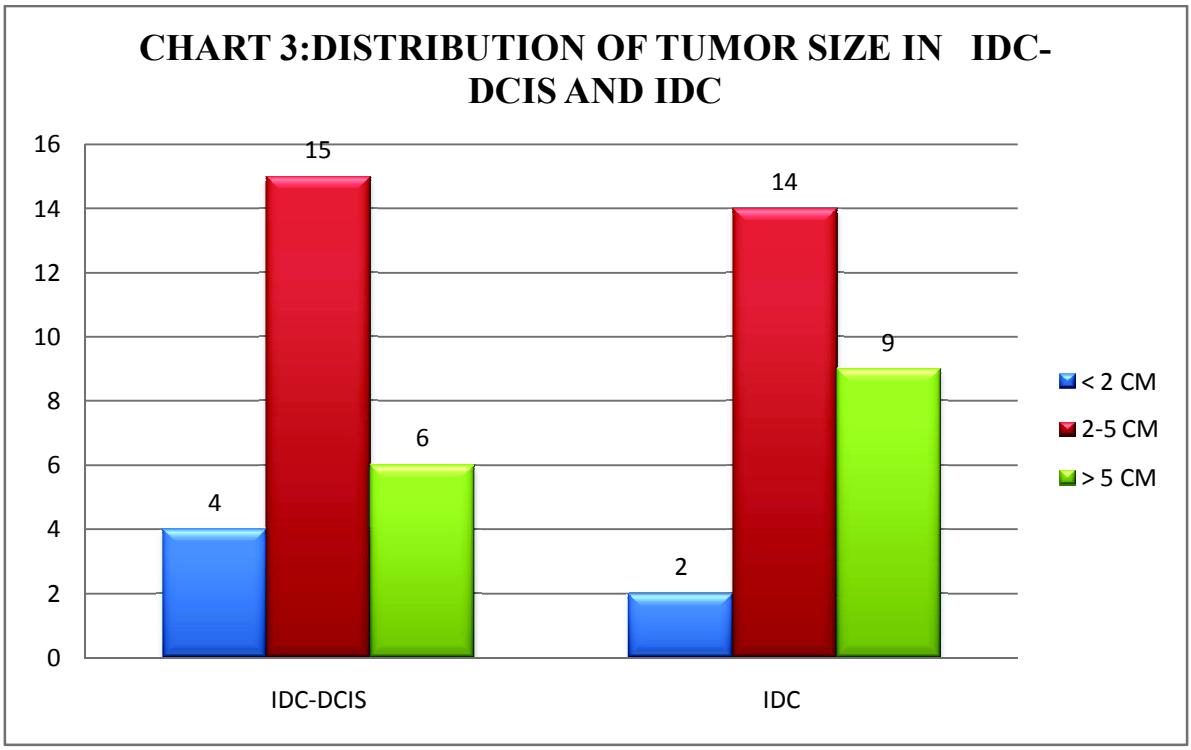


CHART 3:DISTRIBUTION OF TUMOR SIZE IN IDC-DCIS AND IDC
16
14
12
10
8
6
4
2
0
15
14
6
4
2
9
IDC-DCIS
IDC
< 2 CM
2-5 CM
> 5 CM

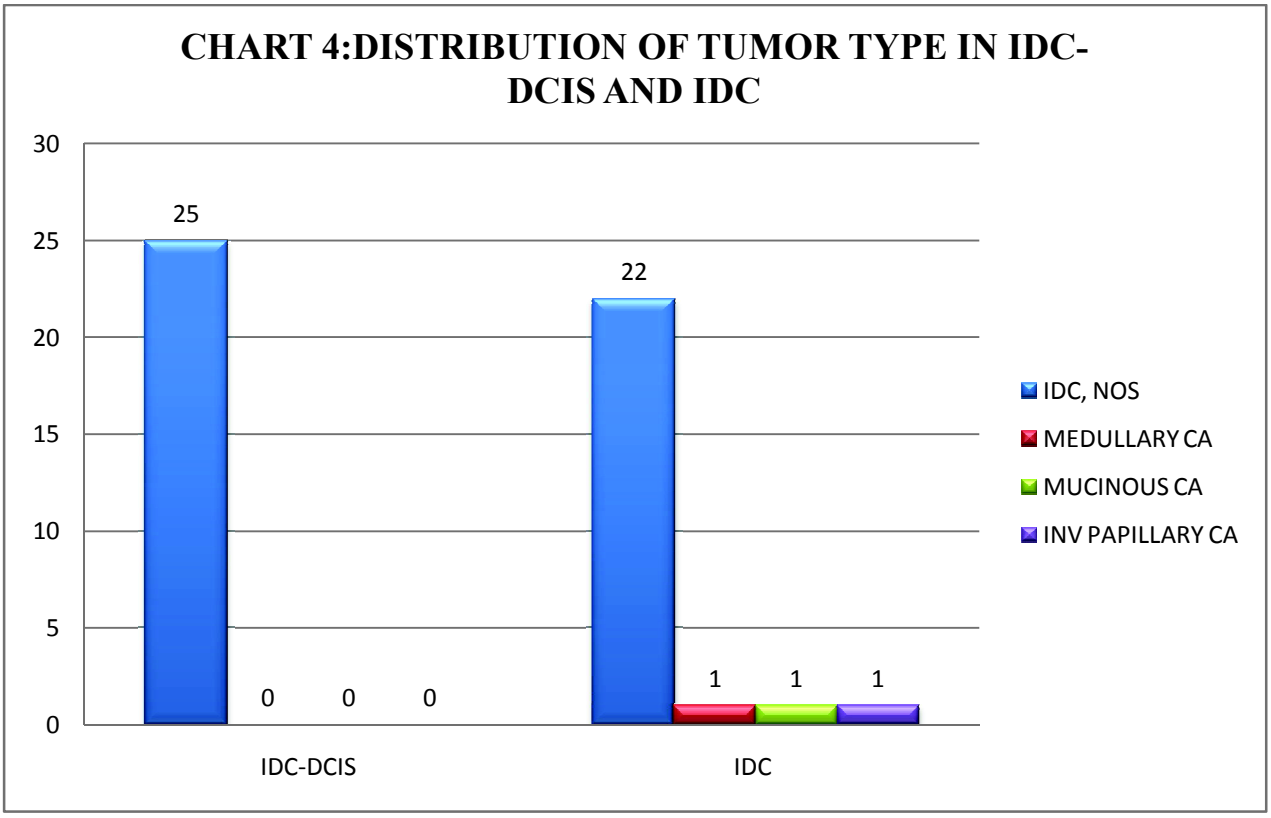


CHART 4:DISTRIBUTION OF TUMOR TYPE IN IDC-DCIS AND IDC
30
25
20
15
10
5
0
25
0
0
0
22
1
1
1
IDC-DCIS
IDC
IDC, NOS
MEDULLARY CA
MUCINOUS CA
INV PAPILLARY CA

CHART 5 :DISTRIBUTION OF VARIOUS TYPES OF DCIS IN IDC-DCIS

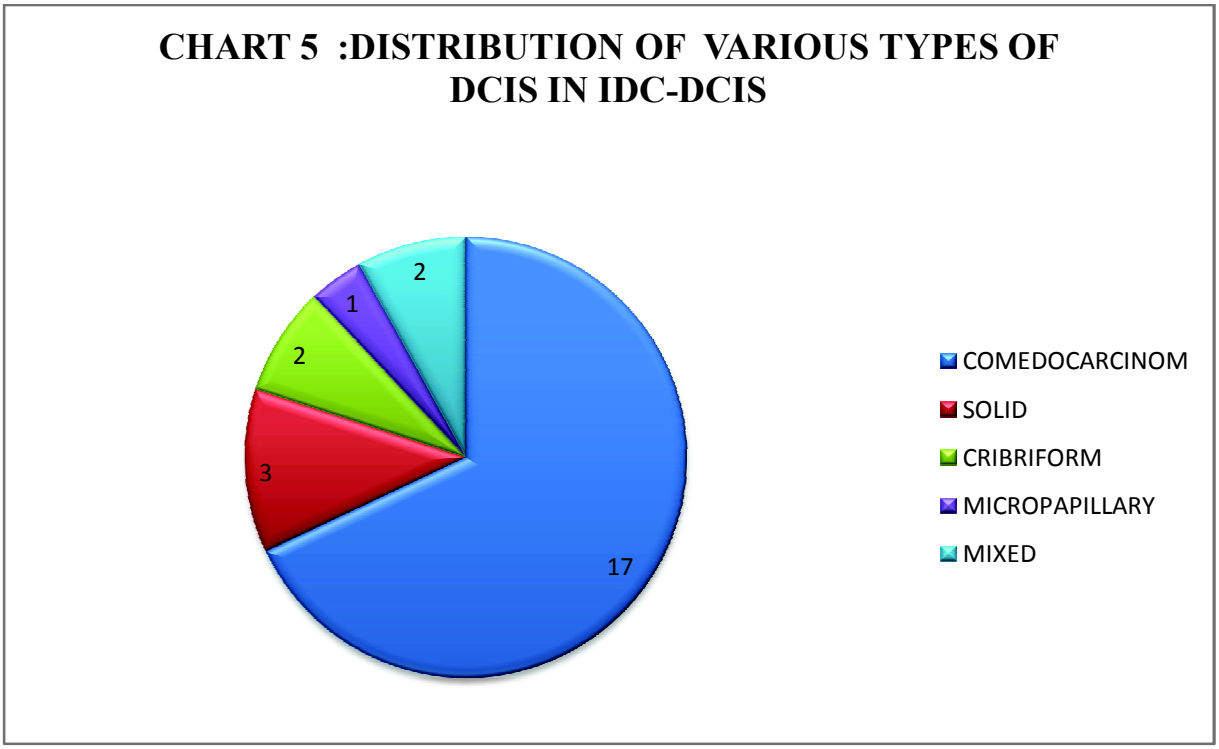


CHART 6 :DISTRIBUTION OF HISTOLGICAL GRADE OF DCIS IN IDC-DCIS

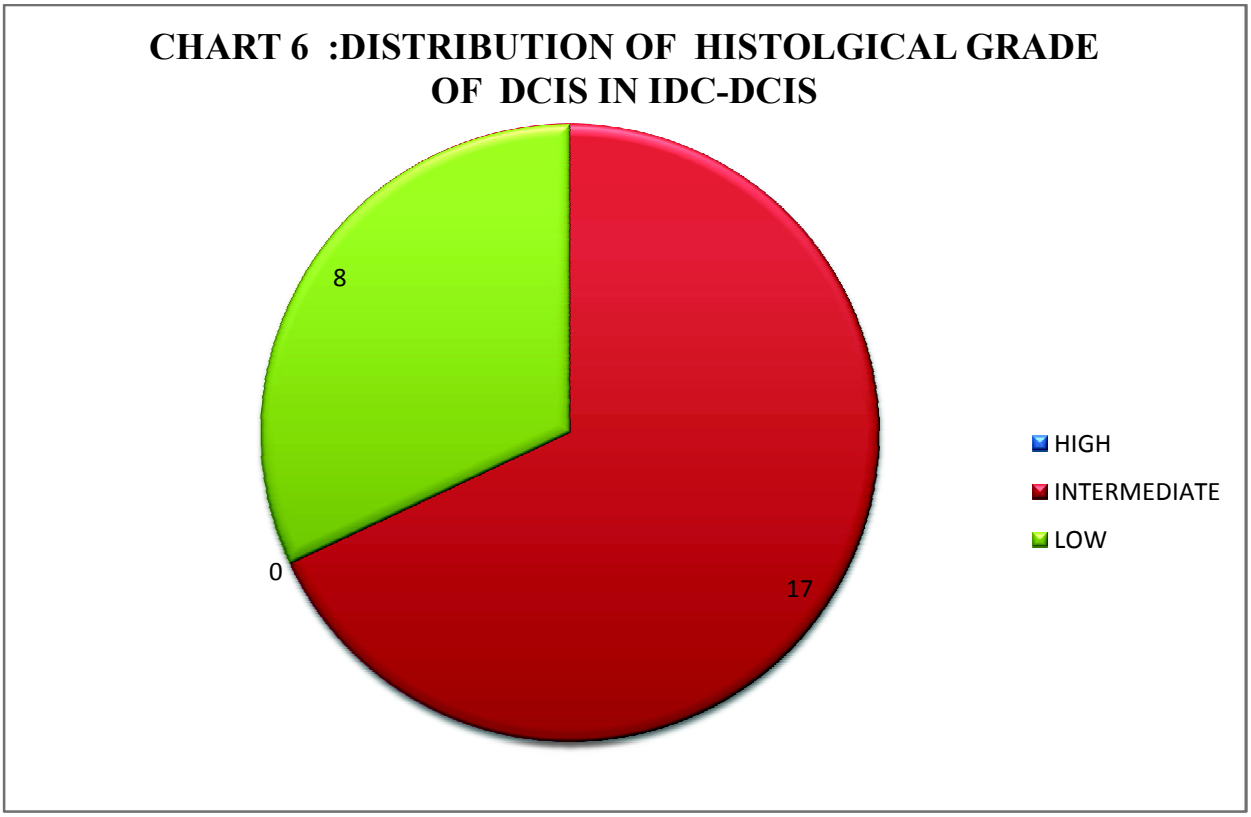


CHART 7:DISTRIBUTION OF TUMOR GRADE IN IDC-DCIS AND IDC .

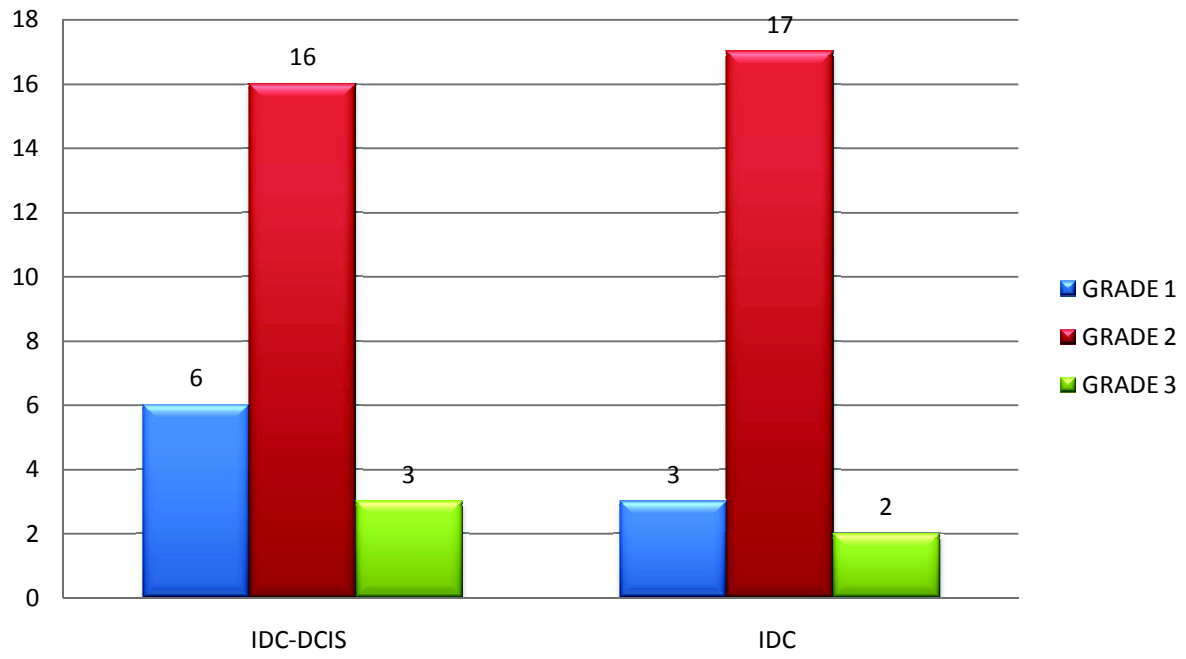


CHART 8 :DISTRIBUTION OF TUMOR NECROSIS IN IDC-DCIS AND IDC

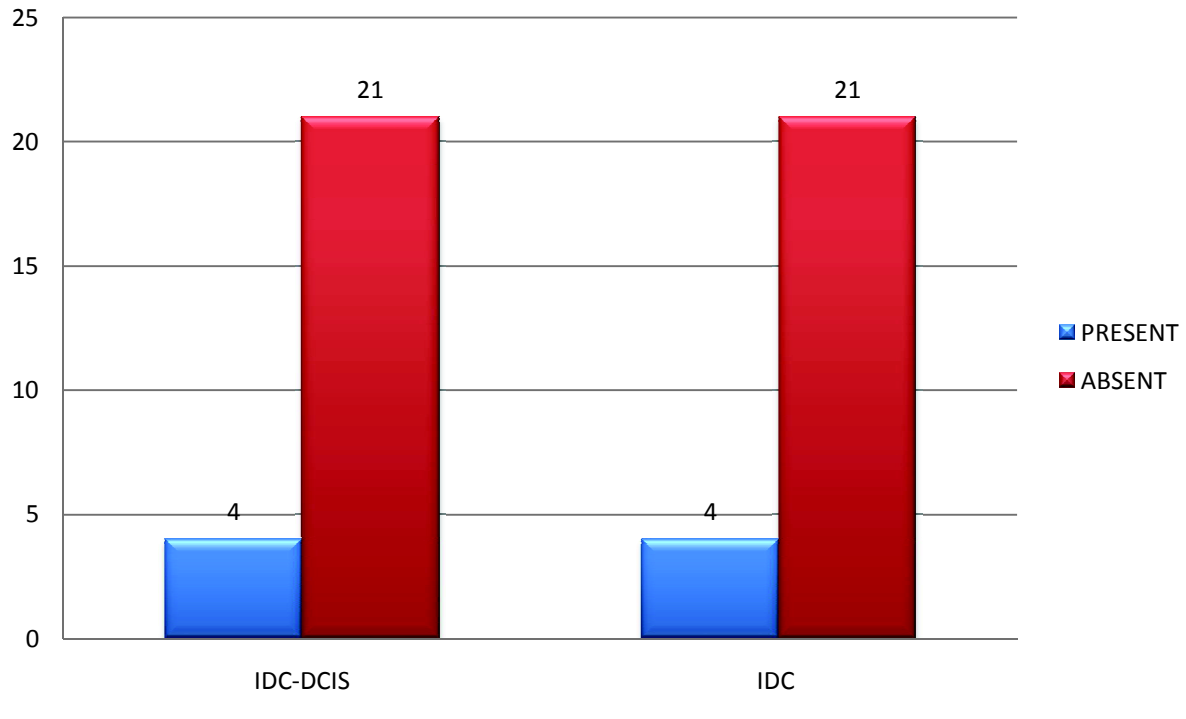


CHART 9 :NIPPLE INVASION IN IDC-DCIS AND IDC

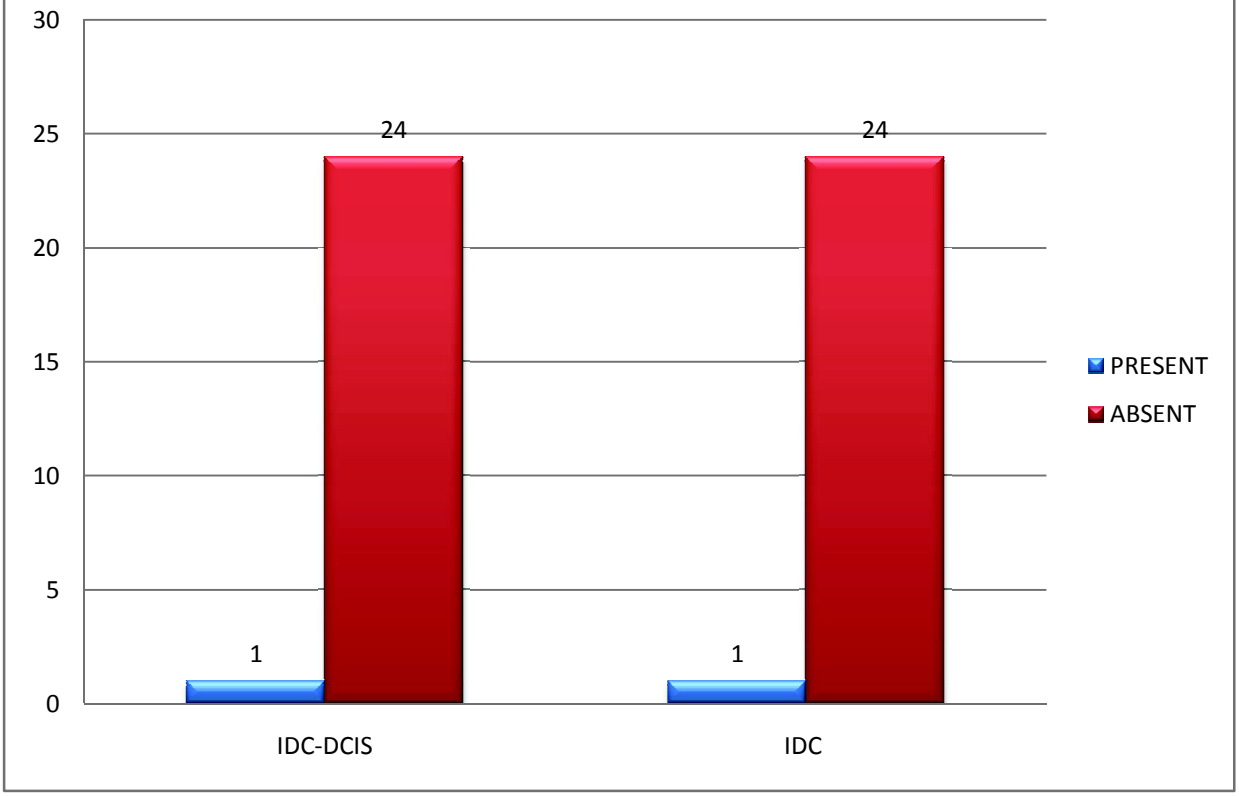


CHART 10: DISTRIBUTION OF LYMPHOVASCULAR INVASION IN IDC-DCIS AND IDC

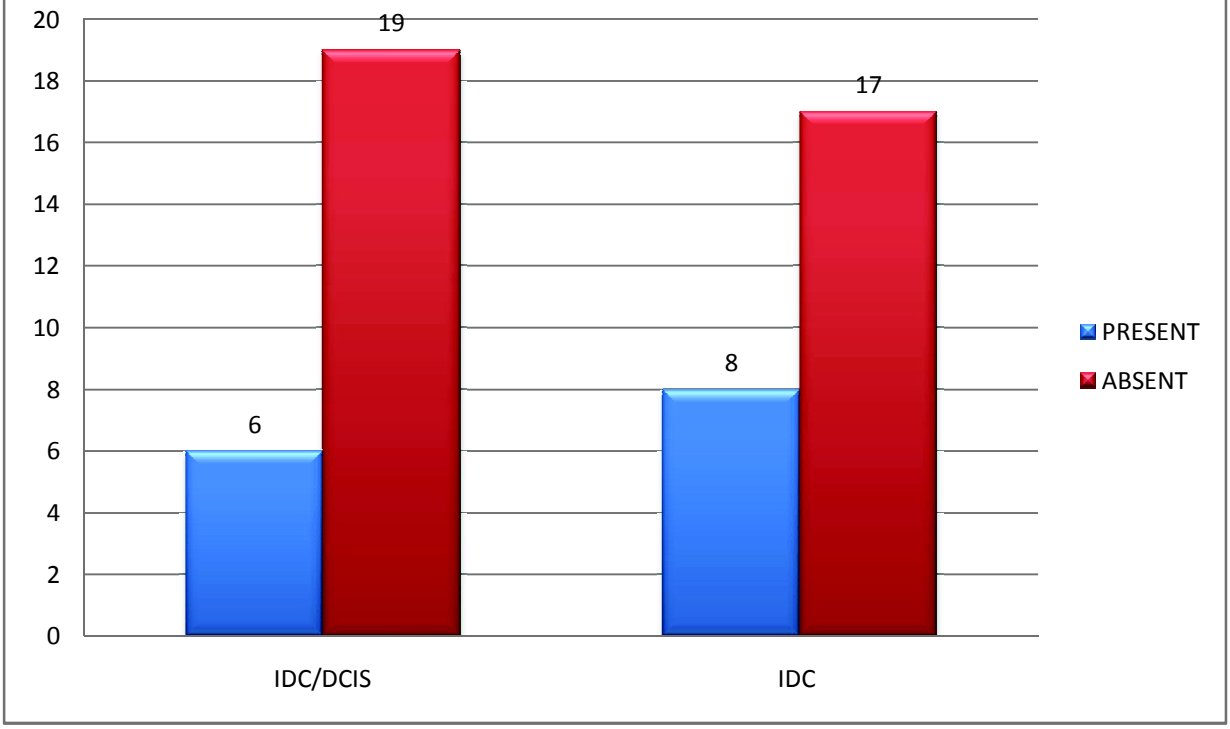


CHART 11 :DISTRIBUTION OF LYMPH NODE STATUS BETWEEN IDC-DCIS AND IDC

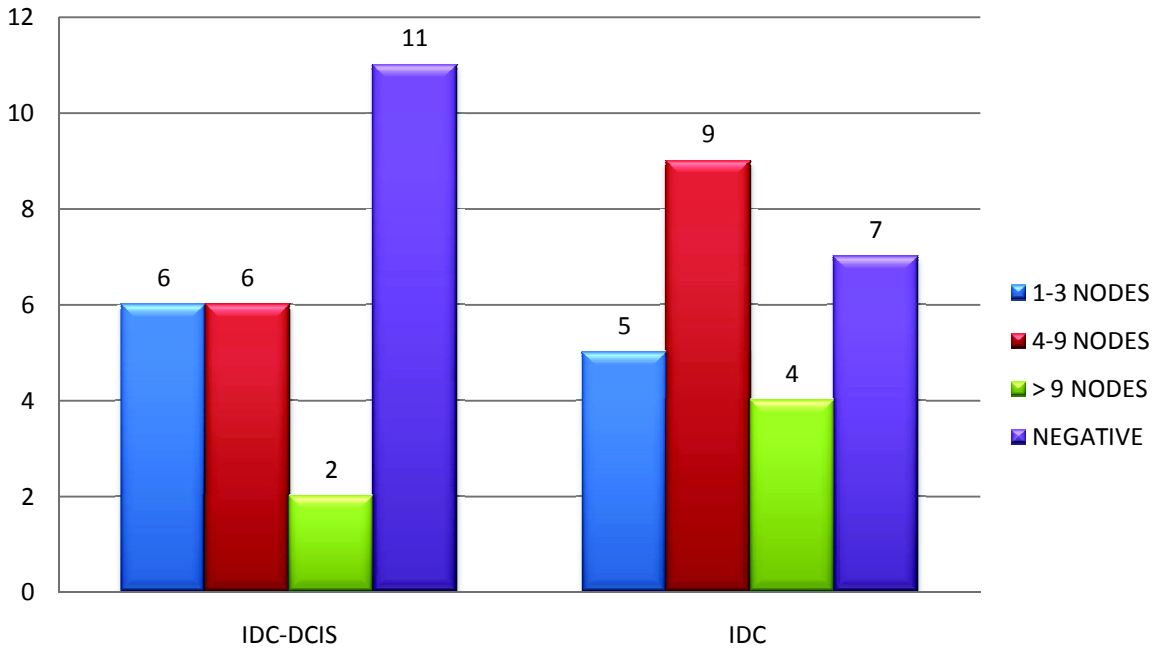


CHART 12 :ESTROGEN RECEPTOR STATUS IN IDC - DCIS AND IDC

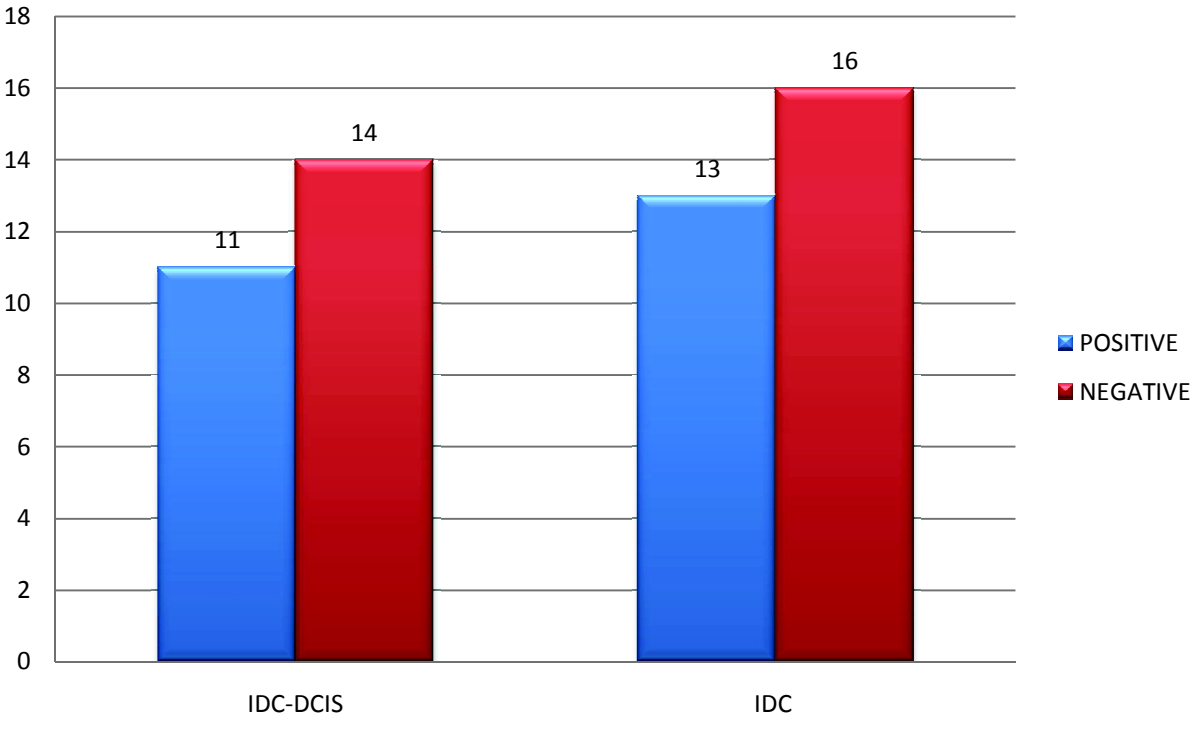


CHART 13 :PROGESTERONE RECEPTOR STATUS IN IDC-DCIS AND IDC

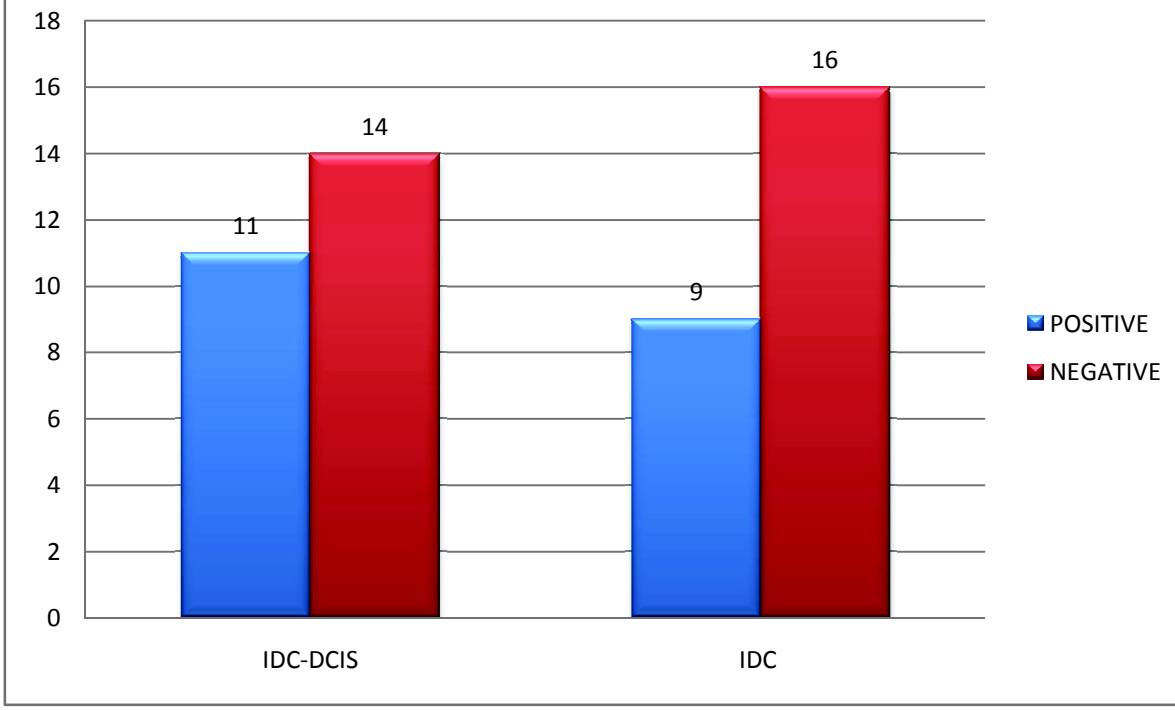


CHART 14: HER2/ NEU EXPRESSION IN IDC-DCIS AND IDC

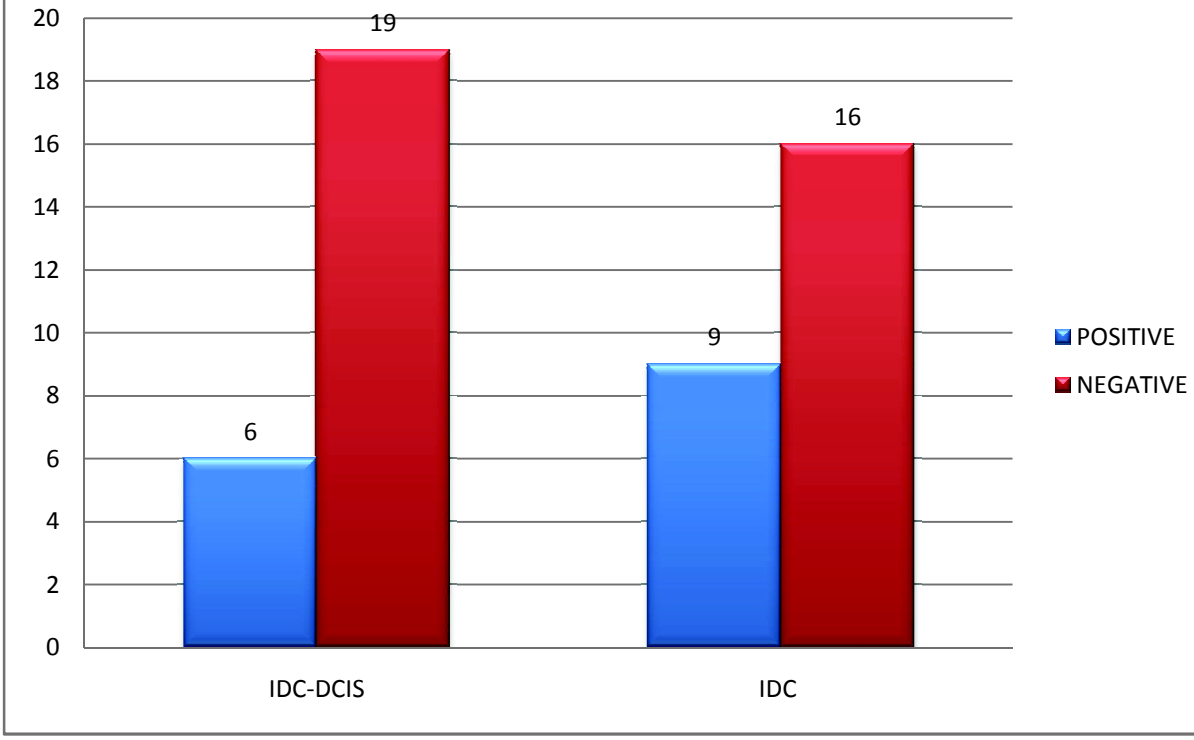


CHART 15:DISTRIBUTION OF Ki 67 EXPRESSION IN IDC-DCIS AND IDC

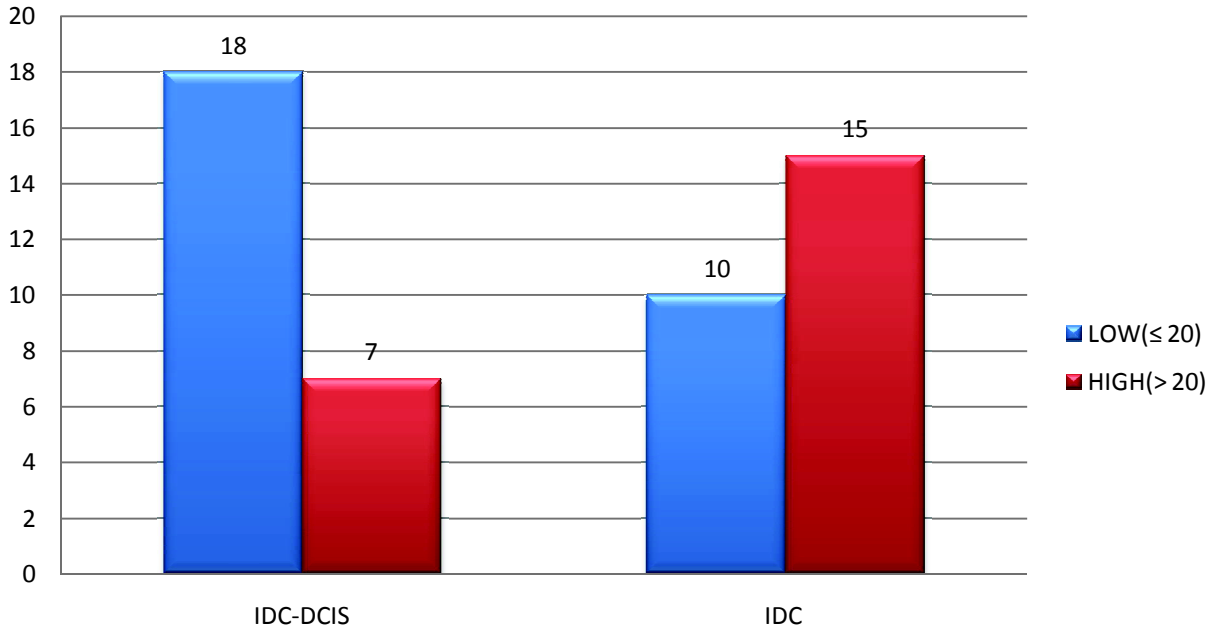


CHART 16:CORRELATION OF HER2 /NEU WITH AGE IN IDC-DCIS

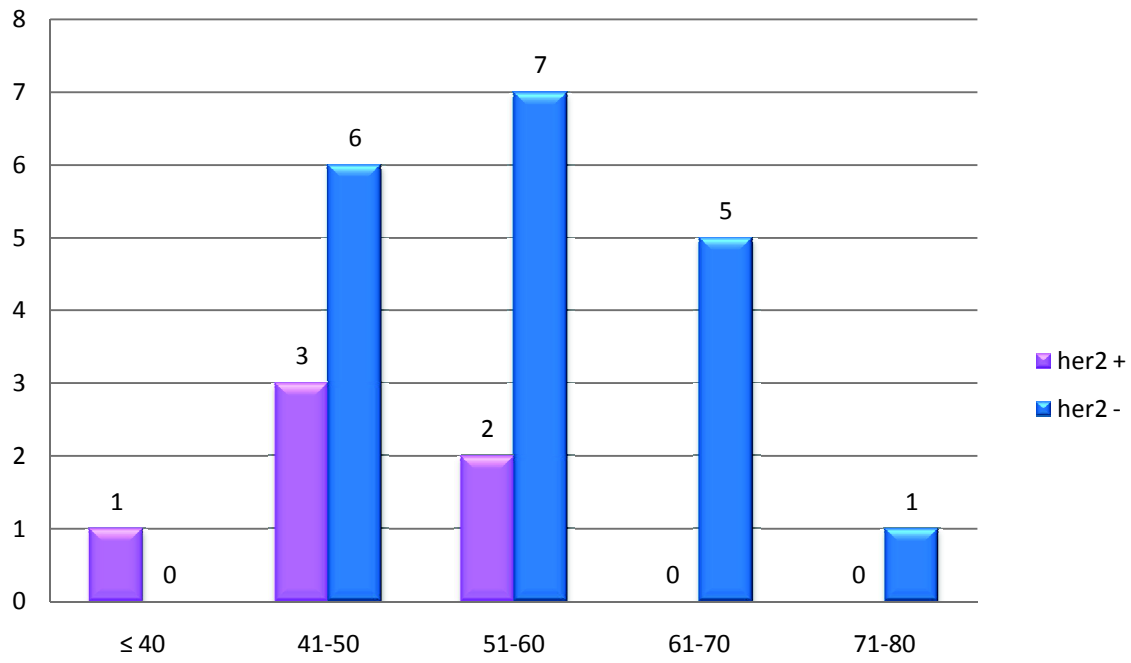


CHART 17:CORRELATION OF HER2NEU WITH AGE IN IDC

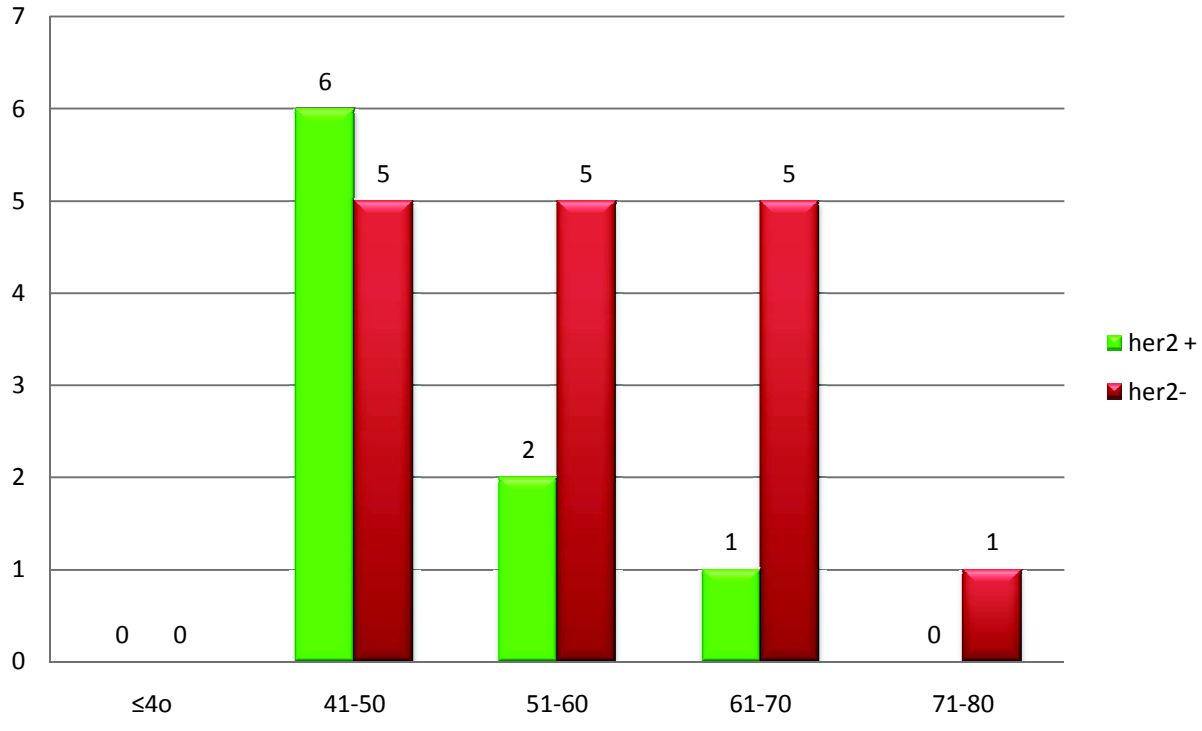


CHART 18 :ASSOCIATION OF HER2 /NEU WITH MENOPAUSAL STATUS IN IDC-DCIS

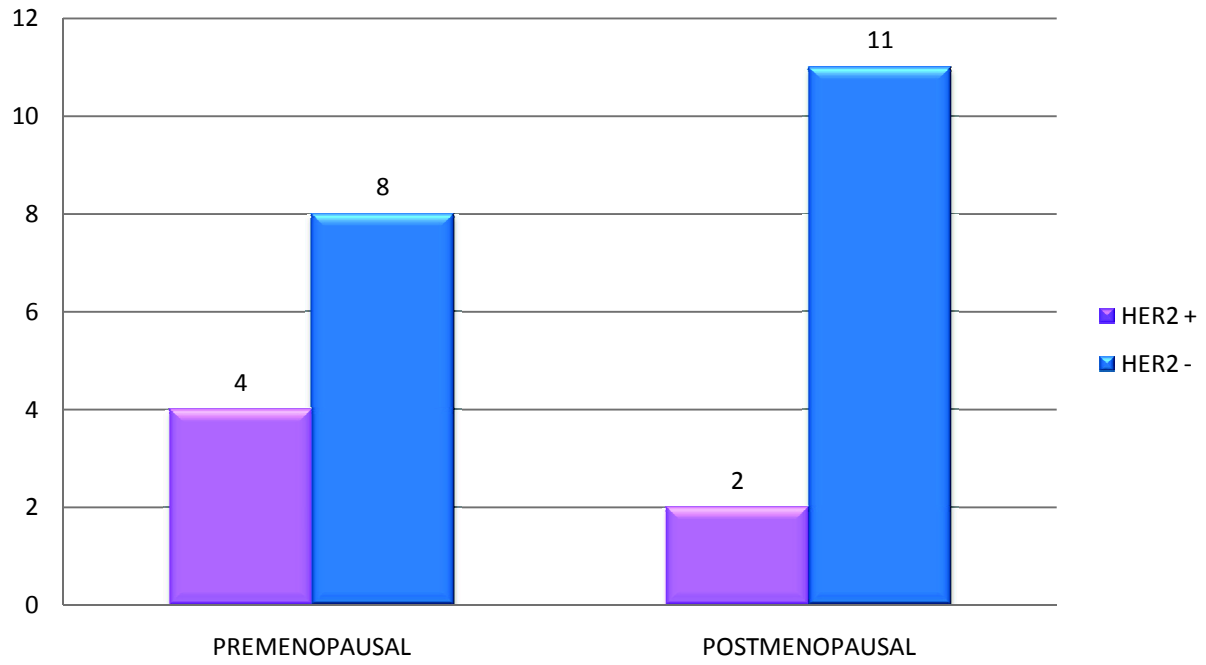


CHART 19:ASSOCIATION OF HER2/neu WITH MENOPAUSAL STATUS IN IDC

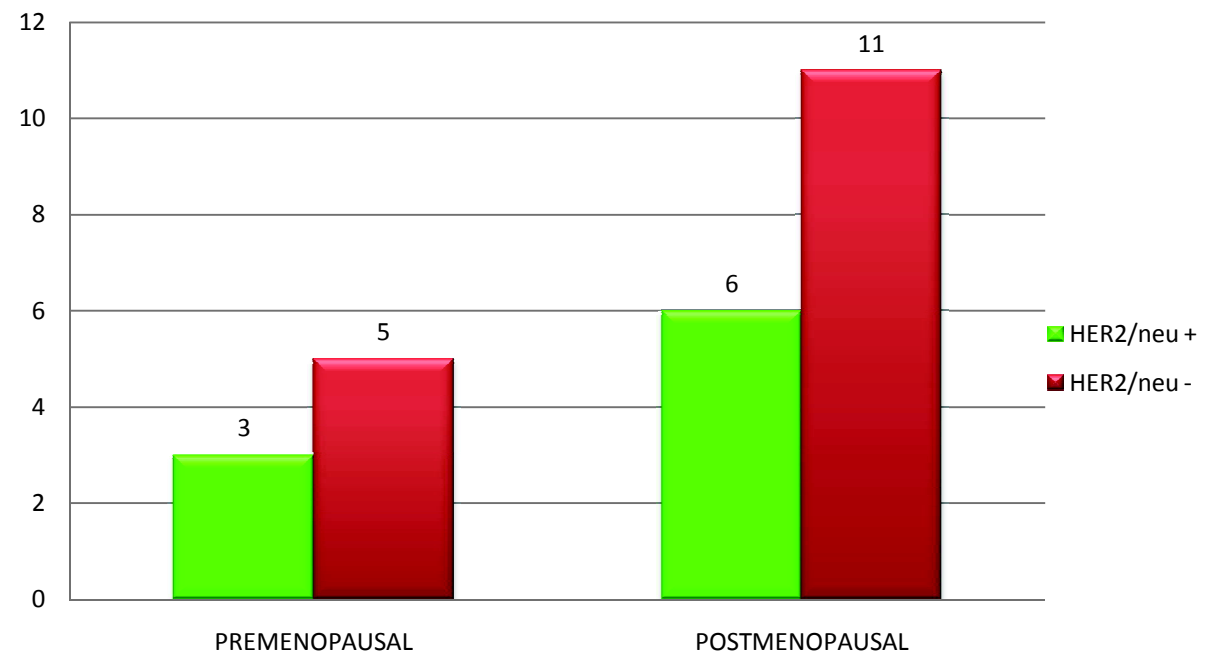


CHART 20 :ASSOCIATION OF HER2 /NEU WITH TUMOR SIZE IN IDC-DCIS

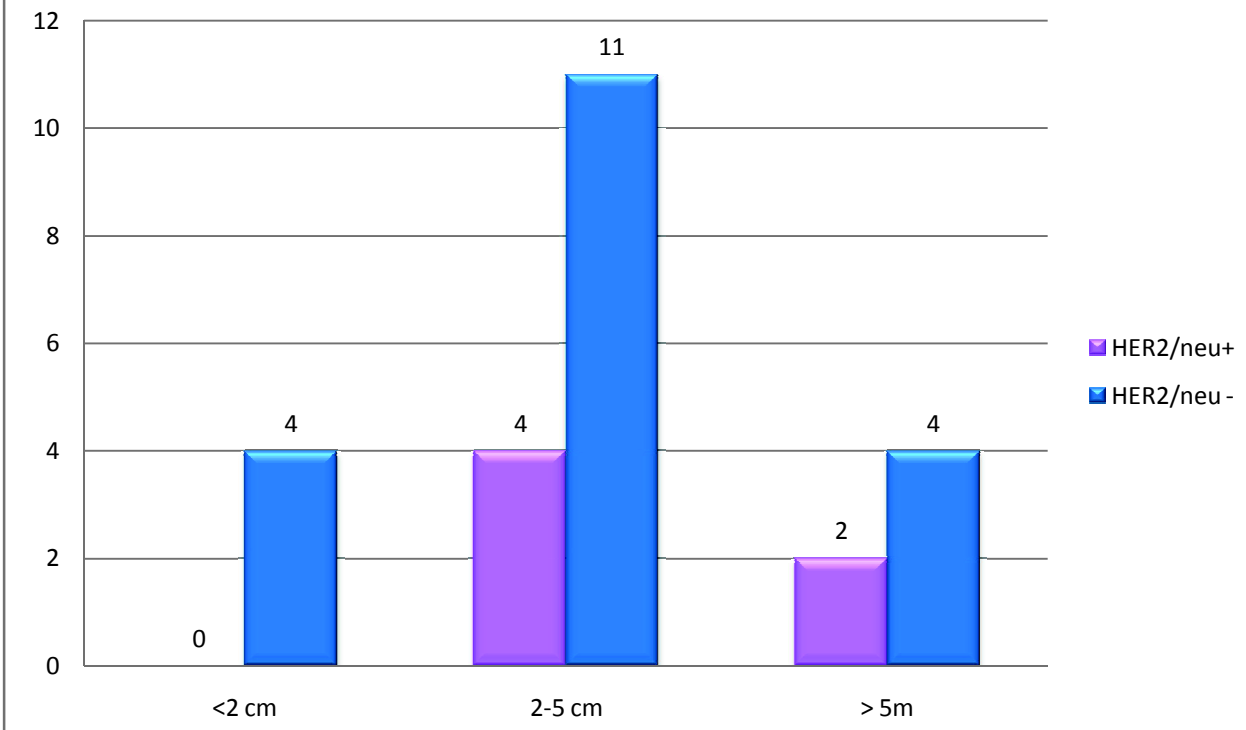


CHART 21 :ASSOCIATION OF HER2/ NEU WITH TUMOR SIZE IN IDC

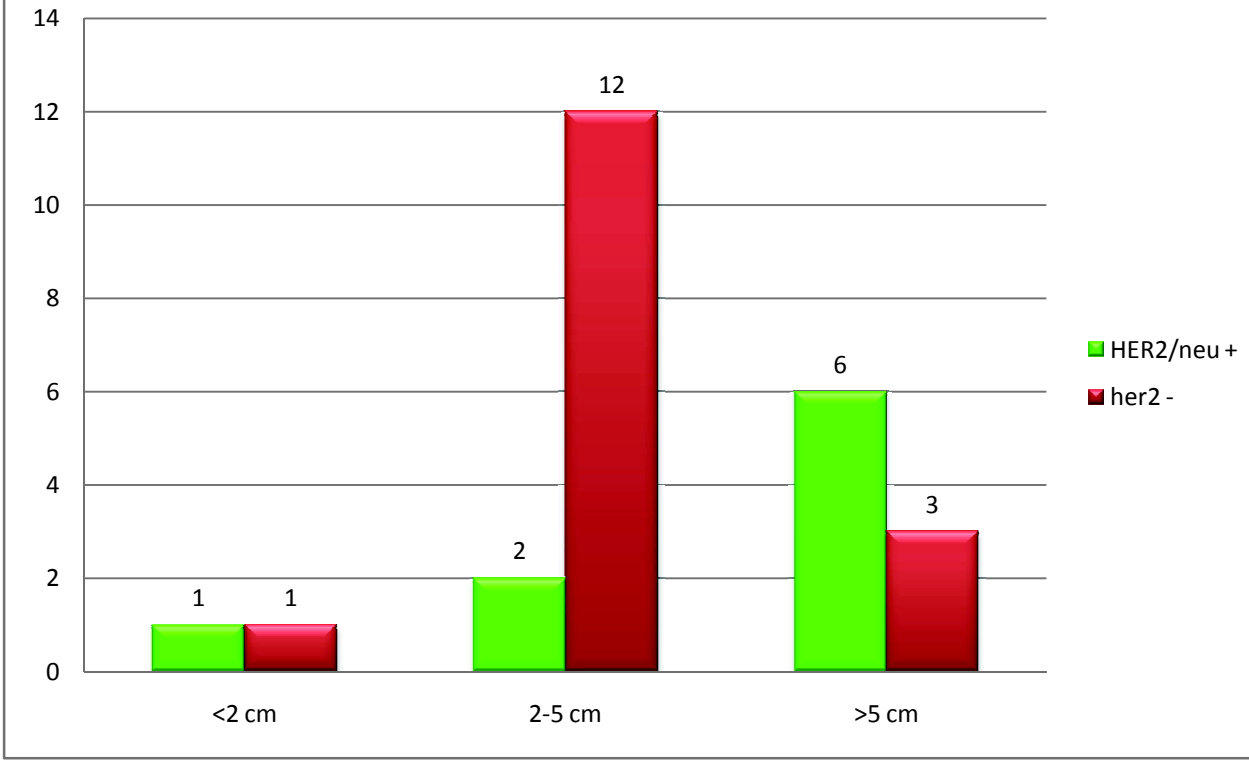


CHART 22:ASSOCIATION OF HER2/NEU WITH TUMOR GRADE IN IDC-DCIS

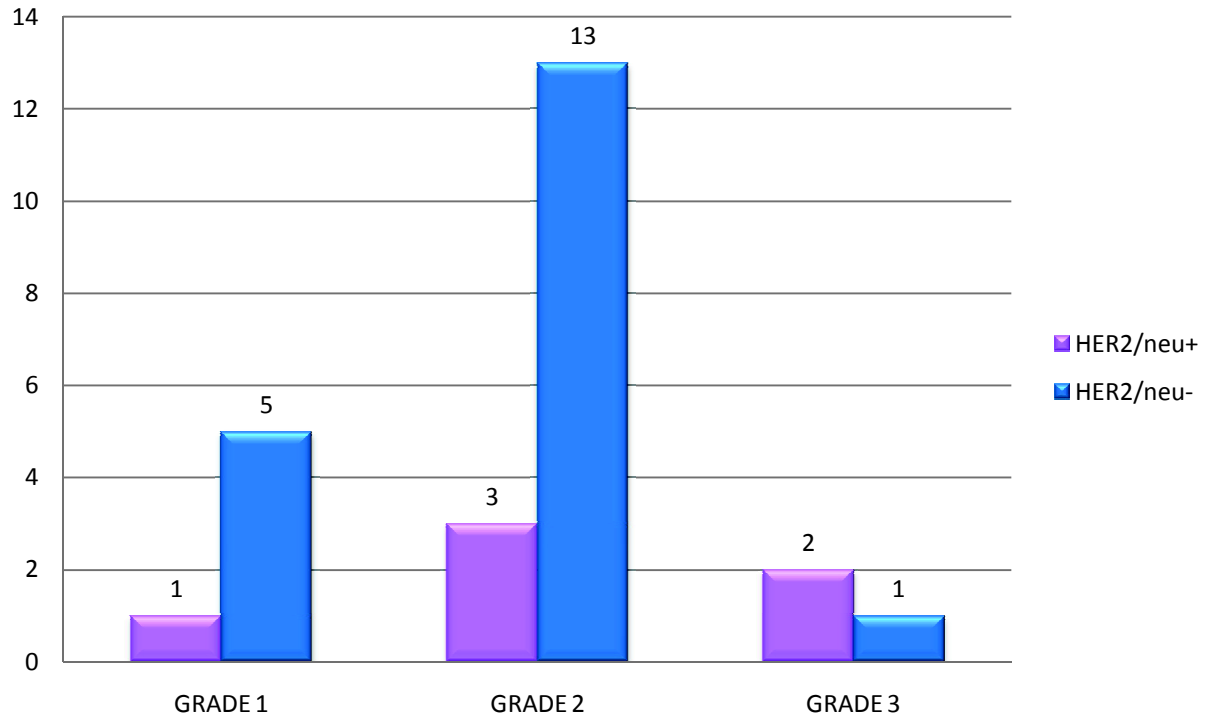
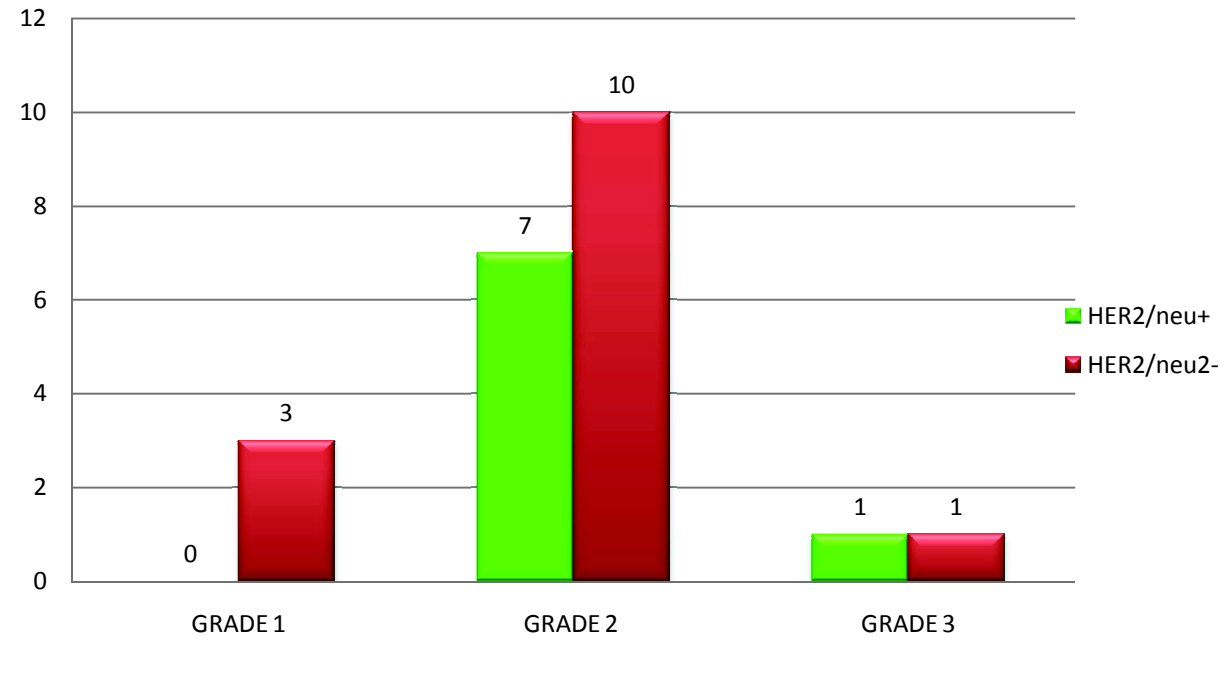


CHART 23:ASSOCIATION OF HER2/NEU WITH TUMOR GRADE IN IDC



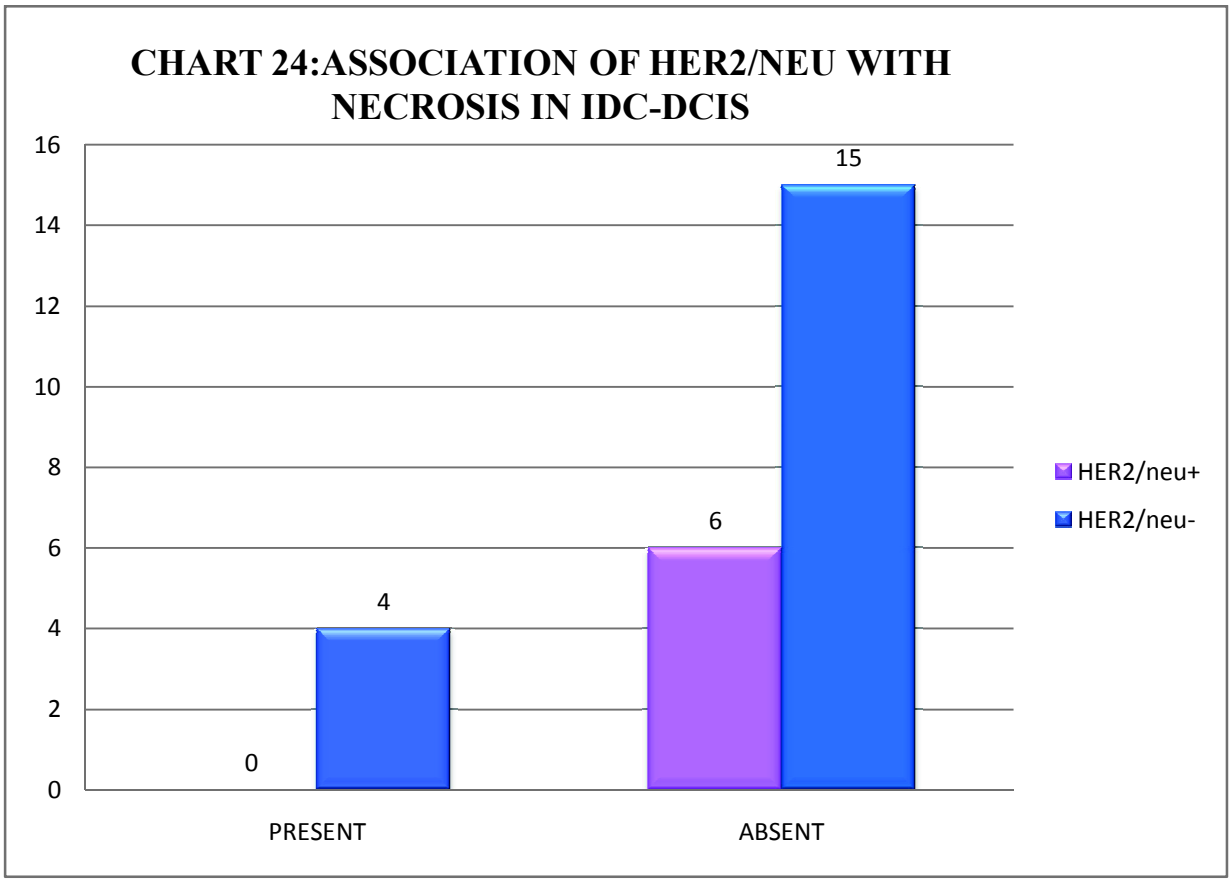


CHART 24:ASSOCIATION OF HER2/NEU WITH NECROSIS IN IDC-DCIS
16
14
12
10
8
6
4
2
0
0
4
6
15
PRESENT
ABSENT
HER2/neu+
HER2/neu-

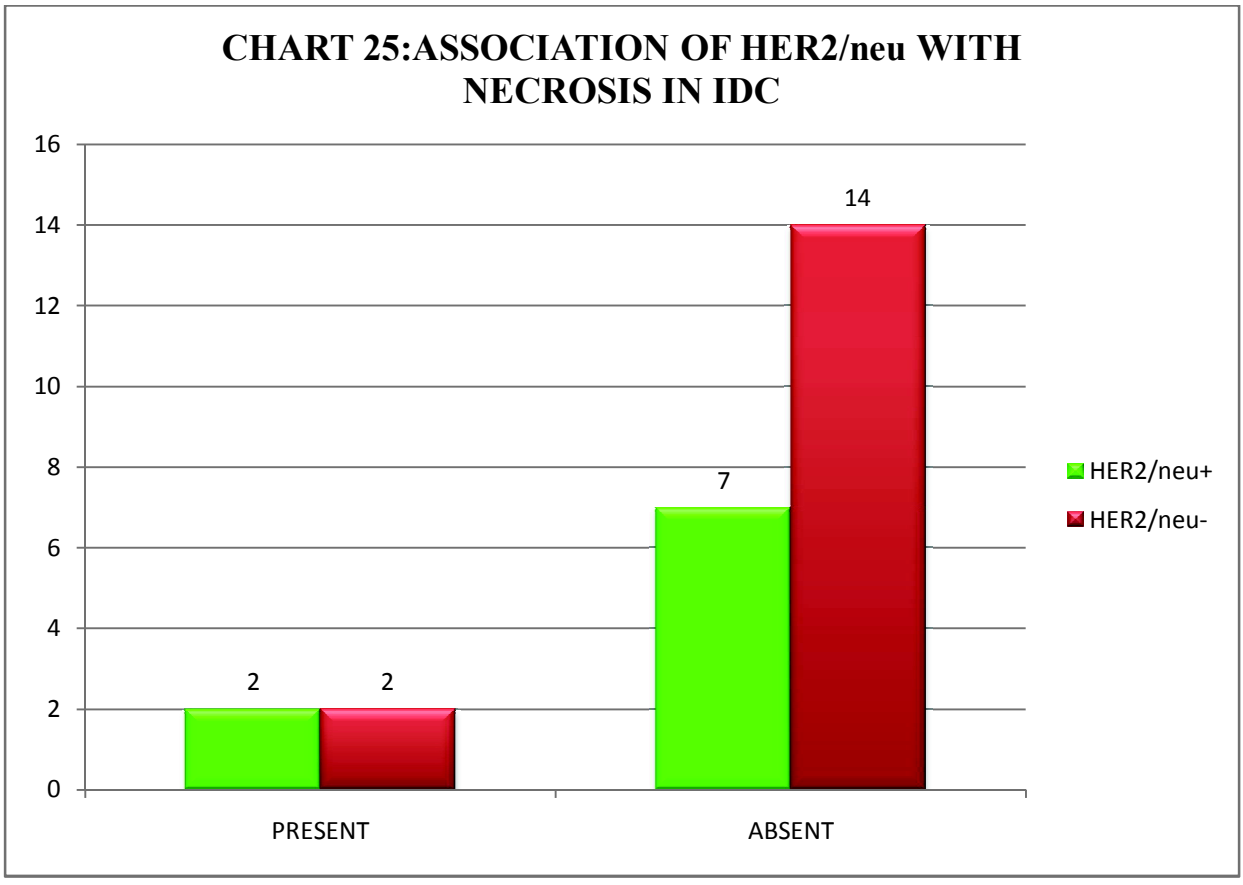


CHART 25:ASSOCIATION OF HER2/neu WITH NECROSIS IN IDC
16
14
12
10
8
6
4
2
0
2
2
7
14
PRESENT
ABSENT
HER2/neu+
HER2/neu-

CHART 26 :ASSOCIATION OF HER2/neu WITH NIPPLE INVASION IN IDC-DCIS

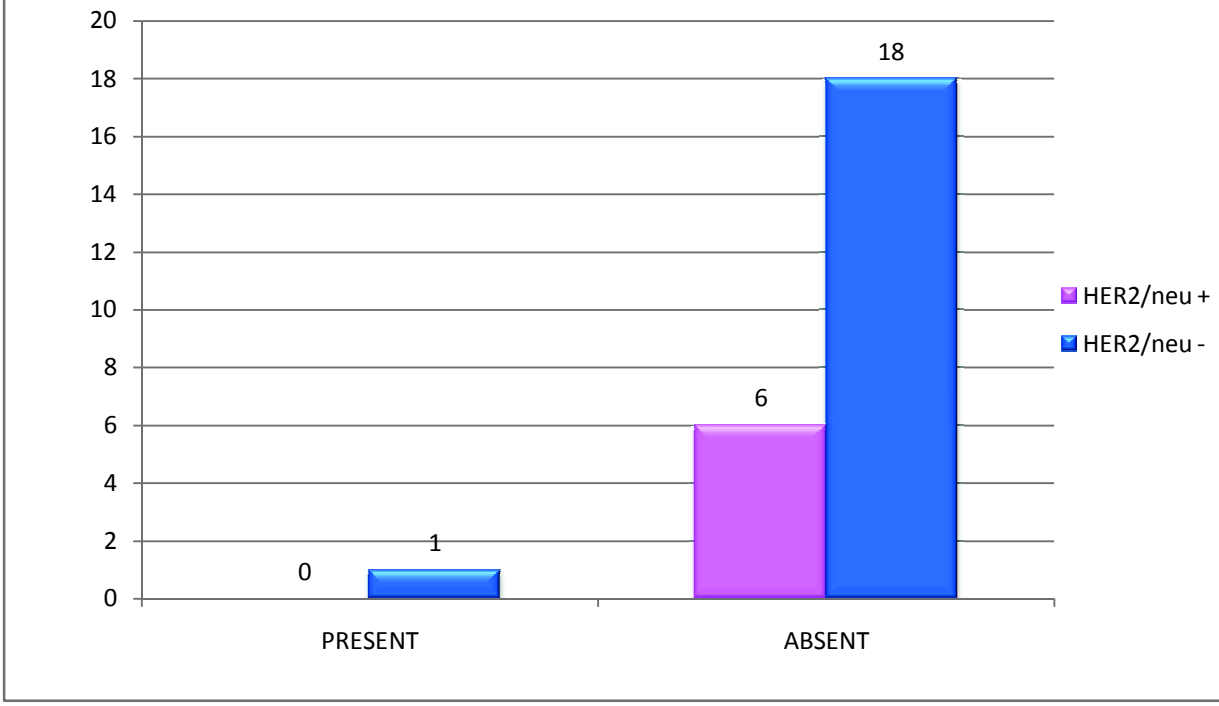


CHART 27:ASSOCIATION OF HER2/neu with NIPPLE INVASION IN IDC

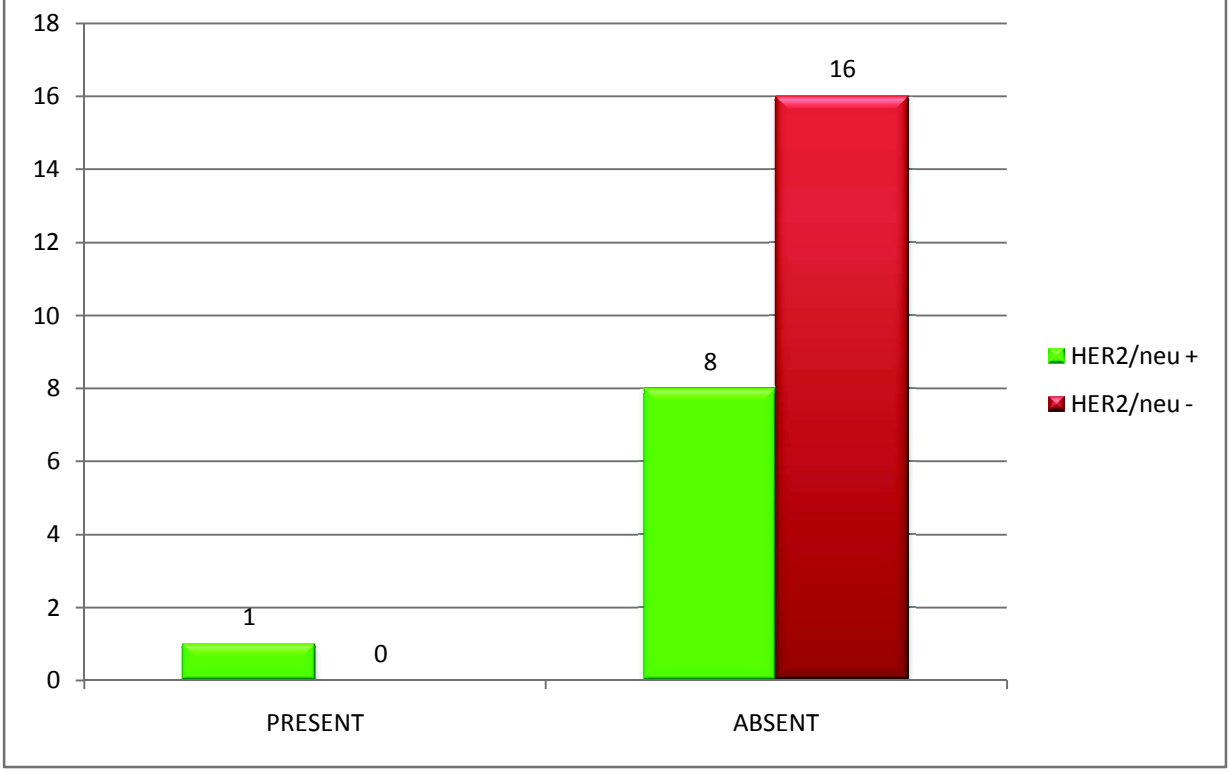


CHART 28 :ASSOCIATION OF HER2/neu WITH LVI IN IDC -DCIS

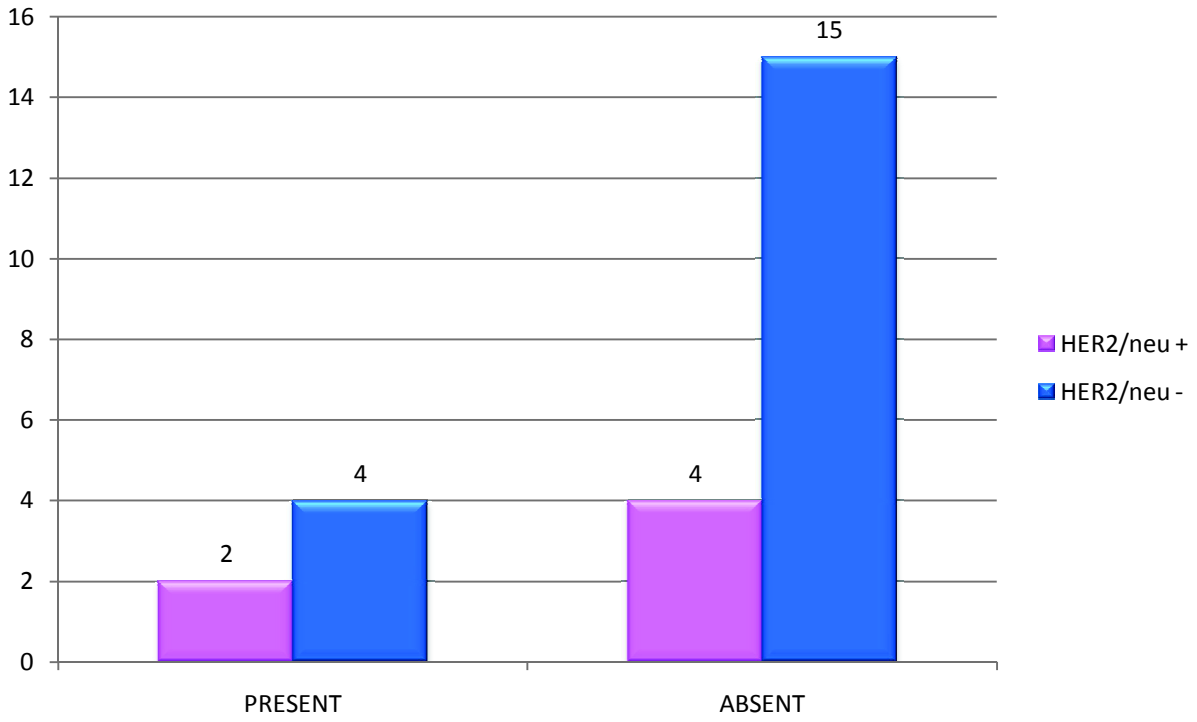


CHART 29:ASSOCIATION OF HER2/neu WITH LVI IN IDC

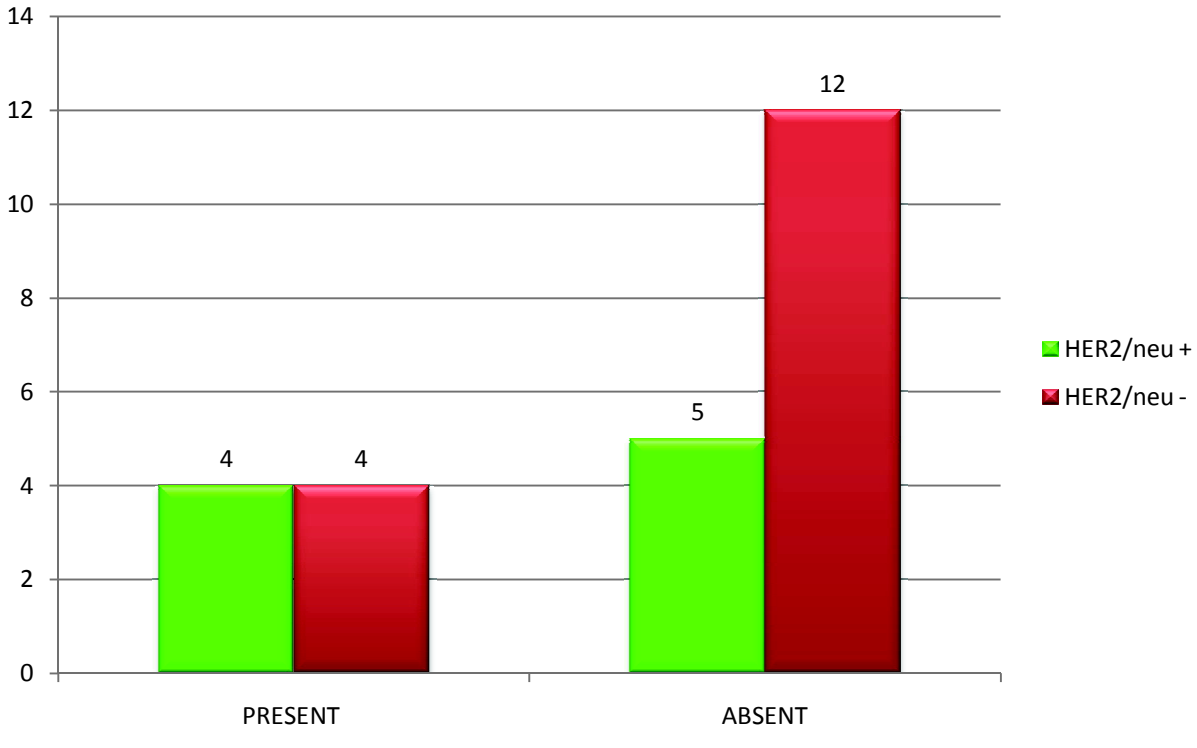


CHART 30 :ASSOCIATION OF HER2/neu WITH LYMPH NODE STATUS IN IDC-DCIS

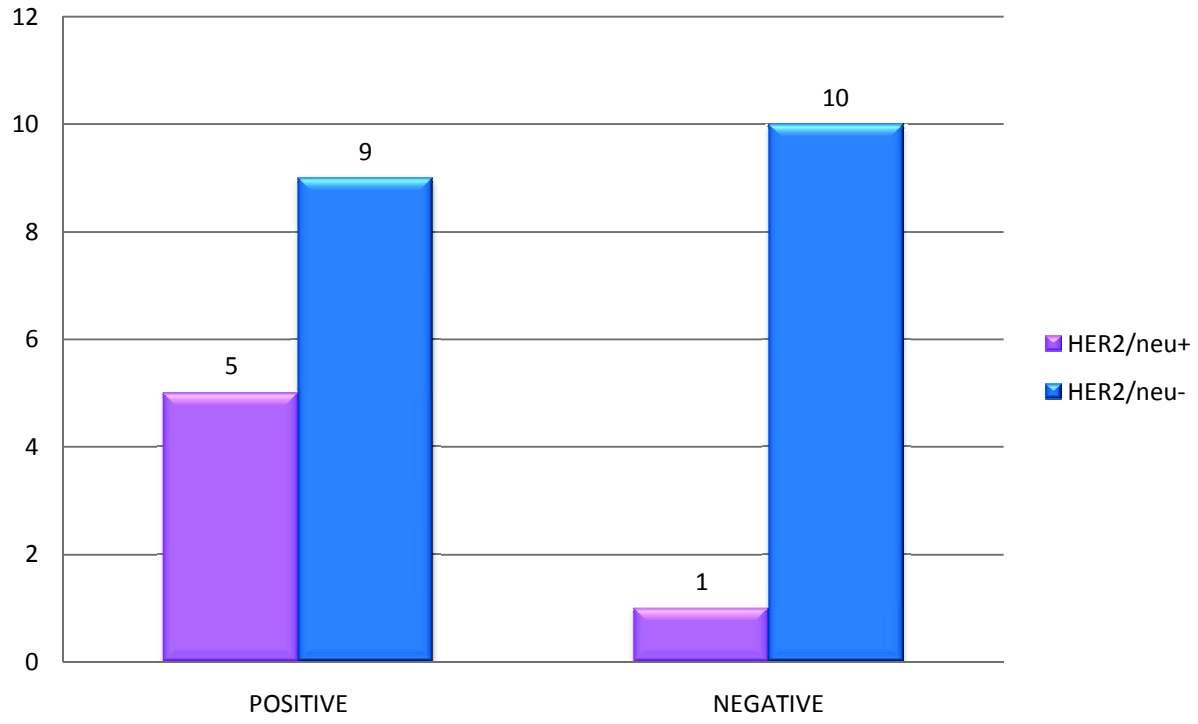


CHART 31 :ASSOCIATION OF HER2/neu WITH LYMPHNODE STATUS IN IDC

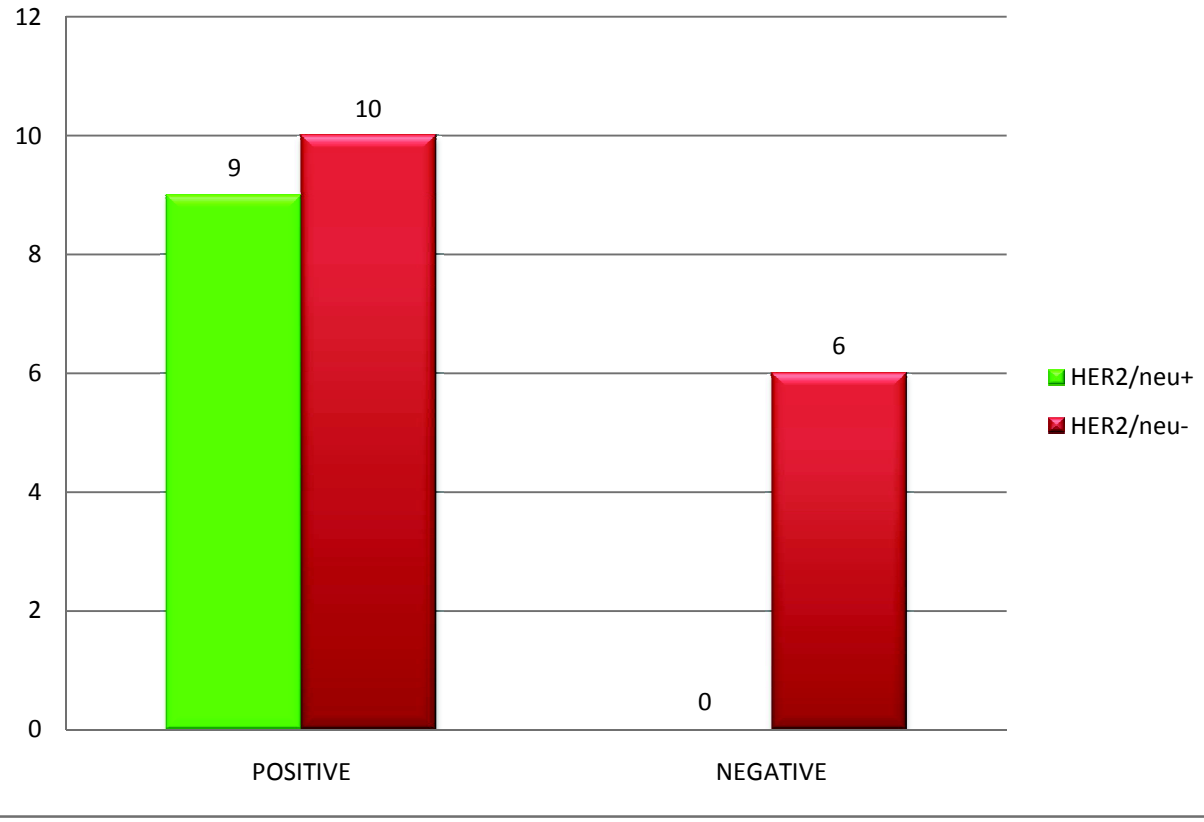


CHART 32:ASSOCIATION OF HER2/neu WITH ER RECEPTOR IN IDC-DCIS

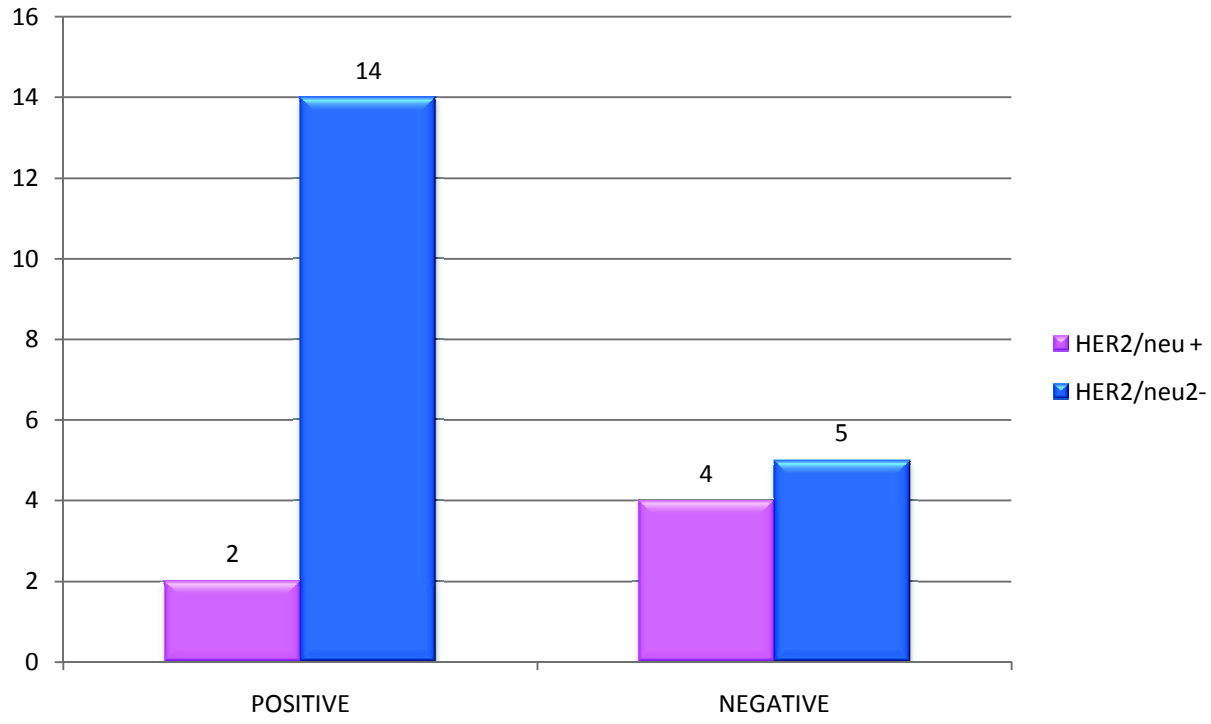


CHART 33:ASSOCIATION OF HER2/neu WITH ER RECEPTOR IN IDC

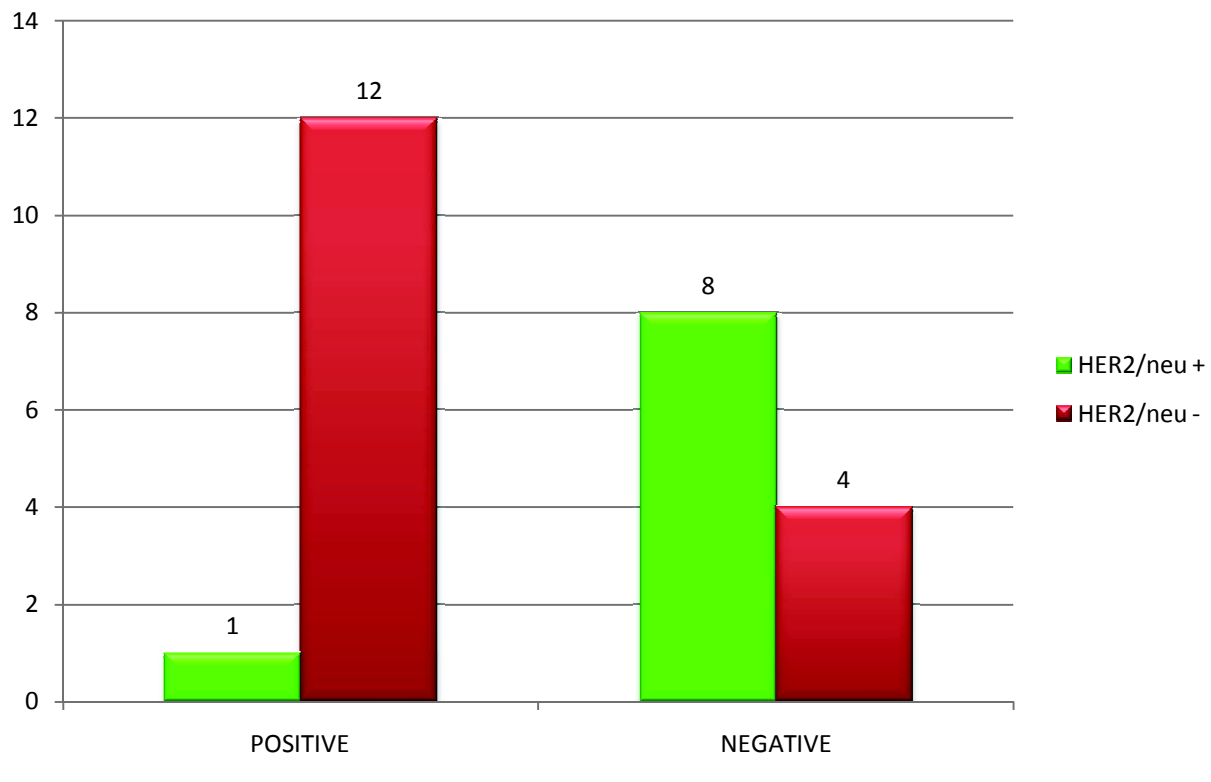


CHART 34 :ASSOCIATION OF HER2/neu WITH PR IN IDC-DCIS

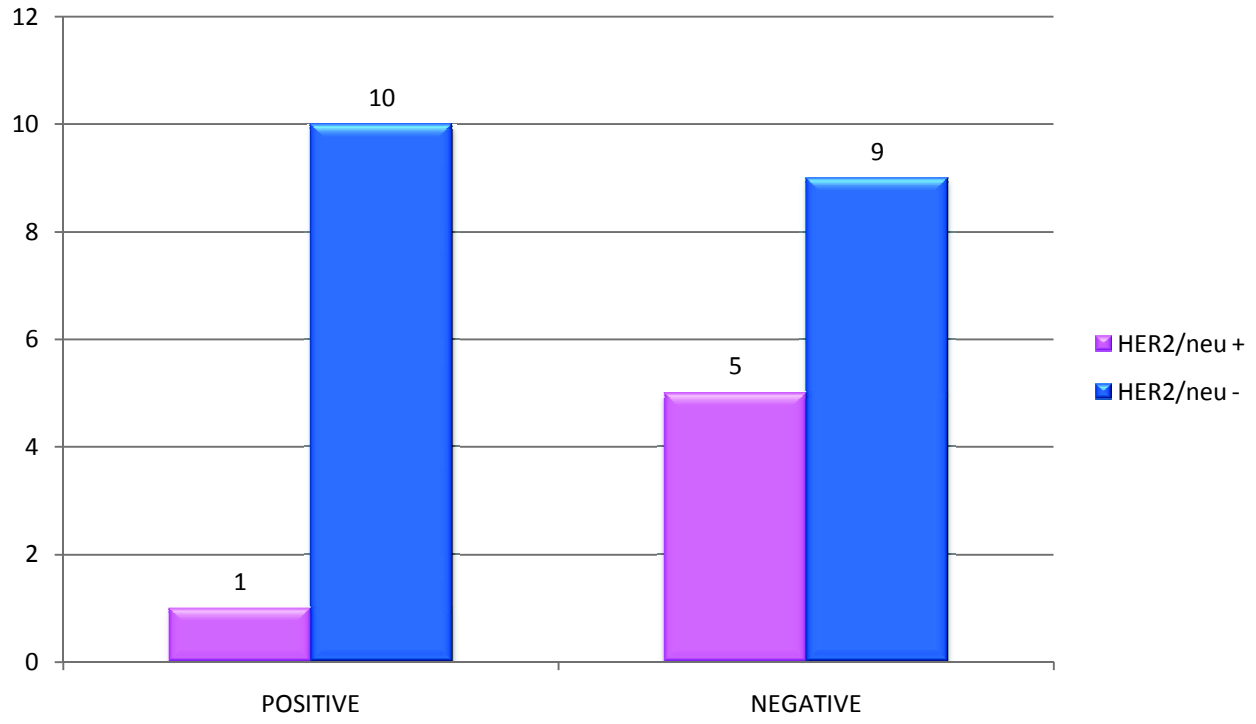


CHART 35:ASSOCIATION OF HER2/neu WITH PR IN IDC

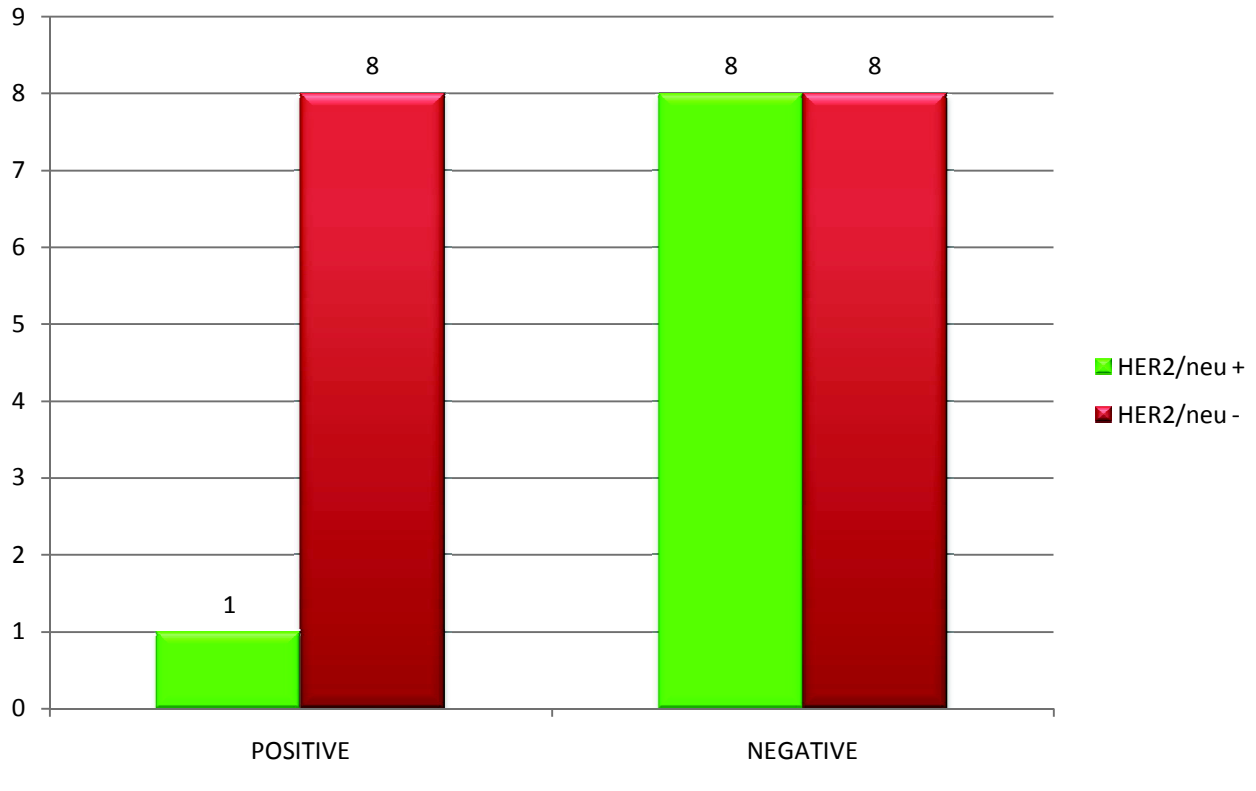


CHART 36 :ASSOCIATION OF HER2/NEU WITH KI 67 IN IDC-DCIS

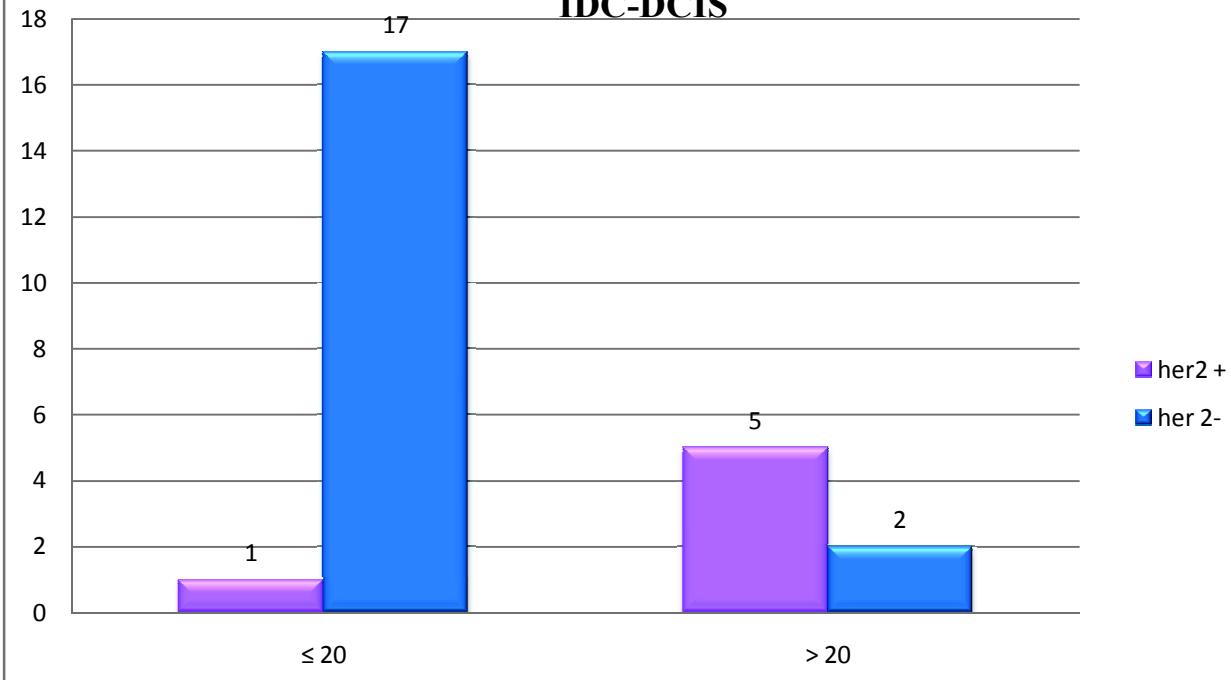


CHART 37 :ASSOCIATION OF HER2/NEU WITH Ki67 IN IDC

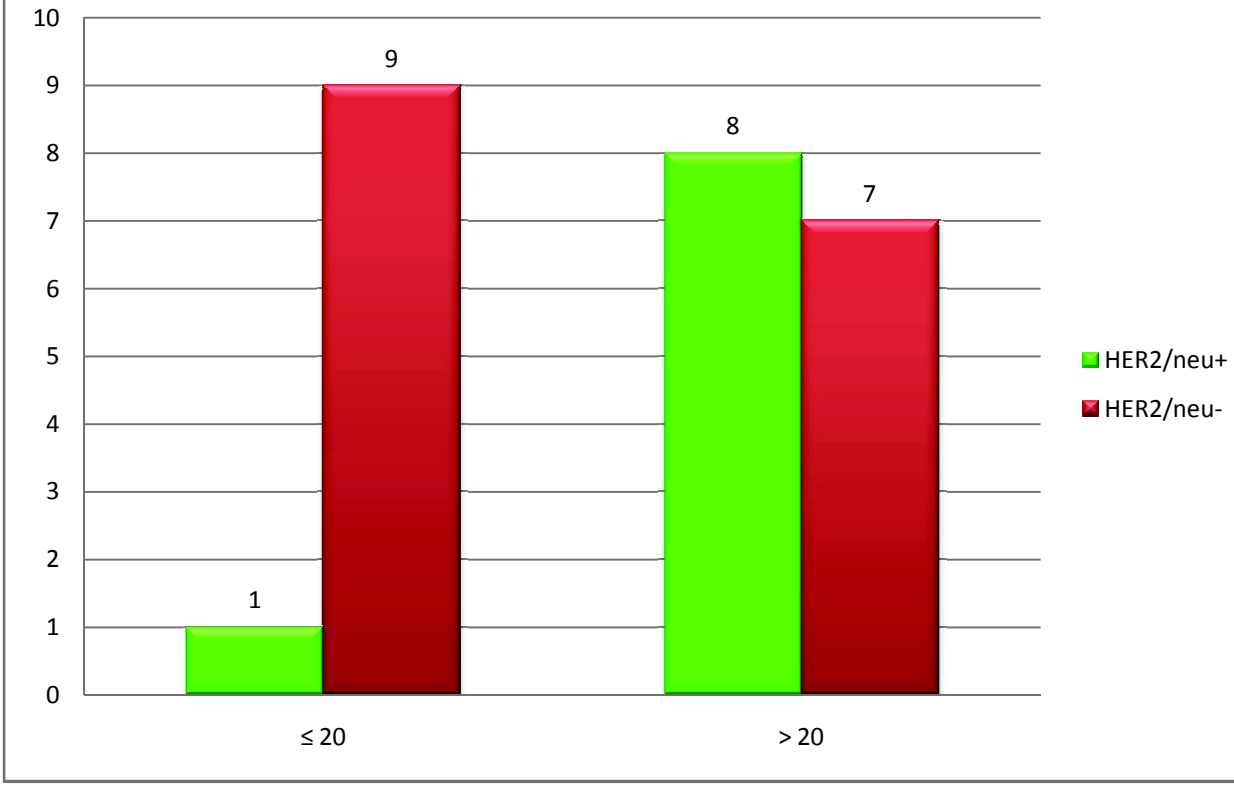


Fig 2:Gross Photograph Of Mastectomy Specimen C/S Shows A Irregular Greyish White Mass

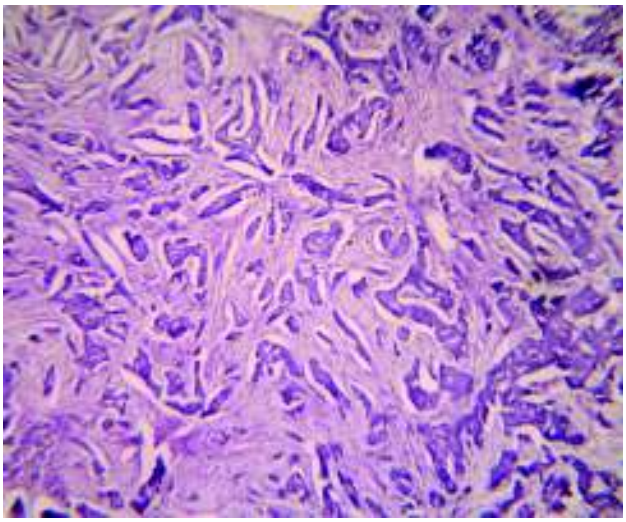


Fig 3:Invasive Ductal Carcinoma,Nos Type (H & E,X 100)

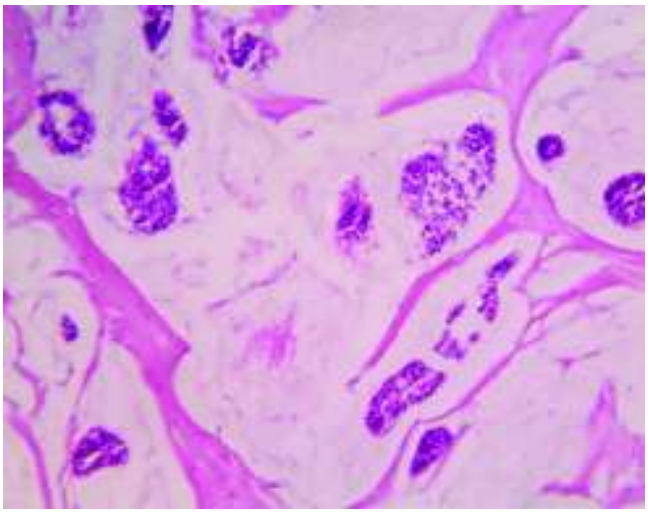


Fig 4: Mucinous Carcinoma Of Breast (H & E,X 100)

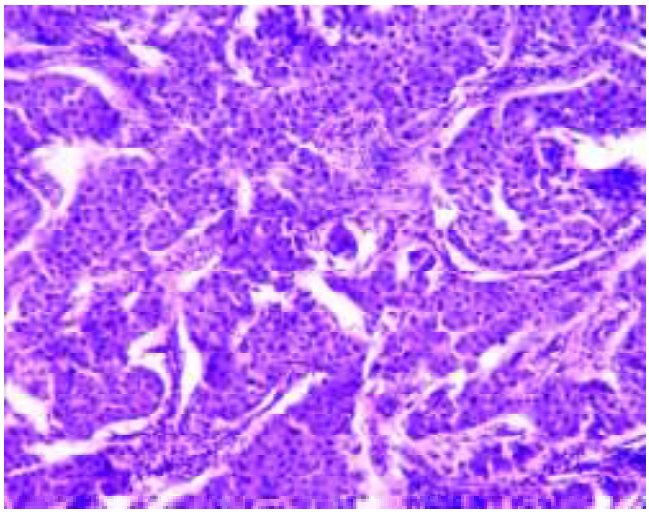


Fig 5 : Atypical Medullary Carcinoma Of Breast (H & E,X 100)

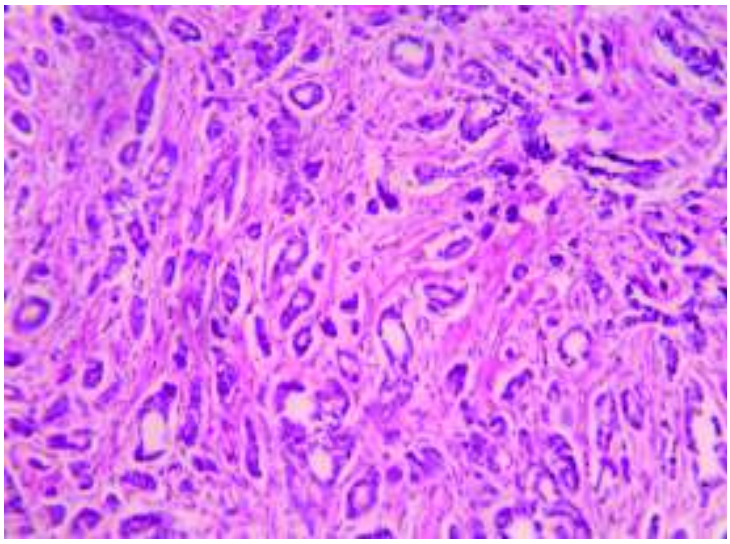


Fig 6:IDC, Nos Type- Grade 1 (H & E,X 100)

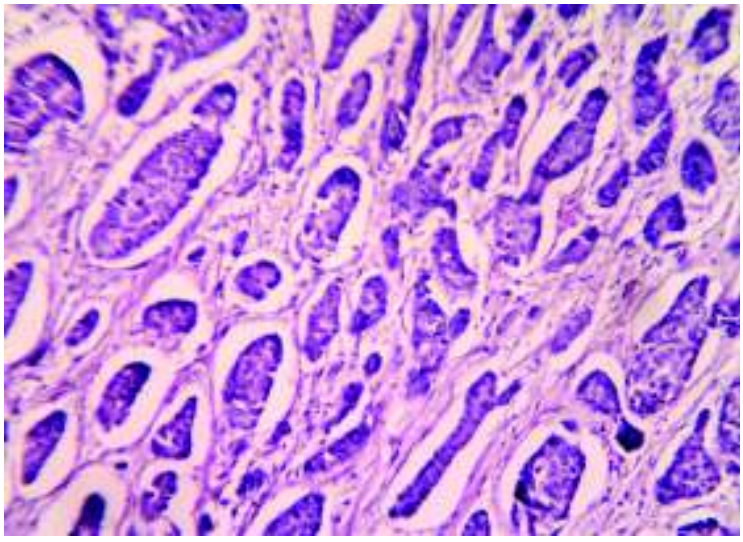


Fig 7: IDC, Nos Type –Grade 2(H & E, X 100)

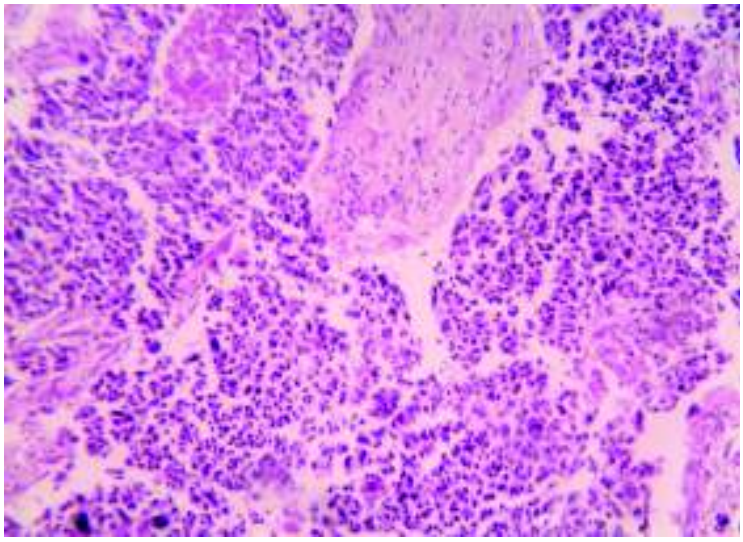


Fig 8 : IDC, Nos –Grade 3(H & E,X 100)

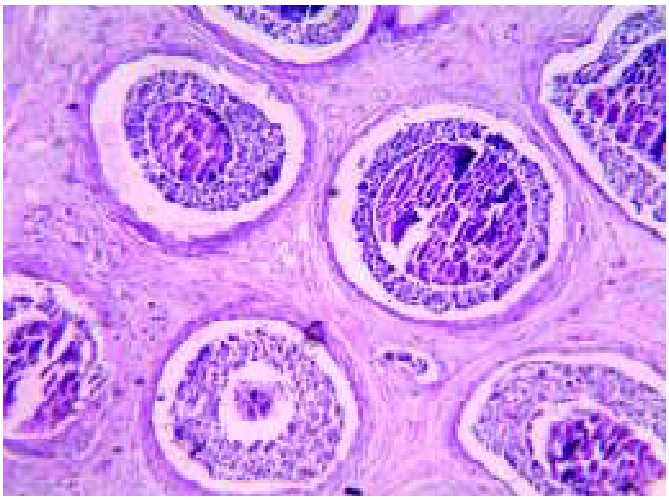


Fig 9 : Comedocarcinoma Type Type DCIS
In IDC-DCIS (H & E,X100)

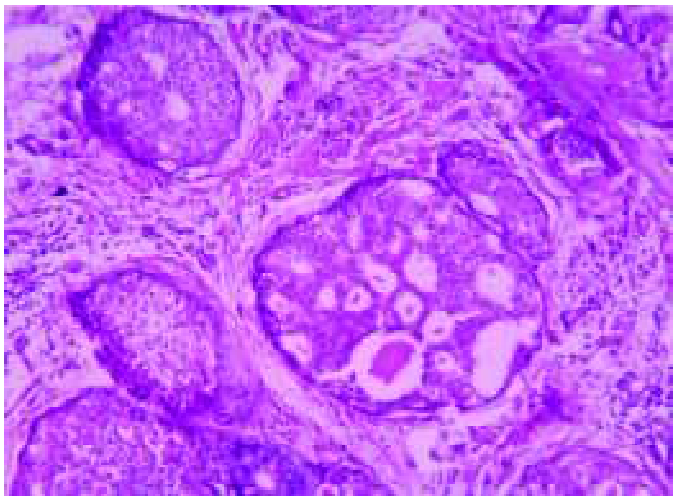


Fig 10: Cribriform Type Type DCIS
In IDC-DCIS (H &E,X100)

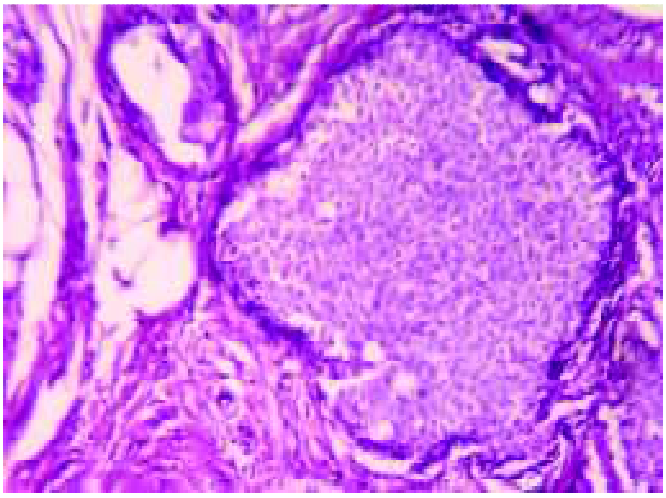


Fig 11 : Solid Type Type DCIS
In IDC-DCIS (H & E,X 100)

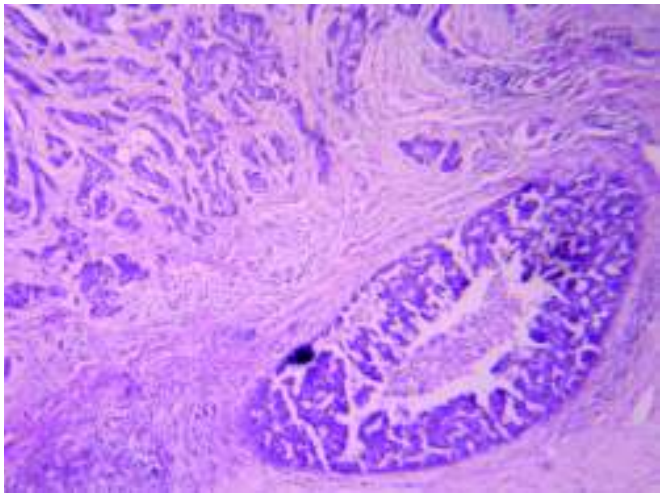


Fig 12: Micropapillary Type DCIS
In IDC-DCIS (H & E,X 100)

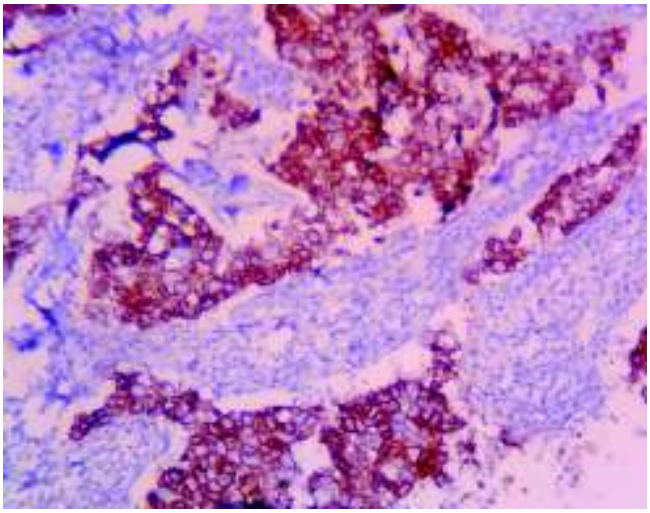


FIG 13: HER2/Neu Positivity With Score 3+ (DAB, X 400)

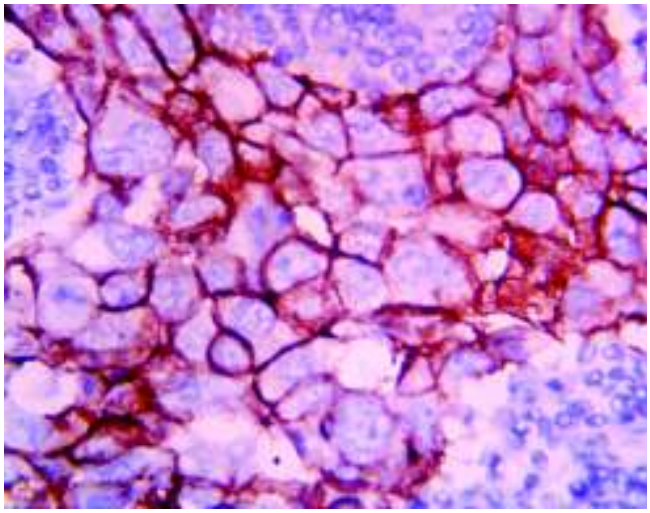


FIG 14: Strong Complete Membranous Staining In HER2/Neu (DAB,X 100)

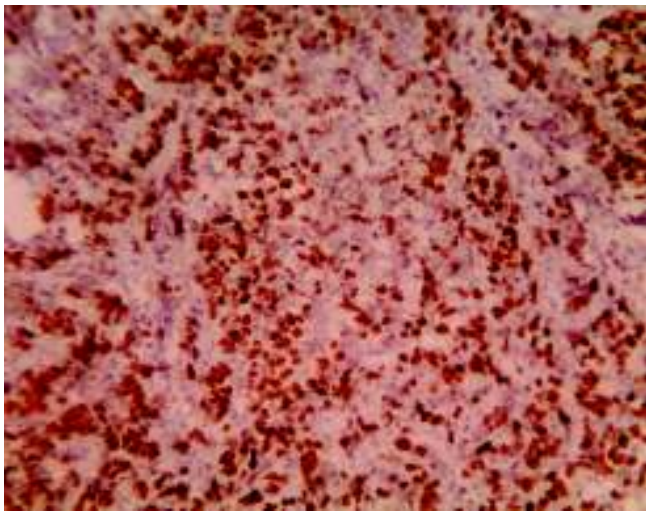


FIG 15:Ki 67 –High Proliferative Index (DAB,X 100)

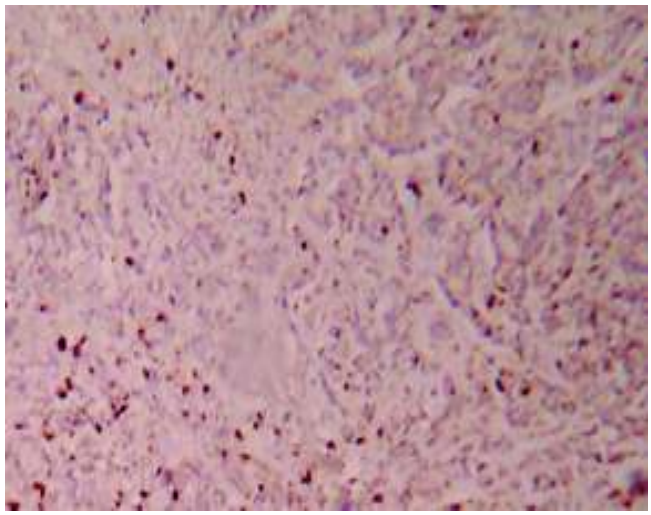


Fig 16 : Ki 67 –Low Proliferative Index (DAB,X 100)

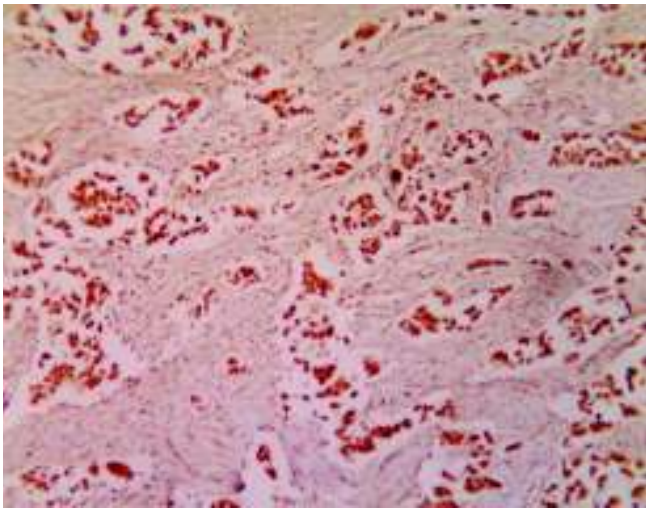


Fig 17:Estrogen Receptor Positivity In IDC, Nos –Score 8 (DAB, X 100)

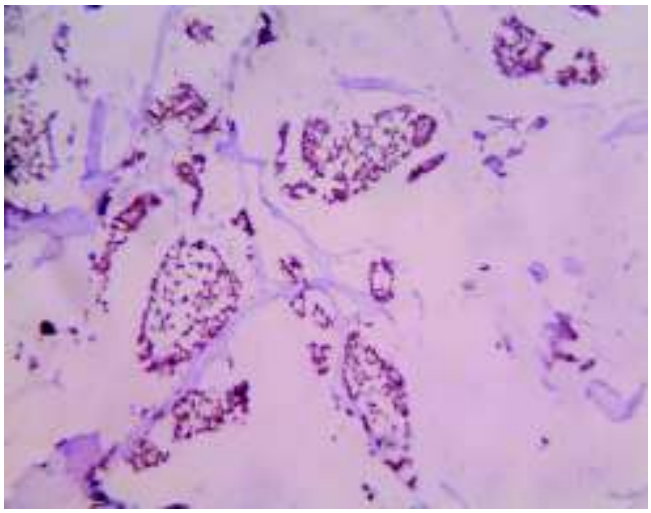


Fig 18: ER Positive In Mucinous Carcinoma Of Breast (DAB,X 100)

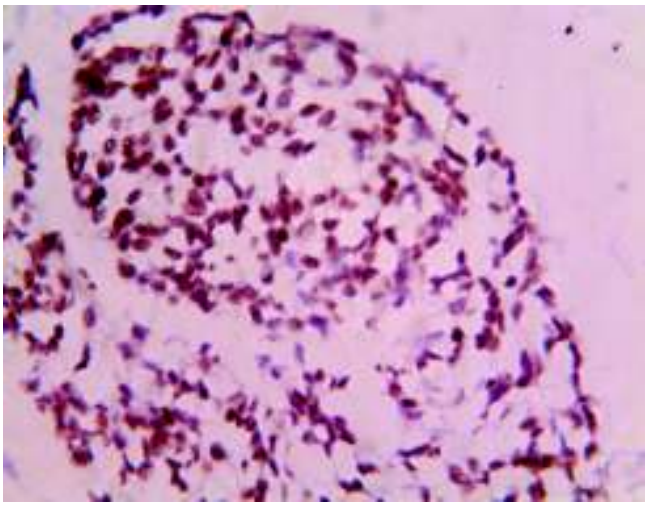


Fig 19:ER Positive-Mucinous Carcinoma Of Breast (DAB, X 400)

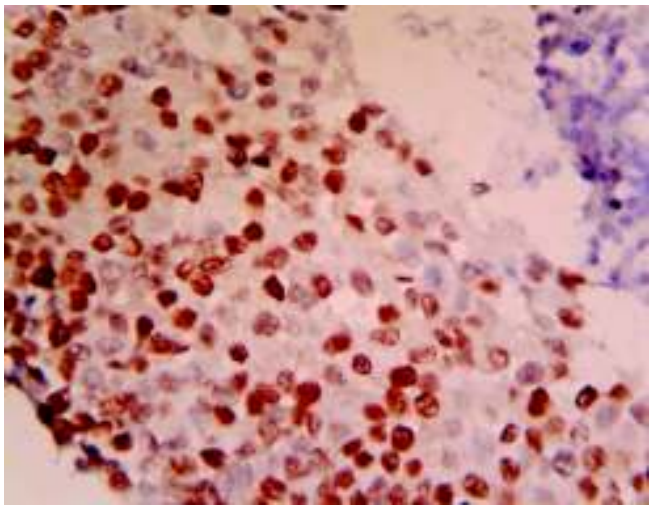


Fig 20 : Progesterone Receptor Showing Nuclear Positivity In IDC, Nos (DAB, X 400)

DISCUSSION

Breast carcinoma is the most common malignant tumor in females. The clinical outcome of breast carcinoma varies in every individual due to its molecular heterogeneity. Recent days there is an rising interest whether the association of DCIS will affect the prognosis and overall survival of the patient. Only a very few studies have been conducted so far to assess the expression of HER2/neu in IDC-DCIS and IDC without DCIS(IDC).

This present study evaluate the expression of HER2/neu between invasive ductal carcinoma associated with and without DCIS and also to assess the correlation of HER2/neu with other prognostic factors between IDC-DCIS and IDC. This study includes totally 50 cases of invasive ductal carcinoma of breast in which 25 cases were of IDC associated with DCIS (IDC-DCIS) and the other 25 cases were of invasive ductal carcinoma without DCIS.(IDC)

Table 45: Number of cases studied in various studies

Study	Year and duration	IDC-DCIS	IDC without DCIS
B H Jo et al[5]	1996-1997	144	84
H Wong et al[6]	2000-2008	616	543
Mylonas et al[7]	1999-2002	36	130
Rana S Aziz et al[101]	2008-2009	12	7
Present study	2012-2014	25	25

Age:

In the present study the median age of the total cases was 54 years. Median age of IDC-DCIS is 54 years and IDC is 53 years. Median age was higher compared to studies of B H Jo et al[5] ,H Wong et al [6].

Menopausal status:

Most cases are in the postmenopausal status. In our study premenopausal status is higher in IDC-DCIS (48%) compared to that of IDC(32%). Studies by B H Jo et al[5], H Wong et al[6] showed a higher proportion of premenopausal women in IDC-DCIS.

Tumor size:

In our study the median tumor size is 4cm. The median size of present study was comparatively higher than the results of H Wong et al[6] and Chagpar et al.[8]

Table 46 :Comparison of tumor size with other study

Study	**IDC-DCIS**	**IDC**
Chagpar et al[8]	1.37 cm	1.44 cm
Present study	3.5 cm	4 cm

Studies by Chagpar et al[8] and Dieterich et al[102] showed that IDC-DCIS had a small tumor size and a significant difference in tumor size between the two groups. In our present study we found a relatively small tumor size in IDC-DCIS compared to that of IDC.

Tumor type:

In our study in IDC-DCIS all the cases are of IDC,NOS which showed 24% positivity. In IDC without DCIS 9 cases showed positivity among 22 cases of IDC, NOS. HER2/neu positivity is not seen in cases of medullary, mucinous and invasive papillary carcinoma.

DCIS type :

In our study in IDC-DCIS 68 % of the cases show comedocarcinoma type DCIS. This is similar to the studies of Collins et al[29] and Sanders et al.[30]

Tumor grade:

In our present study most patients present with histological grade 2 with 68 % in IDC-DCIS and 76 % in IDC. There is no significant difference between the IDC with and without DCIS.A significant increase in tumor grade was observed by Mylonas et al[7] and Chagpar et al.[8]

Tumor necrosis :

In the present study necrosis is seen in 16 % of the cases. Studies done by Rashed et al[103] showed tumor necrosis in 24 % of the cases. In our study there is equal number of cases showing tumor necrosis in IDC with and without DCIS. There is no statistical significance in relation to tumor necrosis between IDC-DCIS and IDC.

Nipple invasion:

In our study only 4% of the cases showed nipple invasion. Studies done by Laronga et al[61] showed 6 % of cases and Wang et al[62] showed 9.5 % of cases with nipple involvement. In studies done by Verma et al[60] nipple involvement is not seen. In both IDC with and without DCIS only 4% of the cases showed nipple invasion.

Lymphovascular invasion:

In our study 28 % of the cases showed lymphovascular invasion. Studies done by Ejlertsen et al[104] showed lymphovascular invasion in only 15% of the cases and Brachtel et al[105] showed 41% of the cases with lymphovascular invasion.

In IDC-DCIS 24% of the cases showed lymphovascular invasion and 32% of the cases showed lymphovascular invasion in IDC without DCIS. This was similar to the results of Wong et al[6] who showed lymphovascular invasion increases as the size of the invasive component increase.

Table 47: Comparison of Lymphovascular invasion with other study

Study	IDC-DCIS	IDC
Wong et al[7]	Small IDC-Large DCIS- 27% Large IDC-small DCIS -57.7%	39.2%
Present study	24%	32%

Lymph node status:

In the present study 72 % of the IDC cases showed lymph node positivity compared to that of 56 % in IDC-DCIS. In pure IDC i.e.without DCIS there is a higher number of lymph node involvement compared to that of IDC-DCIS .This results were similar to that of H Wong et al[6] where there is higher number of node involvement in pure IDC than that of IDC-DCIS.

Estrogen receptor:

In the present study 58% of the cases cases showed estrogen receptor positivity. Studies done by Vasudha et al[106] and Azizun Nisa et al[107] showed 48.2% and 32.7% of cases with ER positivity. A higher rate of positivity (73%) is seen in the study done by Ivkovic-Kapickl et al.[108]

In IDC-DCIS there is a higher rate of ER positivity with 64 % compared to 52 % in IDC without DCIS. This was similar to the results of Wong et al[6] and Mylonas et al[7].There is no statistical significance in the expression of ER between IDC with and without DCIS.

Progesterone receptor

In the present study 40 % of the cases showed PR positivity.Studies done by Vasudha et al[106] and Wang et al[62] showed 37.9% and 66 % of the cases with PR positivity. In IDC-DCIS 44 % of the cases showed PR positivity and 36 % of the cases showed positivity

in IDC without DCIS. There is no significant difference in relation to progesterone status between IDC with and without DCIS

Table 48:Comparison of ER,PR in IDC-DCIS and IDC in other studies.

STUDY	ER +(%)		PR+(%)	
	IDC-DCIS	IDC	IDC-DCIS	IDC
Mylonas et al[7]	48.4	54.1	46.7	71
Wong et al[6]	81.5	74.0	74.7	70.5
Present study	64	52	44	36

HER2/neu

In the present study IDC-DCIS showed amplification in 24 % of cases.IDC alone showed amplification in 36 % of the cases. This result was concurrence to the results of Mylonas et al[7] , which showed 31 % positivity in IDC-DCIS and 49.6 % in IDC. He suggested that since the expression of HER2/neu is lower in IDC-DCIS compared to that of IDC, IDC-DCIS may be a precursor for the development of a more aggressive and malignant IDC.[7]

In our study there is no statistical difference in the expression of HER2/ neu between the IDC-DCIS and IDC.This was similar to the study of Rana S Aziz et al.[101] They concluded that the presence or absence of in situ component had no effect in the expression of HER2 /neu.

But in contrary H Wong et al[6] showed higher HER2/neu amplification of 25.5% in IDC-DCIS compared to 16.2 % in pure IDC

Table 49:Comparison of HER2/neu expression in IDC-DCIS and IDC in various studies.

Study	IDC-DCIS	IDC	P value	significance
Present study	24%	36%	0.354	Not significant
Mylonas et al[7]	31%	49.6%	P<0.05	significant
H Wong et al[6]	25.5%	16.2%	P<0.0005	significant
R S Aziz et al[101]	-	-	0.243	Not significant

Ki 67:

In the present study IDC showed a higher Ki 67 index of 60 % compared to that of 28 % in IDC-DCIS.Our study showed a significant difference in Ki 67 expression between IDC-DCIS and IDC with a p value of 0.02. This was similar to the studies of H Wong et al[6], Mylonas et al[7] which showed a significant difference between the two groups.

Table 50 : Comparison of proliferative rate with other study

Study	IDC-DCIS	IDC (without DCIS)
Mylonas et al[7]	49.7 %	64%
Present study	28 %	60%

CORRELATION OF HER2 NEU WITH OTHER PROGNOSTIC FACTORS

Many studies have correlated the expression of HER2/neu with other prognostic factors in breast carcinoma .In our study we correlated the association of HER2/ neu with other prognostic factors in the 50 cases of invasive ductal carcinoma. In addition to it we have also attempted to compare the correlation of HER2/neu with other prognostic factors between IDC-DCIS and IDC without DCIS.

In our study HER2/neu amplification was found in 30 % of the cases.

Table 51 : Comparison of HER2/neu amplification in various studies.

Study	HER2/neu amplification
Ivkovic-Kapickl et al[108]	20%
Ahmed et al et al[109]	37%
Azizun –Nisa et al[107]	37.4%
Present study	30%

Age:

In our study HER2/neu positivity is peak in the age group of 41-50 years.HER2/neu amplification starts declining with increasing age from 60% in 41-50 years to 26% in 51-60 years. Studies by Azizun Nisa[107] showed HER2/neu amplification decreases with age. But however statistically there is no association of HER2/neu with age .Studies by

Khorshid et al[110],Al Moundhri et al[111] also showed that age is not associated with HER2/neu amplification.

In our study HER2/neu positivity was more common in the age group of 41-50 years and positivity decreased with increasing age in both IDC-DCIS and IDC.

Menopausal status:

In the present study HER2/neu overexpression was higher in postmenopausal patients. But there was no significant association between HER2/neu overexpression and menopausal status. This results were similar to that of Rana S.Aziz et al[101], Khorshid et al[110], Al Moundhri et al.[111]There is no difference in the association on HER2/neu with menopausal status between IDC-DCIS and IDC without DCIS.

Tumor size

In our study HER2/neu positivity increases from 16 % in tumors <2 cm size to 20% among tumors with 2-5cm and 53% among tumors with size >5cm.This was similar to the study of Ivkovic-Kapickl et al.[108]

It shows HER2/neu positivity increases with increase in size of the tumor. Similar to our study Bhatavdekar et al[112] also found higher rate of HER2/neuexpression in large sized tumors.

Table 52:comparison of HER2/neu positivity with tumor size

Tumor size	Kapickl et al[108]	Present study
<2 cm	8%	16%
2-5 cm	30%	20%
>5cm	50%	53%

But there is no statistical significance between HER2/neu amplification and tumor size. Studies done by Arigar[113], Prati R[114], Huang HJ et al[115] also showed that tumor size is not associated with HER2/neu overexpression. In contrast studies by Rana S Aziz et al[101], kapicl et al[108], Vijver et al[116] showed HER2/neu overexpression is significantly associated with large tumor size.

In IDC-DCIS there is no association of HER2/neu positivity with tumor size. In pure IDC (without DCIS) 66.6% of the cases showed positivity in tumor size > 5cm .IDC without DCIS showed a statistical significant association between HER2/neu overexpression and tumor size.

Tumor grade:

In our study among grade 1 tumors 10% of the tumors showed HER2/neu positivity, grade 2 tumors show 31% of positivity and 60% of the grade 3 tumors showed HER2/neu overexpression. But in the present study there is no significant correlation between the grade of the tumor and HER2/neu amplification. Al Moundhri et al[104] also found similar results. But the results were in disagreement with the studies of Rana S

Aziz et al[101] ,Kapickl et al[108], and Hoff et al[117] .They showed a positive correlation between the grade of the tumor and HER2/neu overexpression.

There is no significant association in the expression of HER2/neu and tumor grade in both IDC-DCIS and IDC.

Necrosis

In our study 25 % of the cases with necrosis showed HER2/neu positivity. Among tumors without necrosis 30.9 % of the cases showed HER2/neu positivity. There is no significant association between tumor necrosis and HER2/neu overexpression. Rashed et al[103] also concluded that necrosis and HER2/neu overexpression is not statistically significant.There is no significant association of HER2neu with tumor necrosis between IDC-DCIS and IDC.

Nipple invasion

There is no significant relation between nipple invasion and HER2/neu positivity. This was contrary to the results of Brachtel et al[105] who showed 16% amplification in cases with nipple involvement and Wang et al[62] who showed 18 % amplification in cases with nipple involvement.

Lymphovascular invasion

In our study 42.8 % of the cases showed HER2/neu positivity among tumors with lymphovascular invasion. There is no significant

association between lymphovascular invasion and HER2/neu positivity.This is similar to the results of Al-Ahwal[118].In his study 29.3 % cases showed HER2/neu amplification in cases with lymphovascular invasion which is not statistically significant.

Among cases with lymphovascular invasion there is a higher rate of positivity (60%) in IDC without DCIS compared to 33.3 % of in IDC-DCIS.

Lymph node status:

Among the total 32 node positive cases 13 cases are HER2/neu positive with 40% positivity. Among the node negative cases only 11.1 % shows HER2/neu positivity. Among the 15 cases with HER2/neu amplification, 13 cases i.e, 86.6% of the cases show lymph node metastasis. This results are similar to the results of Slamon et al[91] and Tiwari et al[119].

Table 53 : Association of HER2/neu with lymph node status in other studies.

Study	No of HER2/neu+ cases	Cases with lymph node metastasis	% of positivity
Tiwari et al[119]	16	14	88%
Present study	15	13	86.6%

There is a significant association between the nodal and involvement and HER2/neu amplification. The results are similar to that of Tiwari et al[119] and Berger et al[120] .In contrary Ivkovic-kapicl et al[108] Azizun-Nisa et al[107] and Al moundhri[111]did not find a significant correlation between lymph node status and HER2/neu expression.

There is higher rate of HER2/neu overexpression in pure IDC (47.3%) compared to 35.7% in IDC -DCIS in cases showing lymph node metastasis. IDC without DCIS showed a significant association between lymph node metastasis and HER2/neu overexpression.

Estrogen receptor status

In our study HER2/neu positivity is higher (63.1%) in tumors which are negative for ER. There is inverse relationship between ER and HER2/neu overexpression which is statistically significant. This results were similar to that of Vasudha et al.[106] and Ivkovic-kapickl[108] .In both IDC-DCIS and IDC without DCIS HER2/neu positivity is higher in tumors with ER negativity but a statistically significant association is observed in IDC without DCIS.

Progesterone receptor

In our study among PR negative tumors HER2/neu positivity is higher with 35.7%.There is a significant inverse association between PR and HER2/neu overexpression which is similar to the studies of Vasudha et al.[106] and Ivkovic-kapickl et al[108] .In both IDC with and without DCIS

HER2/neu positivity is higher in tumors with PR negativity but there is no statistically significant relation.

Ki 67 :

In our present study significant correlation was found between HER2/neu and ki67.Among the 21 cases of high proliferative index,13 cases i.e, 59% showed positivity for HER2/ neu overexpression. Among 28 cases of low proliferative rate only 2 cases i.e, 7 % showed HER2/neu positivity. The results were similar to that of Kilickap et al [81] and Ivkovic-kapickl et al.[108]The percentage of positivity is higher in our study compared to the study of Ivkovic- kapickl et al.[108]

Table 54 :Association of HER2/neu with Ki67 in other studies.

Study	% of HER2 + in Low proliferative cases	% of HER2 + in high proliferative cases
Kapickl etal[108]	0	25%
Present study	7%	59%

HER2/neu is significantly associated with Ki 67 in both IDC associated with and without DCIS

SUMMARY

- This study includes a total 50 cases of invasive ductal carcinoma with 25 cases of IDC-DCIS and 25 cases of IDC without DCIS.
- Most common age group in IDC-DCIS is 41-60 years and 41-50 years in IDC.
- 60 % of the cases are in postmenopausal status .Patients with premenopausal status are higher in IDC-DCIS compared to IDC.
- Most common tumor size is between 2-5 cm with 58% of the cases.
- IDC-DCIS showed a smaller tumor size (< 2cm) in 20% of cases compared to 8 % in IDC.
- Higher rate of lymph node metastasis was seen in 72% of the cases in IDC compared to 56 % in IDC-DCIS. Higher number of node involvement (>9 nodes) is seen in 16 % of cases in IDC and 8% of cases in IDC-DCIS.
- Higher proliferative rate was in 60% of cases in IDC than 28% in IDC-DCIS. There is a statically significance in the proliferation rate between IDC-DCIS and IDC.
- HER2/neu amplification is seen in 36 % of IDC and 24% in IDC-DCIS. No statistical significance was seen in the expression of HER2/neu between IDC-DCIS and IDC.
- Only expression of Ki 67 is significant between the two groups.

- HER2/neu is significantly correlated with lymph node status and Ki 67 expression in the total 50 cases .Significant inverse association was seen between HER2/neu and ER, PR status.
- There is no significant correlation of HER2/neu with other prognostic factors like age, menopausal status, tumor size and histological grade, tumor necrosis , nipple invasion and lymphovascular invasion.
- In IDC-DCIS significant association was seen between HER2/neu and Ki 67.No association was seen between HER2/neu and other prognostic features.
- In IDC without DCIS significant association was found between tumor size, lymph node status, estrogen receptor (inverse association) and Ki 67 expression. No significant association was seen between HER2/neu and other prognostic factors.

CONCLUSION

In our study it has been found that HER2/neu expression is not significantly different between IDC with (IDC-DCIS) and without DCIS(pure IDC). However the expression of HER2/neu was found to be high in the IDC group and significantly associated with the larger tumor size and positive lymph node status. The proliferative index (Ki 67) which is considered as a marker of chemotherapy response was significantly high in the pure IDC group. This could implicate the less malignant behavior of the IDC- DCIS compared to pure IDC.

Since molecular markers play an important role in carcinogenesis and cancer progression, further studies at a large scale to be done to substantiate the finding of this study which might help in identifying the subgroup for targeted therapy.

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ANNEXURE 1

WHO HISTOLOGICAL CLASSIFICATION OF TUMORS OF BREAST

Epithelial tumors

Invasive ductal carcinoma,not otherwise specified

- Mixed type carcinoma
- Pleomorphic carcinoma
- Carcinoma with osteoclast like giant cells
- Carcinoma with choriocarcinomatous features
- Carcinoma with melanotic features

Invasive lobular carcinoma

Tubular carcinoma

Invasive cribriform carcinoma

Medullary carcinoma

Mucinous carcinoma and other tumors with abundant mucin

- Mucinous carcinoma
- Cystadenocarcinoma and columnar cell mucinous carcinoma
- 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

Apocrine carcinoma

Metaplastic carcinoma

- Pure epithelial metaplastic carcinoma
    - Squamous cell carcinoma
    - Adeno carcinoma 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 clear cell carcinoma

Sebaceous carcinoma

Inflammatory carcinoma

Lobular neoplasia

- Lobular carcinoma in situ

Intraductal proliferative lesions

- Usual ductal hyperplasia
- Flat epithelial atypia
- Atypical ductal hyperplasia
- Ductal carcinoma on situ

Microinvasive carcinoma

Intraductal papillary neoplasm

- Central papilloma
- Peripheral papilloma
- Atypical papilloma
- Intraductal papillary carcinoma
- Intracystic papillary carcinoma

Benign epithelial proliferations

Adenosis including variants

- Sclerosing Adenosis
- Apocrine Adenosis
- Blunt duct Adenosis
- Microglandular Adenosis
- Adenomyoepithelial Adenosis

Radial scar/complex sclerosing lesion

Adenomas

- Tubular Adenoma
- Lactating Adenoma
- Apocrine Adenoma
- Pleomorphic Adenoma
- Ductal Adenoma

Myoepithelial lesions

Myoepitheliosis

Adenomyoepithelial adenosis

Adenomyoepithelioma

Malignant myoepithelioma

Mesenchymal tumors

Hemangioma

Angiomatosis

Hemangiopericytoma

Pseudoangiomatous stromal hyperplasia

Myofibroblastoma

Fibromatosis

Inflammatory myofibroblastic tumor

Lipoma

Angiolipoma

Granular cell tumor

Neurofibroma

Schwannoma

Angiosarcoma

Liposarcoma

Rhabdomyosarcoma

Osteosarcoma

Leiomyoma

Leiomyosarcoma

Fibroepithelial tumors

Fibroadenoma

Phyllodes tumor

- Benign
- Borderline
- Malignant

Periductal stromal sarcoma,low grade

Mammary hamartoma

Tumors of the nipple

Nipple adenoma

Syringomatous adenoma

Pagets disease of nipple

Malignant lymphoma

Diffuse large B cell lymphoma

Burkitt’s lymphoma

Extranodal marginal zone B Cell lymphoma

Follicular lymphoma

Metastatic tumors

Tumors of the male breast

Gynaecomastia , Carcinoma :invasive, insitu

ANNEXURE 2

TNM CLASSIFICATION OF BREAST CARCINOMA

Primary tumor (T):

TX	:	Primary tumor cannot be assessed
T0	:	No evidence of primary tumor
Tis	:	Carcinoma in situ; intraductal carcinoma, lobular carcinoma in situ, or Paget's disease of the nipple with no associated tumor.
T1	:	Tumor 2.0 cm or less in greatest dimension T1mic- Microinvasion 0.1 cm or less in greatest dimension T1a- Tumor more than 0.1 but not more than 0.5 cm in greatest dimension T1b-Tumor more than 0.5 cm but not more than 1.0 cm in greatest dimension T1c-Tumor more than 1.0 cm but not more than 2.0 cm in greatest dimension
T2	:	Tumor more than 2.0 cm but not more than 5.0 cm in greatest dimension
T3	:	Tumor more than 5.0 cm in greatest dimension
T4	:	Tumor of any size with direct extension to

T4a: Extension to chest wall

T4b: Edema (including peau d'orange) or ulceration of the skin of the breast or satellite skin nodules confined to the same breast

T4c: Both of the above (T4a and T4b)

T4d: Inflammatory carcinoma

Regional lymph nodes (N):

NX	:	Regional lymph nodes cannot be assessed (e.g., previously removed)
N0	:	No regional lymph node metastasis
N1	:	Metastasis to movable ipsilateral axillary lymph node(s)
N2	:	Metastasis to ipsilateral axillary lymph node(s) fixed to each other or to other structures
N3	:	Metastasis to ipsilateral internal mammary lymph node(s)

Pathologic classification (pN):

pnx	:	Regional lymph nodes cannot be assessed (not removed for pathologic study or previously removed)
pN0	:	No regional lymph node metastasis
pN1	:	Metastasis to movable ipsilateral axillary lymph node(s)

pN1a	:	Only micrometastasis (none larger than 0.2 cm)
pN1b	:	Metastasis to lymph node(s), any larger than 0.2 cm
pN1bi	:	Metastasis in 1 to 3 lymph nodes, any more than 0.2 cm and all less than 2.0 cm in greatest dimension
pN1bii	:	Metastasis to 4 or more lymph nodes, any more than 0.2 cm and all less than 2.0 cm in greatest dimension
pN1biii	:	Extension of tumor beyond the capsule of a lymph node metastasis less than 2.0 cm in greatest dimension
pN1biv	:	Metastasis to a lymph node 2.0 cm or more in greatest dimension
pN2	:	Metastasis to ipsilateral axillary lymph node(s) fixed to each other or to other structures
pN3	:	Metastasis to ipsilateral internal mammary lymph node(s)

Distant metastasis (M):

MX	:	Presence of distant metastasis cannot be assessed
M0	:	No distant metastasis
M1	:	Distant metastasis present (includes metastasis to ipsilateral supraclavicular lymph nodes)

TNM STAGING

Stage	T	N	M
0	Tis	N0	M0
IA	T1	N0	M0
IB	T0	N1mi	M0
	T1	N1mi	M0
IIA	T0	N1	M0
	T1	N1	M0
	T2	N0	M0
IIB	T2	N1	M0
	T3	N0	M0
IIIA	T0	N2	M0
	T1	N2	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
IIIB	T4	N0	M0
	T4	N1	M0
	T4	N2	M0
IIIC	Any T	N3	M0
IV	Any T	Any N	M1

ANNEXURE 3

PROCESSING FOR IMMUNOHISTOCHEMISTRY

- 3µm thickness sections were cut using microtome from the selected paraffin blocks.
- The sections are taken in poly L-lysine coated adhesive slides.The slides are incubated at 60º C for one hour.
- The slides are subjected to 2 changes of xylene 5 minutes each for deparaffinization.
- They are then transferred to absolute alcohol for 5 minutes followed by by 80% and 70% alcohol for 5 minutes to rehydrate the tissue sections.
- Tissue sections are then placed in running tap water for 5 minutes and washed in distilled water
- Antigen retrieval was performed using pressure cooker in specific buffer (citrate buffer for HER2/neu and TRIS EDTA buffer for ER,PR and ki 67)
- Then the sections are cooled to room temperature and the slides are washed with distilled water
- Endogenous peroxidase activity is removed by incubating the tissue sections with enough drops of 3% peroxide block in a humidity chamber for 5 minutes. The sections are then washed in TRIS wash buffer.

- Protein block is then added for 5 minutes followed by wash in TRIS wash buffer
- Primary antibody (Her2 neu/Ki67/ER/PR) is then added over the tissue sections and incubated for 30 minutes .
- The tissue sections are then washed in TRIS wash buffer.
- Followed by that primary amplifier is added for 15 minutes to enhance the process of primary antibody which is then washed in TRIS wash buffer
- Secondary antibody is added and incubated for 20 minutes and then washed with TRIS wash buffer
- DAB chromogen (1ml DAB buffer +1 drop DAB chromogen) is then added over the tissue and incubated for 4 minutes and then washed with 2 changes of distilled water.
- Counterstaining was done with Mayer's haematoxylin for 30 seconds and washed in running tap water.
- Dehydration is done by 2 changes of 100 % alcohol.
- Mounting is done by DPX mountant and observed under microscope.

BUFFER PREPARATIONS

1) Tris – EDTA Buffer: - PH- 9.0

Tris	-	6.05 Gms
EDTA	-	0.744gms
Distilled water	-	1000ml

2) **Citrate buffer :- p H-6.9**

Citrate	-	1.92 gms
Distilled water	-	1000 ml

3) Tris wash buffer

Tris	-	0.605 gm
Sodium chloride	-	8 gm
1 N Hcl	-	4ml
Distilled water	-	1000 ml

PRECAUTIONS

1. The glasswares used should be dry and clean.
2. All the buffers used should be prepared fresh and the p H should be adjusted according to the preferred p H.
3. The staining procedures are never allowed to dry so they are performed under a humidity chamber.
4. DAB chromogen should be handled and disposed carefully as it is a carcinogen.
5. Primary,secondary antibody, DAB chromogen,peroxidase block,amplifier ,everything should be stored at 4-6ºC

While performing IHC every batch should have a positive control slide.

ANNEXURE 4

ALLRED SCORING GUIDELINES FOR ER AND PR

Proportion score

Proportion score is done by calculating the proportion of tumor cells with positive nuclear staining

0 = no nuclear staining

1 = <1% nuclear staining

2 = 1%-10% nuclear staining,

3 = 11%-33% nuclear staining

4 = 34%-66% nuclear staining

5 = 67%-100% nuclear staining

Intensity of staining

0 = no staining

1 = weak staining

2 = moderate staining

3 = strong staining

Total score =proportion score + intensity score (0 to 8)

Interpretation:.

0,2 - Negative

≥ 3 - Positive

ANNEXURE 5

PROFORMA

Case number	:	Name :
HPE number	:	Age :
IP number	:	Gender :

CLINICAL DETAILS

- Presenting complaints and duration
- Family history
- Menstrual and reproductive history
- H/O hormone replacement therapy ,radiation exposure

SURGICAL DETAILS

- Type of surgery
- Lymphnodes submitted or not

HISTOPATHOLOGICAL DETAILS

A.GROSS:

1. Tumor location
2. Tumor size (cms)
3. Skin / Nipple involvement – present / absent
4. Tumor margins and consistency of the tumor.
5. Distance of the tumor from surgical margins.
6. Lymph nodes : present / absent.

MICROSCOPY

1. Tumor type
2. Grade of the tumor
3. Presence or absence of in situ component. If present type and grade of DCIS.
4. Lymphovascular invasion.
5. Presence/absence of necrosis.
6. Surgical margin status.
7. Lymph node involvement

IMMUNOHISTOCHEMISTRY

1. ER, PR
2. HER2/neu
3. Ki 67- < 20 % or ≥ 20.

S. NO	CATEGORY	PATH NO	IP NO	AGE	MENOPAUSAL STATUS	TUMOR SIZE(cm)	TUMOR TYPE	DCIS TYPE	DCIS GRADE	IDC GRADE	TUMOR NECROSIS	NIPPLE INVASION	LVI	LYMPH NODE STATUS	ER STATUS	PR STATUS	HER2 /neu	Ki 67 %
1	1	73/13	1086	49	1	6	IDC,NOS	C	1	2	+	+	+	NEG	+	+	NEG	2
2	1	104/13	2678	41	1	3.5	IDC,NOS	C	1	2	-	NEG	NEG	NEG	+	+	NEG	18
3	1	142/13	3171	55	2	3	IDC,NOS	C	1	3	-	NEG	NEG	1	-	-	3+	30
4	1	521/13	6855	59	2	1	IDC,NOS	CR	3	2	-	NEG	NEG	NEG	+	-	NEG	2.4
5	1	1002/13	21614	45	1	2.5	IDC,NOS	MP	1	1	-	NEG	NEG	1	-	-	1+	14
6	1	1O91/13	19085	50	1	4	IDC,NOS	C	1	2	-	NEG	+	2	+	+	NEG	22.8
7	1	1106/13	17953	54	2	6	IDC,NOS	C	1	1	-	NEG	+	NEG	-	+	2+	2
8	1	1302/13	30121	58	2	6.2	IDC,NOS	C	1	2	+	NEG	+	1	+	+	NEG	18
9	1	1856/13	41235	44	1	5	IDC,NOS	S	1	2	-	NEG	NEG	2	-	-	2+	62
10	1	2040/13	42356	80	2	2.6	IDC,NOS	S	1	1	+	NEG	NEG	NEG	-	-	NEG	2
11	1	2107/13	44985	45	1	4	IDC,NOS	CR	3	2	-	NEG	NEG	2	+	+	NEG	9
12	1	2110/13	46111	51	1	1.5	IDC,NOS	S	3	2	-	NEG	NEG	NEG	+	-	NEG	3.4
13	1	2156/13	47456	58	2	3.2	IDC,NOS	C	1	2	-	NEG	+	3	+	-	NEG	2
14	1	2675/13	56667	55	2	2	IDC,NOS	C	1	1	-	NEG	NEG	NEG	+	+	NEG	16.5
15	1	3420/13	64534	56	2	7.2	IDC,NOS	M	3	1	+	NEG	NEG	NEG	+	-	NEG	2
16	1	3482/13	71220	61	2	5.5	IDC,NOS	C	1	2	-	NEG	NEG	1	-	-	NEG	2
17	1	3400/13	71944	70	2	4	IDC,NOS	M	3	1	-	NEG	NEG	NEG	+	-	NEG	4
18	1	205/14	5028	42	1	1	IDC,NOS	C	1	2	-	NEG	NEG	NEG	+	+	NEG	12
19	1	492/14	8315	62	2	4.5	1DC,NOS	c	1	3	-	NEG	NEG	2	-	-	NEG	16
20	1	858/14	16283	45	1	6	IDC,NOS	c	1	2	-	NEG	NEG	1	+	-	3+	34.8
21	1	1126/14	18967	42	1	4.5	IDC,NOS	C	1	2	-	NEG	+	2	+	-	2+	22
22	1	1279/14	21404	65	2	3	IDC,NOS	C	1	2	-	NEG	NEG	NEG	-	-	NEG	2
23	1	1365/14	22875	34	1	4.5	IDC,NOS	c	1	3	-	NEG	NEG	2	-	-	3+	52
24	1	1422/14	24211	54	1	2.6	IDC,NOS	C	1	2	-	NEG	NEG	3	+	+	NEG	32.6
25	1	1484/14	24732	62	2	1.5	IDC,NOS	c	1	2	-	NEG	NEG	1	+	+	NEG	16
26	2	197/13	4080	65	2	3	IDC,NOS	---	---	2	-	NEG	+	NEG	+	-	NEG	14
27	2	231/13	5132	45	2	6	IDC,NOS	---	---	3	-	NEG	+	3	-	-	3+	62
28	2	792/13	11043	48	1	4	IDC,NOS	---	---	1	-	NEG	NEG	NEG	+	+	NEG	4.5
29	2	1128/13	19800	45	1	4.5	IDC,NOS	---	---	2	-	NEG	+	2	+	+	NEG	40
30	2	1644/13	39784	70	2	9	IDC,NOS	---	---	2	-	NEG	NEG	1	+	-	NEG	22
31	2	1932/13	39468	55	2	5	IDC,NOS	---	---	1	-	NEG	NEG	NEG	+	-	NEG	1
32	2	2101/13	45958	52	2	2	IDC,NOS	---	---	2	-	NEG	+	3	+	+	NEG	22
33	2	2185/13	49876	62	2	6.2	IDC,NOS	---	---	2	+	NEG	NEG	1	+	+	NEG	14.8
34	2	3405/13	70236	55	2	1.8	IDC,NOS	---	---	2	-	NEG	NEG	2	+	+	2+	25
35	2	93/14	181	46	1	7.4	IDC,NOS	---	---	2	-	NEG	NEG	3	-	-	3+	78
36	2	469/14	6983	46	2	9	IDC,NOS	---	---	2	+	+	+	2	-	-	3+	80
37	2	5O2/14	8672	54	2	2.5	ATYPICAL MEDULLARY CA	---	---	NA	-	NEG	NEG	NEG	-	+	NEG	2
38	2	854/14	11797	50	2	11	IDC,NOS	---	0	2	-	NEG	NEG	2	-	-	2+	80
39	2	1173/14	21195	80	2	3	MUCINOUS CA	---	---	NA	-	NEG	NEG	1	+	-	NEG	16
40	2	1238/14	21099	43	1	8.5	IDC,NOS	---	---	2	+	NEG	NEG	1	-	-	3+	25.6
41	2	1384/14	22546	45	1	5	IDC,NOS	---	---	2	-	NEG	NEG	1	-	-	3+	32
42	2	1448/14	24614	43	1	1.5	IDC,NOS	---	---	2	-	NEG	+	2	+	+	NEG	60
43	2	1502/14	28927	67	1	2	IDC,NOS	---	---	1	-	NEG	NEG	NEG	+	+	NEG	2
44	2	1529/14	25551	55	2	2.5	IDC,NOS	---	---	2	-	NEG	NEG	3	+	-	NEG	80
45	2	1591/14	21791	53	2	11.5	IDC,NOS	---	---	2	-	NEG	+	2	-	-	2+	90
46	2	1622/14	28714	55	2	5	IDC,NOS	---	---	2	-	NEG	NEG	NEG	-	-	NEG	18.4
47	2	1651/14	28145	48	2	3	INV PAPILLARY CA	---	---	NA	+	NEG	NEG	NEG	+	+	NEG	25
48	2	2227/14	38001	45	1	7	IDC,NOS	---	---	2	-	NEG	NEG	2	-	-	NEG	25.2
49	2	2228/14	38667	61	2	4	IDC,NOS	---	---	3	-	NEG	+	2	-	-	2+	12
50	2	2280/14	38795	61	2	3	IDC,NOS	---	---	2	-	NEG	NEG	2	-	-	NEG	6

KEY TO MASTERCHART

1.Category : 1-IDC with DCIS (IDC-DCIS)

2-IDC without DCIS(IDC)

2.Menopausal status : 1-Premenopausal

2-Postmenopausal

3.DCIS type : C- Comedocarcinoma

CR-Cribriform type

S- Solid type

MP-Micropapillary type

M – Mixed type.

4.DCIS GRADE : 1-High grade DCIS

2-Intermediate grade DCIS

3-Low grade DCIS

5. IDC histological grade: 1- Grade 1

2-Grade 2

3-Grade 3

6.Necrosis : + : Present

- : Absent

7.Nipple invasion: + : Present

NEG- Absent

8.Lymphovascular invasion(LVI) : + : Present

NEG :Absent

9.Lymph node status : NEG –not involved

1 : 1-3 Nodes involved

2 : 4-9 Nodes involved

3 : > 9 Nodes involved

10.Estrogen receptor status(ER)

\+ : Positive

\- : Negative

11.Progesterone receptor status(PR)

\+ : Positive

\- : Negative

12.HER2/neu

0 , 1 + : Negative

2+,3+ : Positive