"CORRELATION OF E CADHERIN AND VIMENTIN EXPRESSION WITH HISTOLOGICAL TYPE AND GRADE OF DIFFERENTIATION IN BREAST CANCER"

By

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IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF MEDICINE IN

PATHOLOGY

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LIST OF ABBREVIATIONS

DCIS- Ductal carcinoma in situ

EMT- Epithelial mesenchymal transition

E cad/ Ec- E cadherin

ER- Estrogen receptors

H and E – Hematoxylin and Eosin

IDC- Infiltrating ductal carcinoma

IHC – Immunohistochemistry

ILC- Invasive lobular carcinoma

Med Ca/ MC- Medullary carcinoma

MRM- Modified radical mastectomy

n= Number of cases

Vim/Vm- Vimentin

WHO- World Health Organization

TNM- Tumor, nodal, metastasis

ABSTRACT

Background:

Carcinoma breast is the second most common cancer among Indian woman, and an increasing trend in its incidence has been observed in most of the metropolis. Various prognostic parameters in breast cancer is well established in order to identify the patients who will benefit from adjuvant chemotherapy for better survival rate. In spite of the advanced modalities in treatment, not all patients are benefitted equally. Not all tumors behave in the same manner and reason for it is unknown. However one theory that can be put forward is increased aggressiveness and progression in breast cancer due to the epithelial mesenchymal transition (EMT) of the tumor cells. EMT is potentially a new process and investigation that can be targeted against the metastatic progression. This EMT can be investigated by using immunohistochemistry markers like vimentin and E cadherin to show the enhanced invasion and metastatic potential in breast cancers in order to benefit the patients and reduce the mortality and morbidity.

Objective of the study:

To study the expression of vimentin and E cadherin in the breast carcinomas and correlate their expression with the histological parameters.

Methods:

The study was carried at The Department of Pathology, R.L.Jalappa Hospital and Research Centre attached to Sri Devaraj Urs Medical College, Tamaka, Kolar, during the period of 01-01-2014 to 30-07-2015. The study included 58 cases of breast cancers out of which 46 were infiltrating ductal carcinoma, 6 each were medullary carcinoma and invasive lobular carcinoma. Immunohistochemistry was done using antibodies against vimentin and E cadherin. Percentage of positive cells were documented and immunoscore was calculated.

Vimentin and E cadherin expression was correlated with age, tumor size, histopathological type, Bloom Richardson grading, lymph node status, Nottingham Prognostic score, tumor stage, vascular invasion, skin involvement. Statistical analysis used: statistical correlation was done using Pearson's chi-square test. A p value less than 0.05 was considered significant.

Results:

Vimentin expression was seen in 18 cases- 15 infiltrating ductal carcinoma and 3 medullary carcinoma. E cadherin expression was positive in 36 cases and negative in 22 cases- 36 positive and 10 negative in infiltrating ductal carcinoma, 6 each negative in invasive lobular carcinoma and medullary carcinoma. Vimentin expression correlated with tumor grade, tumor stage and skin involvement. E cadherin expression correlated with histopathological type and tumor grade. Vimentin and E cadherin co-expression correlated closely with histopathological type, but was not statistically significant.

Conclusion:

Vimentin expression is associated with higher tumor grade and tumor stage, indicating the aggressiveness the tumor can behave by gaining mesenchymal properties. E cadherin expression aids in differentiating tumors of invasive lobular carcinomas from infiltrating ductal carcinoma with histological variations and its loss confers tumors with increased invasiveness.

Keyword : Vimentin, E cadherin, breast carcinoma.

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INTRODUCTION

INTRODUCTION

Carcinoma breast is the second most common cancer among Indian woman, and an increasing trend in its incidence has been observed in most of the metropolis.[1]

Breast cancer is a heterogeneous disease with a wide array of histological appearances. Greater than 95% of breast cancers are adenocarcinoma, which are divided into in situ carcinoma and invasive carcinoma. The common cause for death in such patients is metastasis.

Worldwide incidence of breast cancer comprises of 10.4% of all cancer incidence among women. The estimated number of new cancers in India per year is about 7 lakhs and over 3.5 lakh people die of cancer each year. **[2]** The incidence of breast cancer in Kolar district is around 6.41%. **[3]**

Various prognostic parameters in breast carcinomas is well established. The parameters being tumor size, histopathological grade, histopathological type, axillary lymph node status, vascular invasion and estrogen receptor status. These factors are essential to identify for the benefit of adjuvant chemotherapy and better survival rate among patients with breast cancer.[4, 5]

In spite of the advanced modalities in treatment, not all patients respond equally to the treatment. Not all tumors behave in the same manner and reason for it is unknown. However one theory that can be put forward is increased aggressiveness and progression in breast cancer due to the epithelial mesenchymal transition (EMT) of the tumor cells. The epithelial cells that undergo EMT, lose their epithelial cell characteristics and acquire a mesenchymal phenotype and achieve migratory and invasive properties. This is

characterized by the loss of E cadherin adhesion molecules and gain in mesenchymal proteins like vimentin, alpha smooth muscle actin.[6]

E cadherin is a transmembrane calcium dependent adhesion protein, and it is a part of the type I classical cadherin family, localized in adherens junction. The normal cell to cell adhesion is maintained by the E cadherin. E cadherin is considered as a tumor suppressor protein, because it was shown that expression and /or functional losses of this protein is associated with tumorigenesis and tumor progression .[7]

Vimentin is an intermediate filament expressed in tissues of normal mesenchymal origin. It is known to express aberrantly in epithelial cancers of prostate, GI tract, breast, central nervous system, lung and malignant melanomas. Vimentin expression in tumors is associated with increased metastatic potential and enhanced invasiveness.[8]

In spite of many progress in the field of diagnosis, prognosis and therapy, the morbidity and specific mortality continues to increase. Therefore, EMT is potentially a new process and investigation that can be targeted against the metastatic progression which may improve the prognosis[6]. There are only few studies and articles done using both E cadherin and vimentin expression in breast cancers to show the EMT process simultaneously and none in our geographic area.

This study will focus on the significance of different histological features of breast cancer and the expression of vimentin and E cadherin, as the expression is potentially a predictor of pathologically aggressive behavior and invasiveness.

AIMS AND OBJECTIVES OF THE STUDY:

To study the expression of vimentin and E cadherin in breast carcinomas and correlate their expression with the histological parameters.

REVIEW OF LITERATURE

Incidence and Epidemiology:

Breast cancer is the second most common cancer affecting woman, following the cervical cancer. Both cervical and breast carcinomas account for nearly 60% of total cases, of which breast carcinoma incidence is 10.4%. In the year 2012, around 1,44,937 woman were newly diagnosed breast carcinoma cases and about 70,218 woman died of breast cancer. [9]

India accounts for nearly six percent of deaths due to breast cancer in the world and also one out of every 22 women in India is diagnosed with breast cancer every year.[10]

Contrary to the west, where it is more common in elderly age group, breast cancer is more common at a younger age in the Indian woman, who present themselves in advanced stage with poor prognostic features and have worst outcome when compared to their counter-parts in western countries.

Immunohistochemical (IHC) markers provide early and accurate information on tumorigenesis, on long-term outcome and help in prediction of the response to treatment in breast carcinoma. The most common IHC markers used in breast cancer is to know the status of estrogen and progesterone receptors which aids in appropriate therapy for better outcome. However with increased availability of other IHC markers like cytokeratin, vimentin, E cadherin, Ki67 etc., all of which are directed for early detection, targeted therapies and better outcome in such patients.

In a study done by Thompson et al to find the relationship between estrogen receptor(ER), vimentin and invasiveness in breast cancer, he found that the tumors with ER+/vimentin- and ER-/vimentin- cell lines were un-invasive, while ER-/vimentin+ cell lines forms

invasive penetrating colonies. Therefore vimentin positivity in breast tumors is associated with increased metastatic potential through enhanced invasiveness.[8]

In another study by Hemalatha et al in 2013 to compare vimentin expression with histological parameters in infiltrating ductal carcinoma cases, they found that vimentin positive cells is associated with high grade tumors, increase proliferation and bad prognostic factors.[11]

In a study by Qureshi et al, show that E cadherin was present in all in situ and invasive ductal carcinomas but in majority of invasive lobular carcinomas and all lobular carcinomas in situ there was complete loss of E cadherin expression. Therefore the loss of E cadherin plays a part in the characteristic morphology of lobular carcinoma and associated with increased invasiveness of malignant cells .[12]

In another study they show that the epithelial- mesenchymal transition in breast cancer is characterized by loss of intercellular adhesions with down regulation of E cadherin and up regulation of mesenchymal markers like vimentin, thus establishing increase in motility, invasiveness and metastatic capabilities of the tumor cells.[13]

In a study done by Hassan et al, on expression of vimentin and cytokeratin in normal and breast cancer cases, they showed that out of 62 cases, 93.75% of normal breast tissues showed positive vimentin staining. In breast tumors, vimentin was positive in 100% cases. Few studies concluded that vimentin is not expressed in lobular carcinomas and mucinous carcinomas, but this study registered its expression in all breast tumors. Therefore they concluded that vimentin is of no clinical use as it is expressed in all breast cancers and most of normal tissue.[14]

In a study done by Liu T et al, to find any correlation between Slug, E-cadherin, vimentin, clinicopathological parameters and prognosis of patients with basal like breast cancer, found that high Slug, low E-cadherin expression in basal like breast cancer patients closely correlated with histological grade, lymph node metastasis, tumor-node-metastasis stage, and lymphatic vessel metastasis. He concluded that high Slug and vimentin expression, and low E-cadherin levels is associated with poor prognosis. [15]

Another study done by Yagasaki et al, to find the clinical significance of E-cadherin and vimentin co-expression in breast cancer in 83 cases, they found the co-expression of E-cadherin and vimentin was associated with axillary metastases, histologic grade and recurrence, and appeared as the most important prognostic factor in disease-free and overall survival. Therefore they concluded that E-cadherin and vimentin expression in together might provide prognostic information for breast cancer patients.[16]

A study done by Aleksandra et al, to find EMT markers in breast cancers, demonstrated high expression of TWIST1 and SNAIL in lymph node metastasis, as well as negative-to-positive conversion of SNAIL, than in primary tumors. They didn't observe much change in E-cadherin and vimentin status between primary tumor and lymph node metastasis. Thus, molecular profiling of lymph node metastasis may be used as surrogate marker for aggressiveness and metastatic potential of primary tumor.[17]

Structural anatomy of Breast:

The breasts or mammary glands are a modified and highly specialized type of sweat glands. It lies between the second & sixth ribs in the vertical axis & between the sternal edge to mid-axillary line in horizontal axis.[18,19,20] The breasts consist of glandular and supporting fibrous tissue embedded within fatty matrix, together with blood vessels, nerves and lymph vessels. The mammary glands are in the subcutaneous tissue overlying the pectoralis major and minor muscles. At the greatest prominence of the breast is the nipple, surrounded by a circular pigmented area, the areola. A small part of the mammary gland may extend along the inferolateral edge of the pectoralis major towards the axillary fossa, forming an axillary process or tail of Spence.[18,19,20]

Architecture of gland:

The breast is composed of acini, which make up lobules, aggregations of which form the lobes of the gland. The lobes are arranged in a radiating fashion like the spokes of a wheel and converge on the nipple, where each lobe is drained by a duct. A single large duct, the lactiferous duct drains each lobe via separate opening on the surface. Ten to fifteen collecting ducts open onto the nipple, each duct draining a segmental system of smaller ducts and lobules. Each breast lobe is sub-divided into a variable number of lobules. The lobule with terminal duct is called "terminal duct lobular unit". Each of these lobes represents the morphofunctional unit of the organ consisting of compound tubuloacinar glands. The ducts are surrounded by connective tissue which is characteristically loose and vascular in the distal ductules. **(Figure 1)**

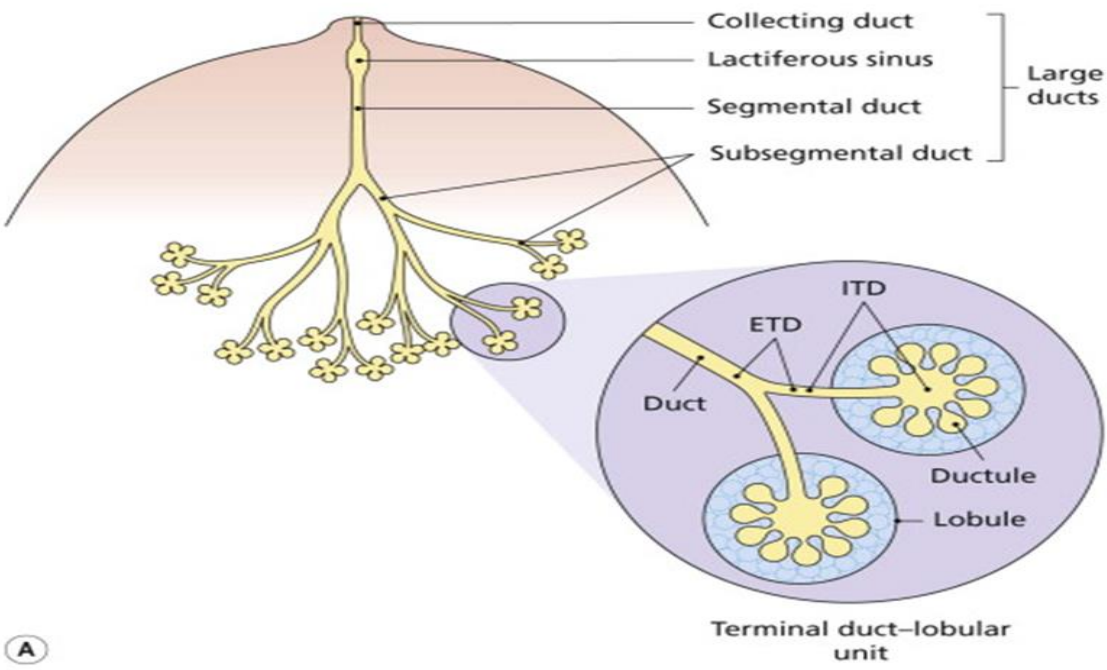


Figure 1: Structural anatomy of breast.

The overlying skin is lined by keratinized squamous epithelium which continues into ducts and then changes in double layered epithelium which rests on a continuous basement membrane. The luminal cuboidal or columnar cells which produce milk rest on the flat discontinuous myoepithelial cells which help in milk ejection. [18,19,20] **(Figure 2)**

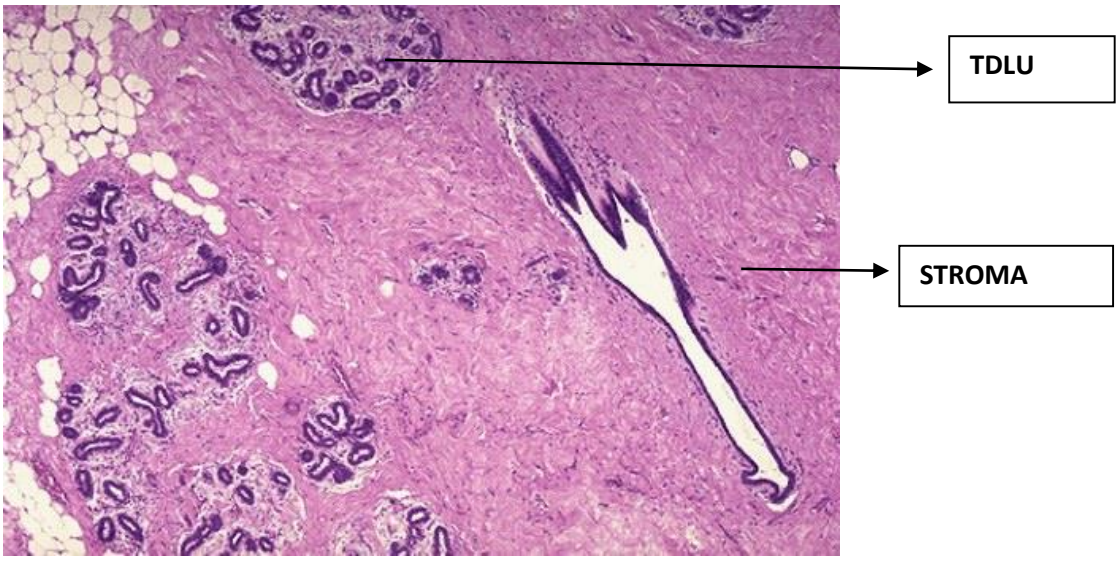


Figure 2: Histology of breast- Terminal duct lobular unit.

Risk factors for developing breast cancer:[21]

1. Geographical: Incidence of breast carcinoma is more in developed countries than in developing countries and high among women in high socio- economic group than compared to that of low socio- economic group.
2. Family history: Women with first degree relatives with breast cancers, have a fourfold risk than that of the general population.
3. Hormonal factors: Increase exposure to endogenous estrogen –early age of menarche, nulliparity or late age at menopause, postmenopausal women, obesity.
4. Diet: High fat diet and alcohol consumption carry increased risk.
5. Environmental: Exposure to ionizing radiations.
6. Inherited Breast cancer

   It accounts to about 5 to 10% of all breast cancers. Mode of inheritance being autosomal dominant inheritance of a mutated gene. However majority of tumors are sporadic. The most common genes involved are due to mutations and accumulation of their products – Proto-oncogenes, Tumor suppressor genes and mismatch repair genes.

   The most commonly involved tumor suppressor genes identified are BRCA 1 and BRCA 2.

   BRCA 1: It is located on chromosome 17. Its mutation is associated with increased risk of developing ovarian and breast cancers. It causes 50% to 85% increase in risk of carcinoma breast.

BRCA 2: It is located on chromosome 13 and is associated with hereditary early onset breast cancer in families. Men with BRCA 2 mutations have increased risk of carcinoma breast.

It is estimated that 55-65% of woman who inherit BRAC1 mutation and about 45% of woman who inherit BRAC2 mutation will develop breast cancer by the age of 70 years.[22,23]

7. Benign breast disease: Atypical hyperplasia is associated with a greater risk of carcinoma.

THE WORLD HEALTH ORGANIZATION (WHO) CLASSIFICATION OF TUMORS OF THE BREAST (2003)[24]

Epithelial Tumors

1. Invasive ductal carcinoma
    - Most are "not otherwise specified (NOS)
    - The remainder are given subtypes
        - Mixed type carcinoma
        - Pleomorphic carcinoma
        - Carcinoma with osteoclastic giant cells
        - Carcinoma with choriocarcinomatous features
        - Carcinoma with melanotic features
2. Invasive lobular carcinoma
3. Tubular carcinoma
4. Invasive cribriform carcinoma
5. Medullary carcinoma
6. Mucinous carcinoma and other tumors with abundant mucin
    - Mucinous carcinoma
    - Cystadenocarcinoma & columnar cell mucinous carcinoma
    - Signet ring cell carcinoma
7. Neuroendocrine tumors
    - Solid neuroendocrine carcinoma
    - Atypical carcinoid tumor
    - Small cell/oat cell carcinoma

- Large cell neuroendocrine carcinoma

8. Invasive papillary carcinoma

9. Invasive micropapillary carcinoma

10. Apocrine carcinoma

11. Metaplastic carcinomas

- Pure epithelial metaplastic carcinomas
  - Squamous cell carcinoma
  - Adenocarcinoma with spindle cell metaplasia
  - Adenosquamous carcinoma
  - Mucoepidermoid carcinoma
- Mixed epithelial/mesenchymal metaplastic carcinomas

12. Lipid-rich carcinoma

13. Secretory carcinoma

14. Oncocytic carcinoma

15. Adenoid cystic carcinoma

16. Acinic cell carcinoma

17. Glycogen-rich clear cell carcinoma

18. Sebaceous carcinoma

19. Inflammatory carcinoma

20. Bilateral breast carcinoma

Mesenchymal Tumors (including sarcoma)

1. Haemangioma
2. Angiomatosis
3. Hemangiopericytoma
4. Pseudoangiomatous stromal hyperplasia (PASH)
5. Myofibroblastoma
6. Fibromatosis (aggressive)
7. Inflammatory myofibrblastic tumor
8. Lipoma
    - Angiolipoma
9. Granular cell tumor
10. Neurofibroma
11. Schwannoma
12. Angiosarcoma
13. Liposarcoma
14. Rhabdomyosarcoma
15. Osteosarcoma
16. Leiomyoma
17. Leiomyosarcoma

Precursor lesions

1. Lobular neoplasia
    - Lobular carcinoma In situ (LCIS)
2. Intraductal proliferative lesions

- Usual ductal hyperplasia
- Flat epithelial hyperplasia
- Atypical ductal hyperplasia
- Ductal carcinoma insitu (DCIS)

3. .Microinvasive carcinoma
4. Intraductal papillary neoplasms
    - Central papilloma
    - Peripheral papilloma
    - Atypical papilloma
    - Intraductal papillary carcinoma
    - Intracystic papillary carcinoma

Benign epithelial lesions

1. Adenosis, including variants
    - Sclerosisng adenosis
    - Apocrine adenosis
    - Blunt duct adenosis
    - Microglandular adenosis
    - Adenomyoepithelial adenosis
2. Radial scar / complex sclerosing lesion
3. Adenomas
    - Tubular adenoma
    - Lactating adenoma

- Apocrine adenoma
- Microglandular adenosis
- Adenomyoepithelial adenosis

Myoepithelial lesions

- Myoepitheliosis
- Adenomyoepithelial adenosis
- Adenomyoepithelioma
- Malignant myoepithelioma

Fibroepithelial tumors

- Fibroadenoma
- Phylloides tumor
    - Benign
    - Borderline
    - Malignant
- Periductal stromal sarcoma, low grade
- Mammary hamartoma

Tumors of Nipple

- Nipple adenoma
- Syringomatous adenoma
- Pagets disease of the nipple

Malignant lymphoma

- Diffuse large B cell lymphoma

- Burkitt lymphoma
- Extranodal marginal zone B –cell lymphoma of MALT type
- Follicular lymphoma

Metastatic tumors

Tumors of male breast

- Gynecomastia
- Carcinoma
    - In situ
    - Invasive

Histopathological types: [20,24]

1. Infiltrating ductal carcinoma

It is the most common type of breast cancer. These tumors vary in size from 0.5cm to 10cm or more. They are usually firm to hard in consistency giving a gritty feel while cutting. Microscopically, the tumor cells are arranged in diffuse sheets, nests, chords or diffusely infiltrating pattern. Nuclei may be uniform to highly pleomorphic with prominent nucleoli.

Few tumors elicit frank desmoplasia. These tumors express low molecular weight keratins, epithelial membrane antigen, gross cystic disease protein 15 and E cadherin. Vimentin is usually expressed in high grade tumors. IDC has a worse prognosis compared to the other types, with 35-50% of 10 year survival rate.

2. Invasive lobular carcinoma

These tumors usually present as bilateral or multifocal lesions in breast. Affect women around 50 year age group. They present as an ill- defined impalpable mass. Microscopically, the tumor cells are small to moderate size cells, arranged in strands or dispersed individually, India file pattern invading into the fibrous stroma. Few cells show occasional intracytoplasmic lumen. These tumors are associated with loss of E cadherin, the gene mutated in them. Prognosis is better than IDC.

3. Tubular carcinoma

The average age affected is around 50 years with favourable prognosis. These tumors are usually poorly circumscribed, hard, small in size. Microscopically, haphazard arrangement of glands having irregular, angulated contours lined by single layer of epithelial cells in a desmoplastic stroma.

4. Invasive cribriform carcinoma

Rare form of breast cancer, with excellent prognosis. Microscopically, the tumor cells are arranged in cribriform patter, that is invasive islands, often angulated with well defined spaces.

5. Invasive papillary carcinoma

Rare form of breast cancer, accounting to less than 1%. Usually occurs in elderly females and it is more frequent in men. These are small, circumscribed, soft tumors with size of 1-3 cm. Microscopically, they show papillary structures with fibrovascular cores, frank invasion into stroma.

6. Metaplastic carcinoma

Heterogeneous group of tumors characterized by admixture of adenocarcinoma with areas of spindle cells and squamous cell carcinoma or mesenchymal differentiation. They are classified into pure epithelial and mixed epithelial and mesenchymal components. These are aggressive tumors, but prognosis is more or less the same.

7. Apocrine carcinoma

These are a rare form of breast malignancy composed of apocrine type of epithelium in more than 90% of the tumor cells. Tumor cells are large, having abundant acidophilic granular cytoplasm which contain golden brown granules that are strongly positive for PAS, glandular differentiation usually found with apocrine snouts.

8. Neuroendocrine carcinoma

WHO defines this type of carcinoma with neuroendocrine marker positivity noted in more than 50% of the cell population. Microscopically, they have an infiltrative pattern, with cells arranged in nests, sheets and trabecular formation and peripheral palisading of cell groups.

STAGING OF BREAST CANCER: [24]

Tumor size

TX - Primary tumor cannot be assessed

T0 - No evidence of primary tumor

Tis - Carcinoma in situ

- Tis (DCIS) Ductal carcinoma in situ
- Tis (LCIS) Lobular carcinoma in situ
- Tis (Paget) Paget's disease of the nipple with no tumor

T1 - Tumor 2 cm or less in greatest dimension

- T1 mic Micro invasion 0.1 cm or less in greatest dimension
- T1a More than 0.1 cm but not more than 0.5 cm in greatest dimension
- T1b More than 0.5 cm but not more than 1 cm in greatest dimension
- T1c More than 1 cm but not more than 2 cm in greatest dimension

T2 - Tumor more than 2 cm but not more than 5 cm in greatest dimension

T3 - Tumor more than 5 cm in greatest dimension

T4 - Tumor of any size with direct extension to (a) chest wall or (b) skin only as described below

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

Lymph Node

There are four lymph node classification values (N0, N1, N2 or N3) which depend on the number, size and location of breast cancer cell deposits in lymph nodes.

PNx - Regional lymph nodes cannot be assessed

pN0- No regional lymph node metastasis

pN1mi - Micro metastasis (larger than 0.2 mm, but none larger than 2 mm in greatest dimension)

pN1 - Metastasis in 1–3 ipsilateral axillary lymph node(s), and/or in internal mammary nodes with microscopic metastasis detected by sentinel lymph node dissection but not clinically apparent

- pN1a -Metastasis in 1–3 axillary lymph node, including at least one larger than 2 mm in greatest dimension
- pN1b - Metastasis in internal mammary lymph nodes with microscopic metastasis detected by sentinel lymph node dissection but not clinically apparent
- pN1c - Metastasis in 1–3 axillary lymph nodes and internal mammary lymph nodes with microscopic metastasis detected by sentinel lymph node dissection but not clinically apparent

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

- pN2a - Metastasis in 4–9 axillary lymph nodes, including at least one that is larger than 2 mm
- pN2b - Metastasis in clinically apparent internal mammary lymph node(s), in the absence of axillary lymph node metastasis

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

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

- pN3b- Metastasis in clinically apparent internal mammary lymph node in the presence of 1 or more positive axillary lymph node; or metastasis in more than 3 axillary lymph nodes and in internal mammary lymph nodes with microscopic metastasis detected by sentinel lymph node dissection but not clinically apparent
- pN3c - Metastasis in supraclavicular lymph node.

Metastasis

There are two metastatic classification values (M0 or M1) which depend on the presence or absence of breast cancer cells in locations other than the breast and lymph nodes (distant metastasis example:-bone, brain, lung) but it includes supraclavicular lymph node.

MX- Distant metastasis cannot be assessed

M0- No distant metastasis

M1 - Distant metastasis

Table 1: Staging of breast carcinoma[24]

STAGE 0	Tis	N0	M0
STAGE 1	T1a	N0	M0
STAGE IIA	T0	N1	M0
	T1a	N1	M0
	T2	N0	M0
STAGE IIB	T2	N1	M0
	T3	N0	M0
STAGE III	T0	N2	M0
	T1a	N2	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
STAGE IIIB	T4	N0	M0
	T4	N1	M0
	T4	N2	M0
STAGE 111C	Any T	N3	M0
STAGE 1V	Any T	Any N	M1

MODIFIED BLOOM RICHARDSON GRADING SYSTEM

The concept of tumor grade was first described by Broders and his predecessors. Tumor grade considers the histopathological qualities of the primary tumor, unlike stage which is based on the extent of disease. It is now an important part of pathology reporting in breast cancer. It is an important determinant of prognosis that allows risk stratification within a given tumor stage. Tumor grade remained a statistically significant prognostic factor for disease free survival and overall survival. [25,26,]

Several histological grading systems were proposed earlier which used parameters like ducto-glandular differentiation or tumor secretory state, nuclear and nucleolar characteristics and others used both duct formation and nuclear abnormalities.[27]

Greenough developed a histological grading system for breast carcinoma which was simplified by Patey and Scarf. Bloom and Richardson made it more simpler by introducing a numerical scoring system to the method described by Patey and Scarf. [28]
Nottingham modification of Bloom Richardson grading combines measurement of differentiation (Tubule formation) with details of cell morphology (Nuclear pleomorphism) and assessment of proliferation (Mitotic activity).[26]

Table 2: **Modified Bloom Richardson Grading system.**

FEATURE	SCORE
TUBULE FORMATION	
>75% OF THE TUMOR	**1**
10-75% OF THE TUMOR	**2**
<10% OF THE TUMOR	**3**
NUCLEAR PLEOMORPHISM	
NUCLEI WITH MINIMAL VARIATION IN SHAPE AND SIZE	**1**
NUCLEI WITH MODERATE VARIATION IN SHAPE AND SIZE	**2**
NUCLEI WITH MARKED VARIATION IN SHAPE AND SIZE	**3**
MITOTIC COUNT (/10HP)- 40X FIELD	
0-5	**1**
6-10	**2**
>11	**3**

Table 3: **Grading of breast tumors.**

GRADE	DIFFERENTIATION	SCORE
Grade I	Well differentiated	3-5
Grade II	Moderately differentiated	6-7
Grade III	Poorly differentiated	8-9

Epithelial to mesenchymal transition:

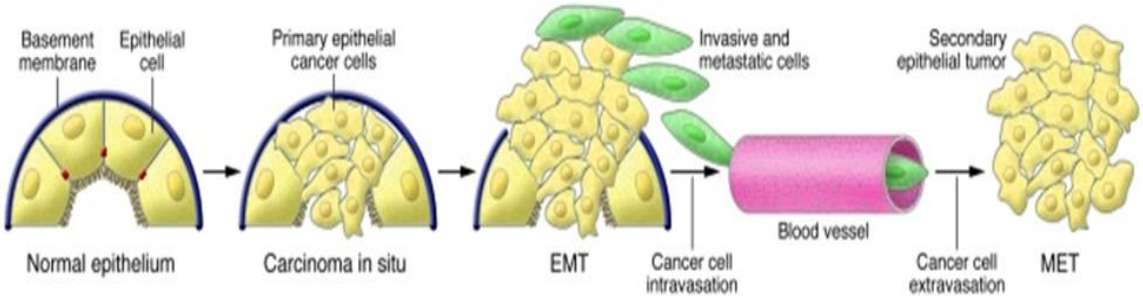
The knowledge of inter-conversion between epithelial and mesenchymal states had been proposed almost a century ago, but it was Greenburg and Hay who first described that epithelial cells in culture may acquire mesenchymal features for the process of epithelial-to-mesenchymal transition. [29]

The role of EMT in carcinoma cell invasion was first described by Thiery and colleagues almost 20 years ago. [30]

EMT also participates in normal processes of the human body. EMT is classified into 3 types based on the functions they assess. These are implantation, embryonic gastrulation and organ development, inflammation and fibrosis, and transformation of cancer cells to mesenchymal phenotype. The epithelial to mesenchymal transition of tumor cells give the tumor cells increased migratory and invasive capabilities associated with metastatic properties.[31] **(Figure 3)**

Signals from the tumor microenvironment trigger the cancer cells to adopt an invasive phenotype through epithelial–mesenchymal transition.

Figure 3: EMT and cancer progression.



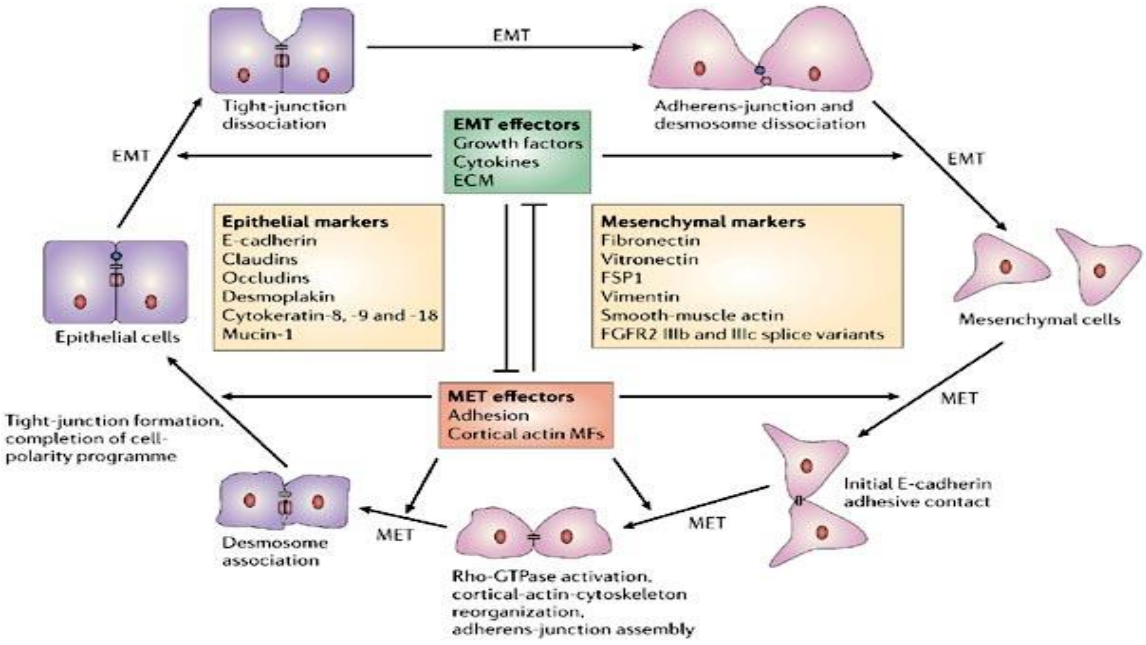
It is a coordinated complex network of extracellular and intracellular signaling factors, required for initiation and feedback mechanisms for the changes that occur within cells during transition from a less epithelial to more mesenchymal phenotype.[30] These include activation of transcription factors, expression of specific cell-surface proteins, reorganization and expression of cytoskeletal proteins, production of ECM-degrading enzymes, and changes in the expression of specific microRNAs.[31]

EMT requires growth factors and signal transductions from the tumor associated stroma such as, transforming growth factor beta, scatter factor/hepatocyte growth factor, epithelial growth factor family members, fibroblast growth factors and insulin-like growth factors 1 and 2 to be responsible for the induction or functional activation in cancer cells of a series of EMT-inducing transcription factors, notably Snail, Slug, zinc finger E-box binding homeobox 1 (ZEB1), Twist, Goosecoid, and FOXC2.[32,33,34,35,36,37] Once expressed and activated, each of these transcription factors can choreograph the complex EMT program. The intracellular signaling pathways involved are ERK, MAPK, PI3K, Akt, Smads, RhoB, β-catenin, lymphoid enhancer binding factor (LEF), Ras, and c-Fos as well as cell surface proteins such as β4 integrins, α5β1 integrin, and αVβ6 integrin.[38] Activation of EMT programs is also facilitated by the disruption of cell-cell adherens junctions and the cell-ECM adhesions mediated by integrins.[31] **(Figure 4)**

Studies have shown that with increased mesenchymal transition in breast cancers, the expression of cell adhesion molecules, E- cadherin is lost and simultaneously there is increased expression of mesenchymal markers like vimentin. This forms the basis for our

study, and therefore the study is done to find the mesenchymal transition and simultaneously loss of E cadherin in breast cancers.

Figure 4: Process of EMT acquisition.



E CADHERIN:

E-cadherin is a transmembrane calcium-dependent adhesion protein, with molecular weight of 120 kDa expressed in normal epithelial tissue. It belongs to type I classical cadherin family, localized in adherens junctions.[39,40,41] E cadherin consists of the following domains: an extracellular domain formed by five repetitive units, which assure the homotypic cell–cell adhesion, a transmembrane domain and an intracellular domain, which is attached to the actin cytoskeleton by catenins (cytoplasmic proteins).[39,40,41] It was first identified as the target of an antibody capable of preventing calcium-dependent compaction of mouse embryos and embryonal carcinoma cells.

E-cadherin is expressed in all epithelial tissues, thereby playing an important role in the pathogenesis of epithelial tumor metastasis. It also helps in maintaining the apical- basal polarity and acts as an important epithelial cell marker.

E-cadherin is a tumor suppressor protein, as studies have shown that expression and/or functional losses of E cadherin is associated with tumorigenesis and tumor progression.[39] Studies have proven that over-expression of E-cadherin in metastatic breast tumor cells inhibits breast cancer invasion and metastases *in vitro* and *in vivo*.[42]

E-cadherin (CDH1) is located on chromosome 16q22.1, which confers loss of heterozygosity in sporadic breast cancer.[43] Also promoter hypermethylation causes transcriptional down-regulation of E cadherin gene.[44] Transcriptional silencing mediated by a class of zinc finger binding proteins that target the promoter region of E-cadherin also suppress its expression. The zinc finger transcription factors such as Snail/Slug are over-expressed in advanced carcinomas.[45] Therefore the loss of E-cadherin confers metastatic ability on breast cancer cells that are otherwise essentially non- metastatic.

VIMENTIN:

Vimentin is a type III intermediate filament expressed normally in cells of mesenchymal origin. Vimentin aids in anchoring the position of the organelles in the cytosol. It is responsible for maintaining cell shape, stabilizing cytoskeletal interactions, integrity of the cytoplasm and protecting against stress.[46] It aids a number of critical proteins involved in migration, attachments and cellular signaling. Vimentin is expressed in various cell types, like pancreatic precursor cells, fibroblasts, sertoli cells, endothelial cells lining blood

vessels, renal tubular cells, trophoblastic giant cells, neutrophils, mesangial cells, leukocytes and renal stromal cells

In recent times, vimentin has gained a lot of importance due to its potential role in EMT. It is characteristically up-regulated in cells undergoing EMT. This EMT is characterized by the expression of vimentin intermediate filaments in epithelial cells, which normally express only keratin intermediate filaments. Increased vimentin expression has been reported in various tumor cell lines and tissues including prostate cancer, endometrial cancer, CNS tumors, malignant melanoma and gastrointestinal tumors including pancreatic, colorectal and hepatic cancers. In recent years, vimentin has gained importance as a marker for epithelial-mesenchymal transition.

The mesenchymal markers expressed in EMT is α-SMA, FSP1, vimentin, and desmin.[47] These vimentin positive cells express more at invasive front of primary tumors and eventually enter into invasion-metastasis cascade, i.e., intravasation, transport through the circulation, extravasation, formation of micrometastases, and finally colonization.[39,48,49]

Vimentin regulates EMT associated induced migration via upregulation of the expression of receptor tyrosine kinase Axl. Histological examination of breast carcinoma In a study they concluded that vimentin expression is predominantly found in high-grade ductal carcinomas with low estrogen receptor levels. It plays a key role in the regulation of *Axl* , Slug- and Ras-induced migration in breast cancer cells.[11]

MATERIAL AND METHODS

MATERIAL AND METHODS:

The study deals with the correlation of expression of vimentin and E cadherin with various histological parameters in breast carcinomas.

A total of 58 cases of breast carcinomas were included in the study. All cases of mastectomy received at department of Pathology, from the department of Surgery, Sri RL Jalappa Hospital and Research Centre attached to Sri Devaraj Urs Medical College and Research Centre, Tamaka, Kolar during the period of January, 2014 to July, 2015 were included in the study.

Also cases of breast cancers were retrieved from archives of department of Pathology from the year 2010 to 2013.

Prior to the study ethical clearance was obtained from the institutional ethical board.

Sample size:

Sample size estimated based on prevalence of breast cancer in Indian population of 18.5% with absolute error of 10% and Zα at 1.96. Sample size n=58

$n = (Z\alpha)2\ p\ q / (d)2$ $Z\alpha = 1.96$

p(prevalence)= 18.5%

q= 100-p : 100-18.5= 81.5

d(absolute error)= 10%

n = (1.96)2 x18.5x81.5/(10)2

n =58 at 95% confidence interval

INCLUSION CRITERIA:

Histologically proven carcinoma of breast were included in the study.

EXCLUSION CRITERIA:

1. Carcinoma of breast in male patients.
2. Sarcomas of breast.
3. Patients on or receiving radiotherapy, chemotherapy or recurrence.

METHOD OF COLLECTION OF DATA:

1. Mastectomy specimens received at department of Pathology, were analyzed and clinical data such as, name, age, history of present illness, personal history, family history, clinical examination and surgical details were obtained.
2. The specimens of mastectomy received were examined and the following details were noted- type of mastectomy, side (right/left), measurements, appearance of nipple areola complex, overlying skin and its measurements, tumor component, axillary tail were noted. The specimens were cut at intervals of 1cm and were allowed for fixation in 10% formalin. After adequate fixation, the tumor component was identified and details such as- size, shape, consistency, multicentricity, quadrant were noted. Axillary clearance received were also grossed and the total number of lymph nodes retrieved, was noted.

3. Tissue bits were taken from the representative areas, processed, blocked and sections taken.
4. Cases from the year 2010-2013 of carcinoma of breast were retrieved from the archives of department of Pathology and above said findings were noted.
5. All slides were reviewed and histological parameters such as, histopathological type, grading according to the modified Scarf- Bloom Richardson grading system, vascular invasion, skin involvement, lymph node status were noted. Tumor stage was assessed. Nottingham prognostic index was calculated for all the cases.
6. Additional sections of 4um thickness were cut from these paraffin blocks and subjected to staining with vimentin and E cadherin according to the standard protocols.

Bloom Richardson grading system:

It consists of the following parameters:

1. **Tubule formation**

   All sections with tumor were scanned and the percentage of the tumor showing tubular structures was assessed .Only tubules with clear lumina were considered in the grading analysis. Artifacts such as clefts formed due to shrinkage artifact were not taken into consideration. Tubule formation was scored 1 to 3 by taking 10 % to 75% as cut off points. Score 1 was assigned when tubule formation were more than 75% , Score 2 when tubule formation was between 10 to 75% and Score 3 when tubule formation was less than 10%.

2. **Nuclear pleomorphism**

Assessment was done based on variation in nuclear size, its chromatin pattern and nucleoli. When the nuclei was small with little increase in size, regular outline and uniform chromatin a score of 1 was assigned .When the cells appeared larger than normal with vesicular nuclei and visible nucleoli , with moderate variability in size and shape , a score of 2 was given. A marked variation in size and shape, especially when there were large bizarre nuclei with presence of prominent nucleoli, a score of 3 was given.

3. **Mitotic counts**

This feature was best assessed in invasive front of tumor. A minimum of ten fields was assessed. Hyperchromatic and pyknotic cells were excluded as they represent apoptosis and not proliferation.

Immunohistochemistry Techniques:

The immunohistochemistry (IHC) was performed on 4-μm thick sections from 10% formalin-fixed paraffin-embedded tissues, according to peroxidase –anti peroxidase method.

The details of the immunohistochemical markers used in the study are as follows:

Table 4: Vimentin

Antigen	Clone	Species	Producer	Dilution	Control	Stain
Vimentin	V9	Mouse	Biogenex	Prediluted	Fibroblast, endothelail cells	Cytoplasmic

Table 5: E cadherin

Antigen	Clone	Species	Producer	Dilution	Control	Stain
E cadherin	36	Mouse	Biogenex	Prediluted	Normal skin	Membranous

IHC Procedure:

- Sections are 3-4mm thickness, floated on to organosialine coated slide and left on hot plate at 60^{o} overnight.
- **Deparaffinization** using Xylene I and II—15 min each
- **Dexylinisation** using absolute alcohol I and II—1 min each
- **Dealcoholisation** using 90% and 70% alcohol—1 min each
- Tap water – 10 min wash.
- Distilled water- 5 min rinsing.
- **Antigen Retrieval technique:** Microwave at power 10 for 6 minutes in TRIS EDTA buffer of pH-9.0 for 2 cycles.

- Transfer to TBS (Tris buffer solution pH- 7.6) - 5minutes x 2 times-wash.
- **Peroxidase block**- 30 minutes to block endogenous peroxidase enzyme.

- TBS buffer for 5 minutes washing for 3 times.
- **Power block**- 30 minutes to block non- specific reaction with other tissue antigen.

- Drain and cover sections with targeted antibody (primary)- 1hr

- TBS buffer- 5min x 3Times.

- **Super Enhancer**- 30 minutes to enhance the reaction between primary and secondary antibodies.

- TBS buffer- 5min x 3 times

- Super sensitive poly- HRP (secondary antibody)- 30 min

- TBS buffer- 5min x 3 times

- Color development with working color development solution (DAB) - 5-8 min

- TBS wash- 5min x 3 times

- Counter stain with Haematoxylin – 1 min.

- Tap water wash for 5 minutes.

- Dehydrate, clear and mount

- Mount with DPX.

Immunohistochemical analysis and counting:

Sections were first examined at low magnification (x40 and x100 magnification) using (Olympus CX 21i) microscope to identify areas of highest positivity (hotspot).
Areas of hotspots which had positivity of tumor cells were selected. Individual cells were counted under x 200 magnification and then average was taken.

1. **Vimentin expression** [14]
    - Vimentin showed cytoplasmic staining of tumor cells.
    - Fibroblasts, endothelial cells adipocytes were taken as internal controls.
    - 500 tumor cells in the area of hotspots were counted and the total number of positive cell expressed were counted and percentage was calculated.
    - **Immunoscore = % of positive cells x staining intensity** [no staining (0), weak (1+), moderate (2+), strong (3+)].
    - A score of more than 30 was considered significant in the study.

2. **E cadherin expression** [50]

- E-cadherin antibody stained the membrane intensely and the cytoplasm of cancer cells weakly.
- Normal skin sections were taken as positive controls.
- E-cadherin expression was semi-quantitatively analyzed according to the percentage of cells showing membrane positivity:
- 0= 0
- 1+ = 1–10%
- 2+ = 11–50%
- 3+ = 51–80%
- 4+ = >80%.

✓ Staining intensity-no staining (0), weak (1+), moderate (2+), strong (3+).

✓ Immunoscore= Staining intensity x % positivity of tumor cells.

✓ E-cadherin expression was considered positive when scores were >2, and negative when scores were <1

✓ A case with cytoplasmic staining only was determined as E-cadherin negative.

STATISTICAL ANALYSIS:

The data was entered into Microsoft excel data sheet. Analysis was done using SPSS 11(Statistical package for social sciences version 11), USA. Chi square test was the test of significance used to find the association between the various histological parameters and the expression of vimentin and E cadherin. p- value <0.05 was considered statistically significant.

RESULTS

RESULTS

The study period was done from January 2014 to July 2015, for a period of 18 months. All cases which satisfied the inclusion criteria were included in the present study. A total of 58 cases were studied.

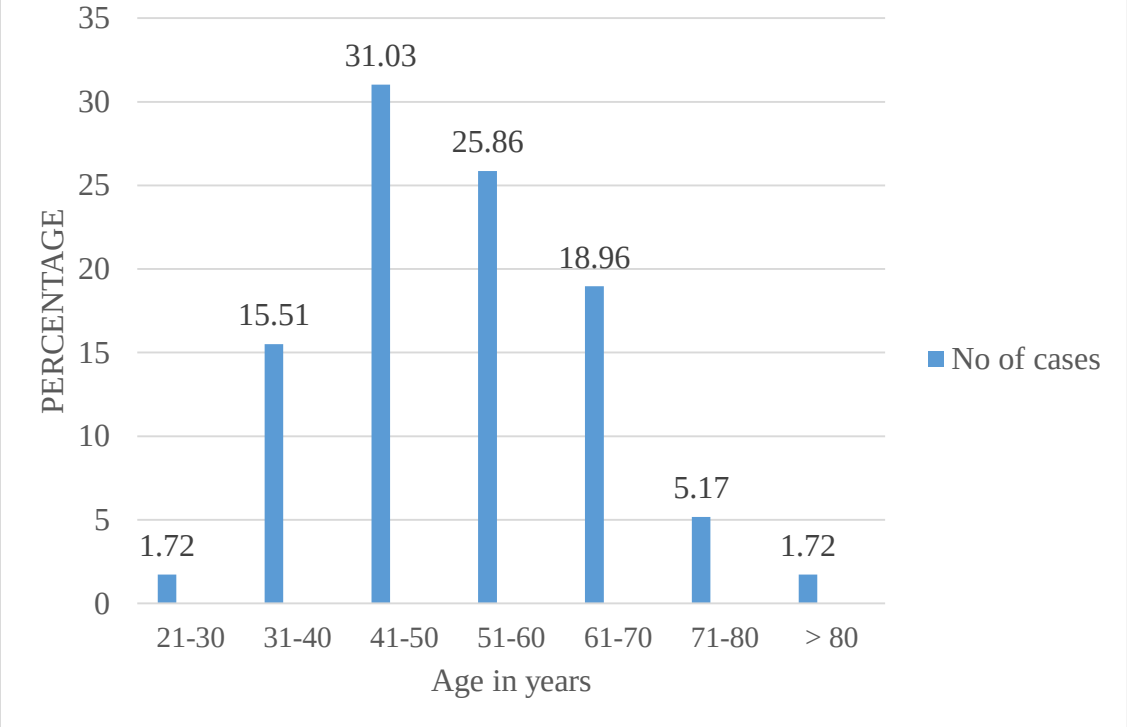
Age Distribution:

In the present study, age group ranged from 23 to 85 years with mean age of 52.3 years. Majority of the patient belonged to 41- 50 years which consisted of 18 cases (31.03%) , followed by 51-60 years constituting 15 cases (25.86 %), 11 cases (18.96%) belonged to 61-70 years age group , 9 cases (15.51%) belonged to 31-40 years age group , 3 cases (5.17%) belonged to 71-80 years age group, 1case(1.72%) was above 80 years age group, and 1 case (1.72%) belonged to 21-30 years age group.

Table 6: Age distribution of the cases

Age (in years)	No. of cases	Percentage (%)
21-30	1	1.72
31-40	9	15.51
41-50	18	31.03
51-60	15	25.86
61-70	11	18.96
71-80	3	5.17
>80	1	1.72
Total	**58**	**100.0**

Chart 1: Case distribution according to age.



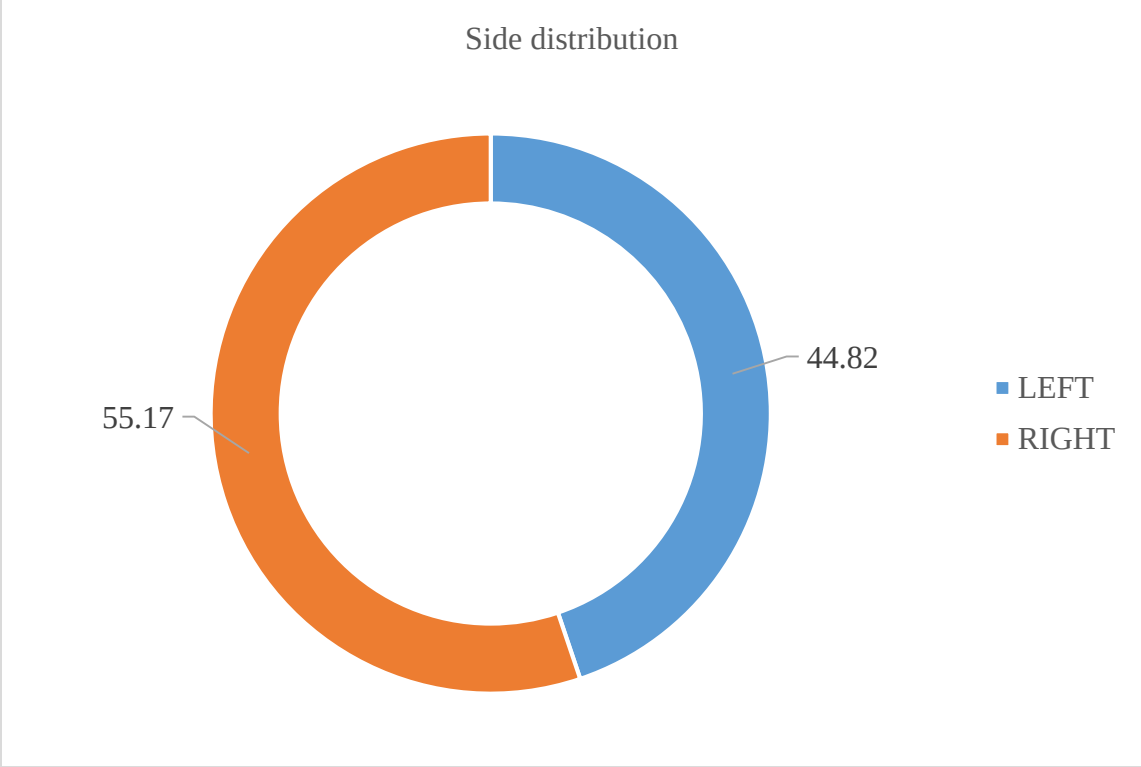
Side of the tumor:

In the present study, tumor was seen more on right breast consisting of 32 cases (55.17%) compared to left breast 26 (44.82%).

Table 7: Distribution of side involved.

Side	No. of cases	Percentage (%)
Left	26	44.82
Right	32	55.17
Total	**58**	**100**

Chart 2: Distribution of side involved



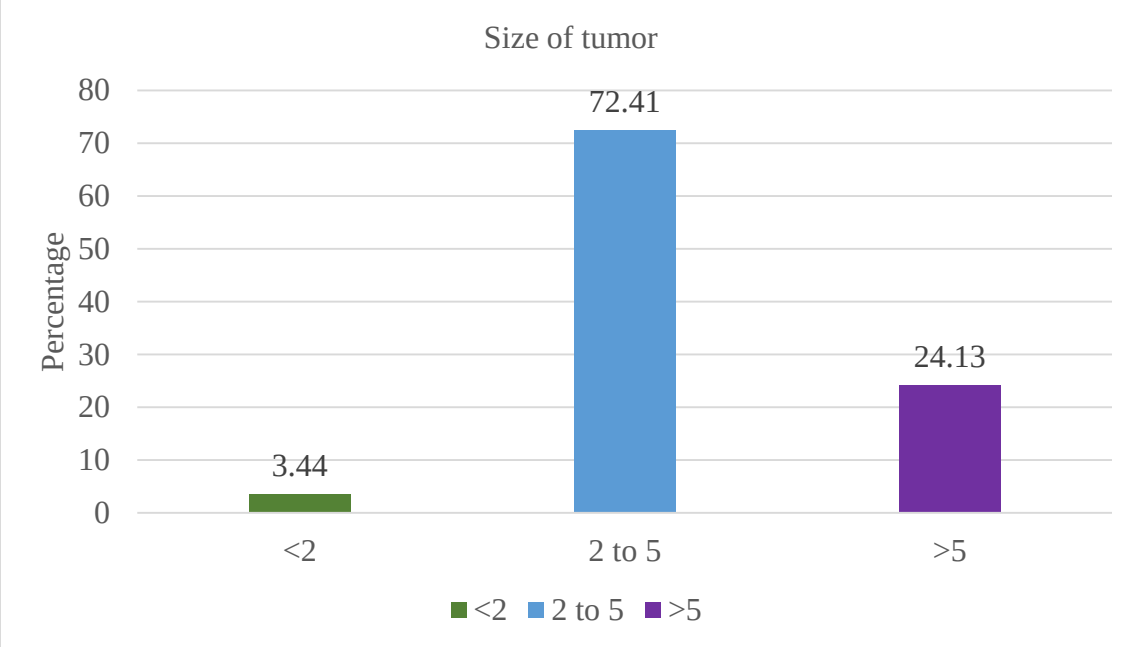
Tumor Size

In the present study, majority of tumor size ranged between 2-5cm, constituting 42 cases (72.41%), 14 patients (24.13%) had tumor size of >5cm and 2 patients (3.44%) of cases had tumor size of <2cm.

Table 8: Distribution of cases with regard to Tumor Size.

Tumour size (in cm)	No. of cases	Percentage (%)
<2	02	3.44
2-5	42	72.41
>5	14	24.13
Total	**58**	**100**

Chart 3: Tumor Size distribution.



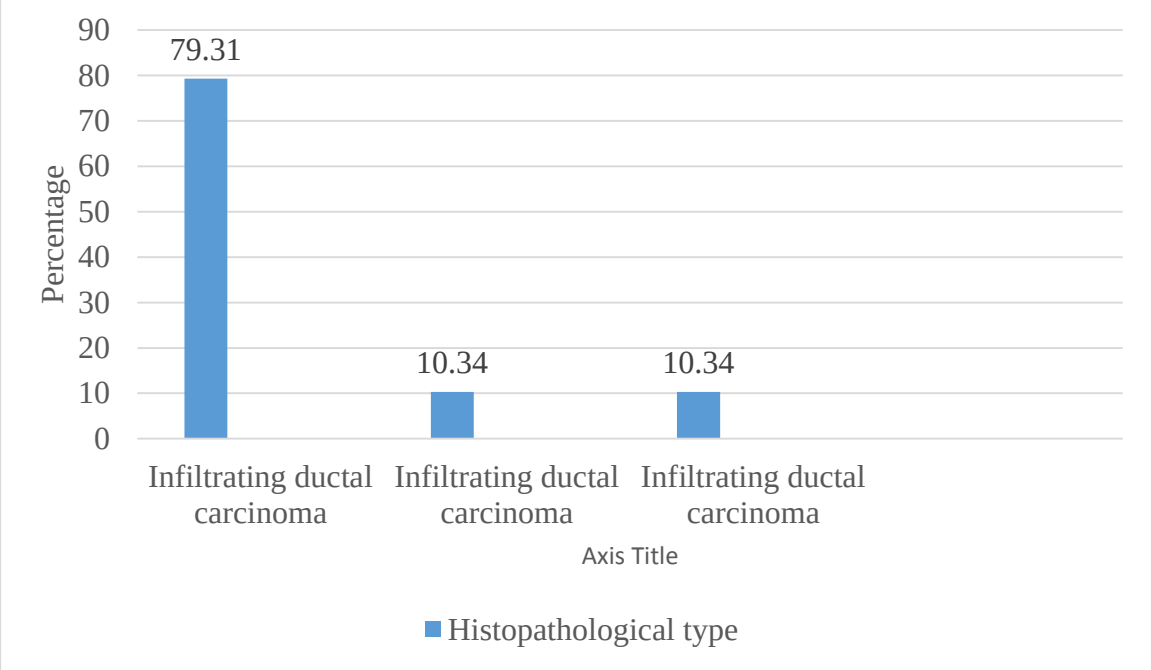
Histopathological Type:

In the present study, Infiltrating ductal carcinoma was seen in majority of our cases comprising of 46 (79.31 %), followed by invasive lobular carcinoma 6 (10.34%) cases and 6 (10.34%) of medullary carcinomas.

Table 9: Distribution of case according to Histological Type.

Histopathology	No. of cases	Percentage (%)
Infiltrating ductal carcinoma(NOS)	46	79.31
Medullary Carcinoma breast	6	10.34
Invasive lobular carcinoma	6	10.34
Total	**58**	**100**

Chart 4: Histopathological types distribution.



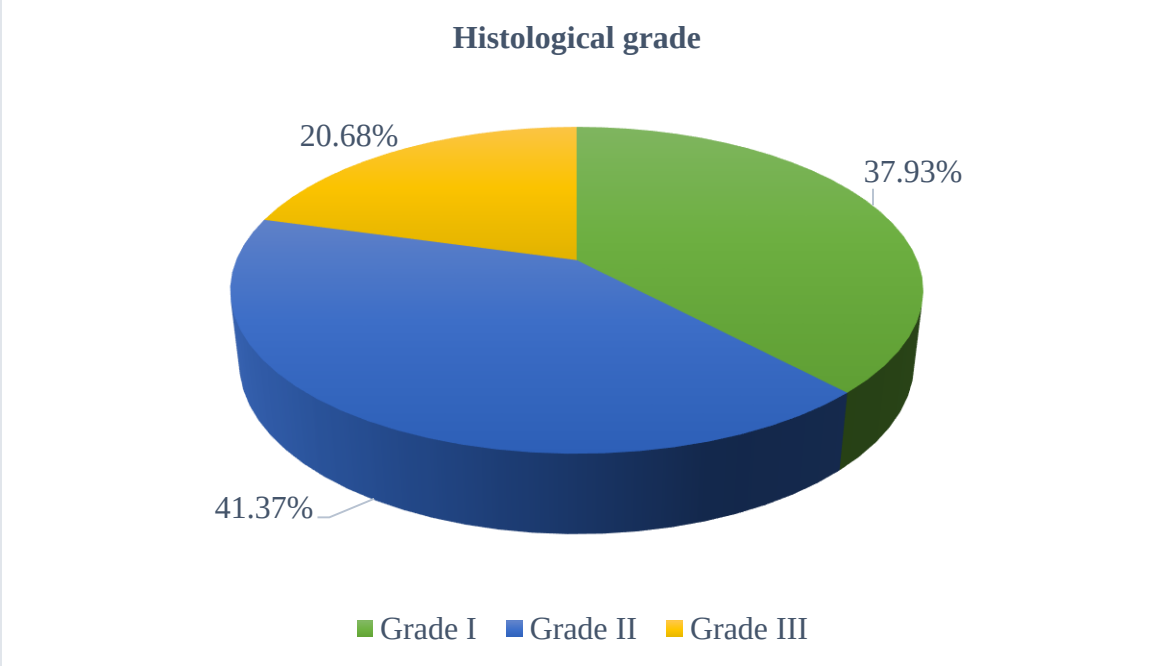
Histological Grade:

In this study, grading was assessed on histology by Modified Bloom Richardson grading system. We found majority of the tumors were grade II tumors constituting 24 (41.37%), followed by grade I tumors 22 (37.93%) and 12 (20.68%) belonged to grade III tumors.

Table 10: Distribution of cases with regard to grade.

Grade	No. of cases	Percentage (%)
I	22	37.93
II	24	41.37
III	12	20.68
Total	58	100

Chart 5: Histological grade distribution.



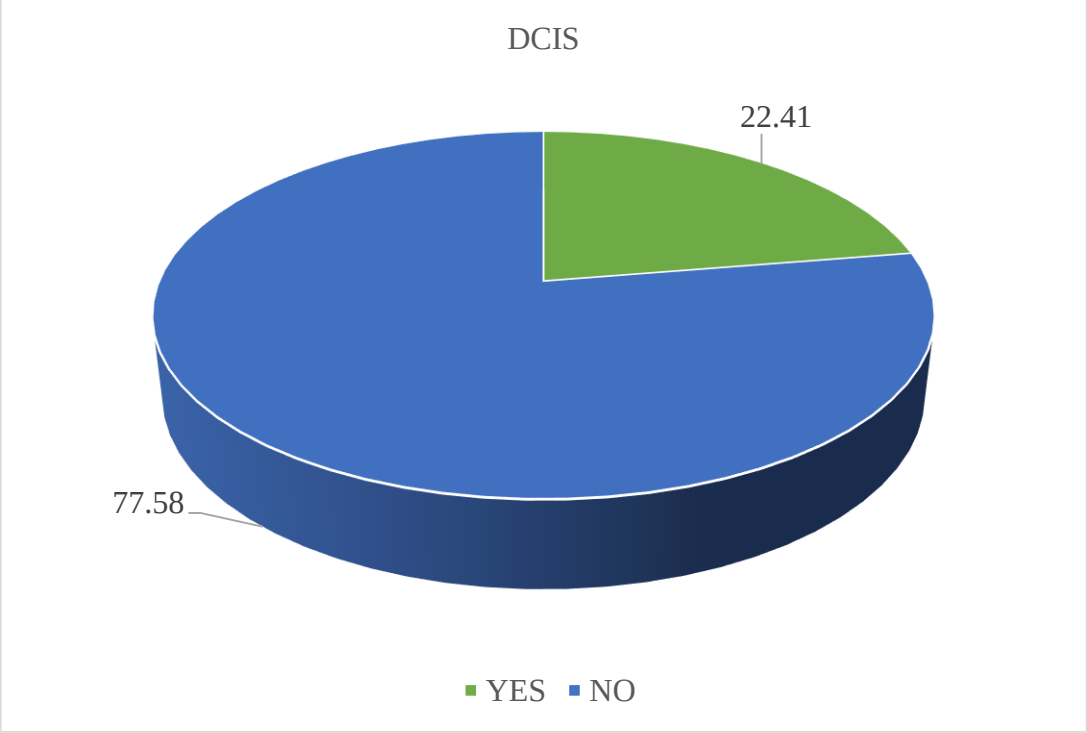
DCIS Association:

In present study, DCIS component was seen in 13 (22.41%) of the cases.

Table 11: Distribution of cases based on associated DCIS.

DCIS association	No. of cases	Percentage (%)
Yes	13	22.41
No	45	77.58
Total	**58**	**100**

Chart 6: Presence of DCIS association.



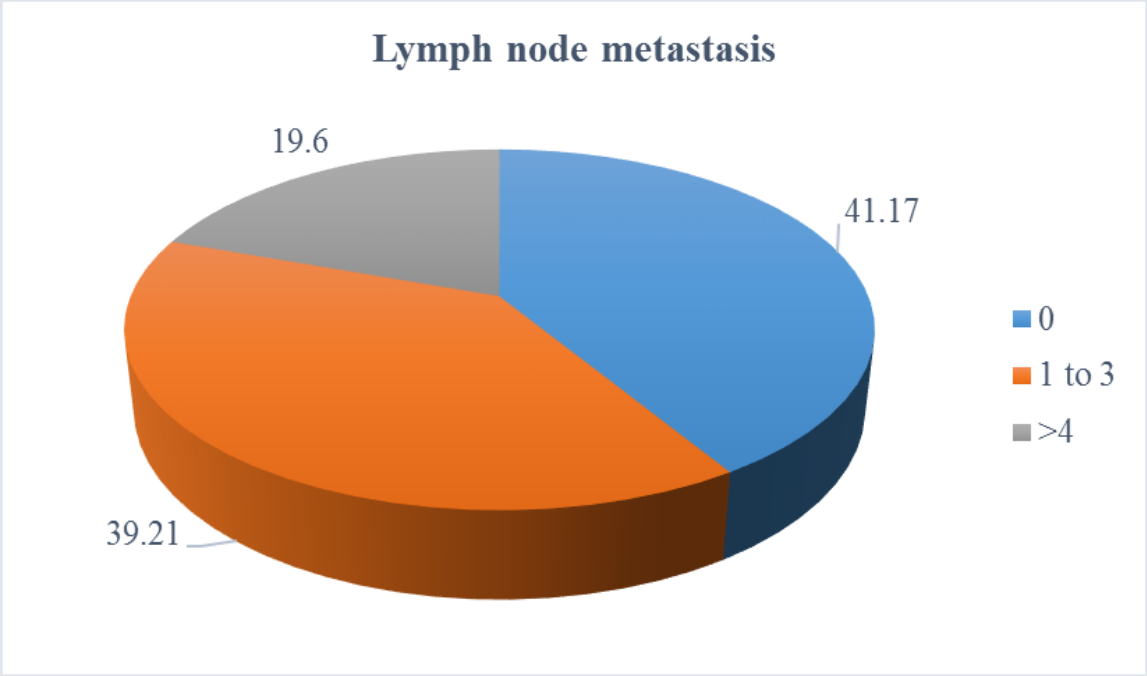
Lymph node Involvement:

In our study out of 58 cases, MRM with axillary clearance was done in 51 cases. Lymph node metastasis was seen in 30 cases. 20 (39.21%) cases had lymph node metastasis in 1-3 lymph nodes, 10 (19.60%) cases had lymph node metastasis in more than 4 lymph nodes. 21 (41.17%) cases didn't show lymph node metastasis.

Table 12: Distribution of cases based on lymph node status.

Lymph node	No. of cases	Percentage (%)
0	21	41.17
1-3	20	39.21
≥4	10	19.60
Total	51	100

Chart 7: Lymph node status distribution.



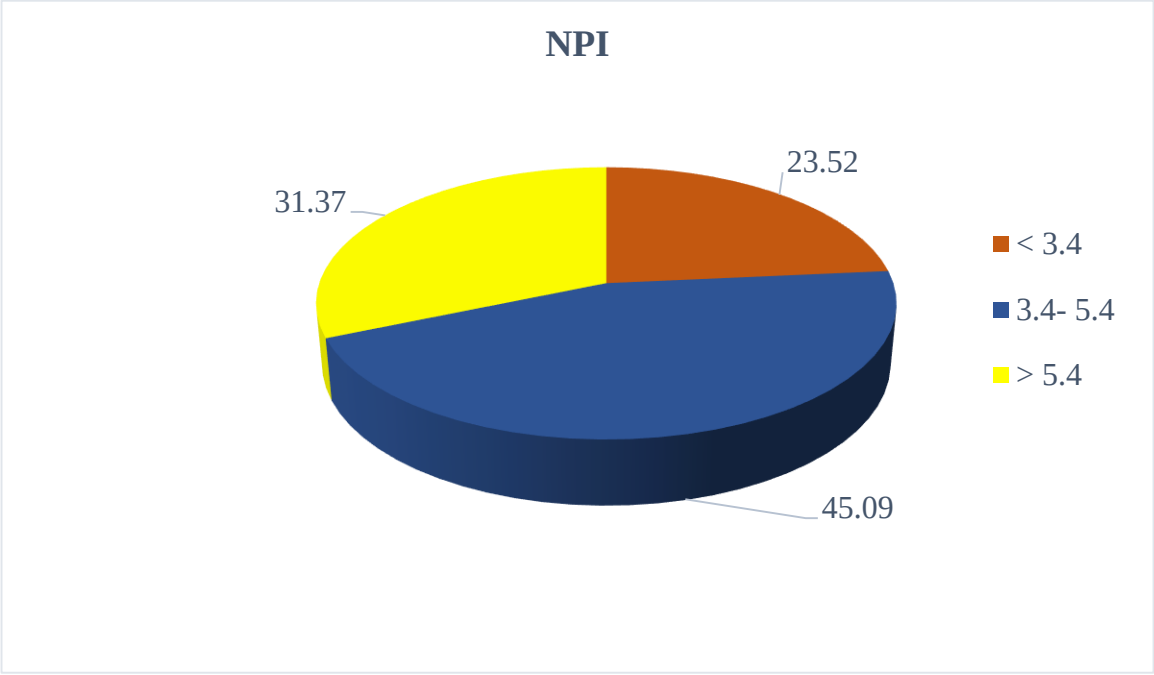
Nottingham prognostic index (NPI)

In our study, according to Nottingham prognostic score, majority of tumors belonged to score of 3.4- 5.4, that is 23 (45.09%) cases. 16 (31.37 %) cases belonged to score of more than 5.4, and 12 (23.52%) cases to score of less than 3.4

Table 13: Distribution of cases based on NPI score.

NPI	No. of cases	Percentage (%)
< 3.4	12	23.52
3.4- 5.4	23	45.09
>5.4	16	31.37
Total	**51**	**100**

Chart 8: Nottingham prognostic index distribution.



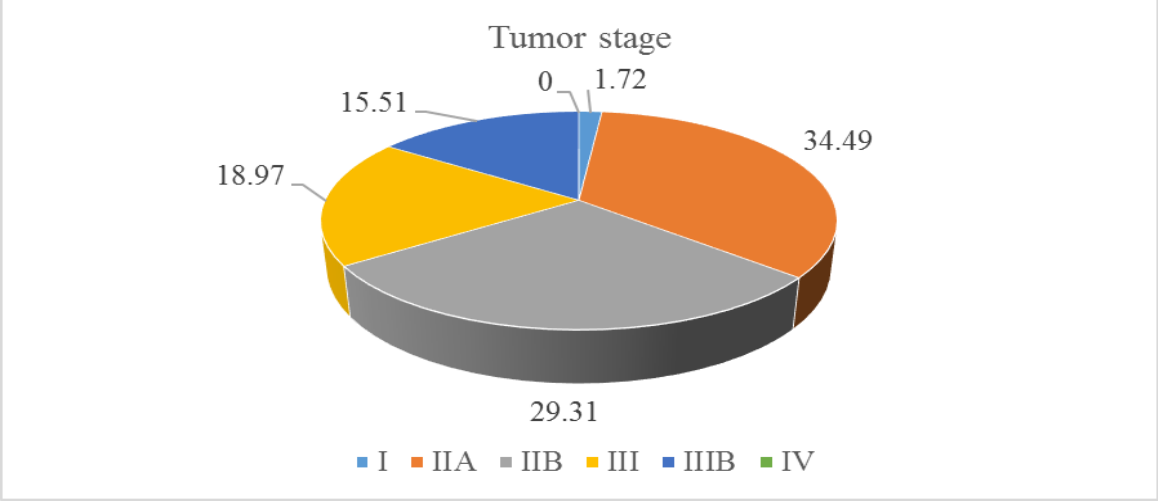
TUMOR STAGE

In this study, majority of tumors belonged to stage IIA (20 cases, 34.49%), followed by stage IIB (17 cases, 29.31%), stage III (11 cases, 18.97%), stage IIIB (9 cases, 15.51%) and 1 tumor belonged to stage I (1.72%).

Table 14: Distribution of cases based on tumor stage.

Tumor stage	No. of cases	Percentage (%)
I	01	1.72
IIA	20	34.49
IIB	17	29.31
III	11	18.97
IIIB	09	15.51
IV	0	-
Total	**58**	**100**

Chart 9: Tumor stage distribution.



Skin involvement

In our study, skin involvement was noted in 9 (15.51%) cases.

Table 15: Distribution of cases based on skin involvement.

Skin involvement	No. of cases	Percentage (%)
Yes	9	15.51
No	49	84.48
Total	**58**	**100**

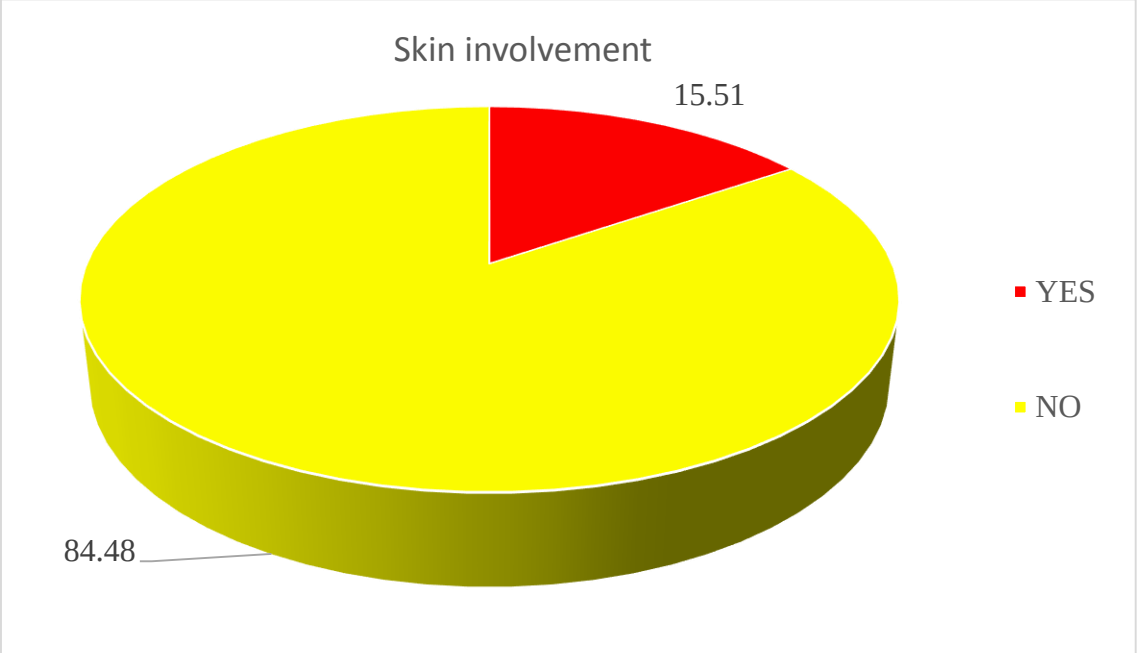


Chart 10: Skin involvement.

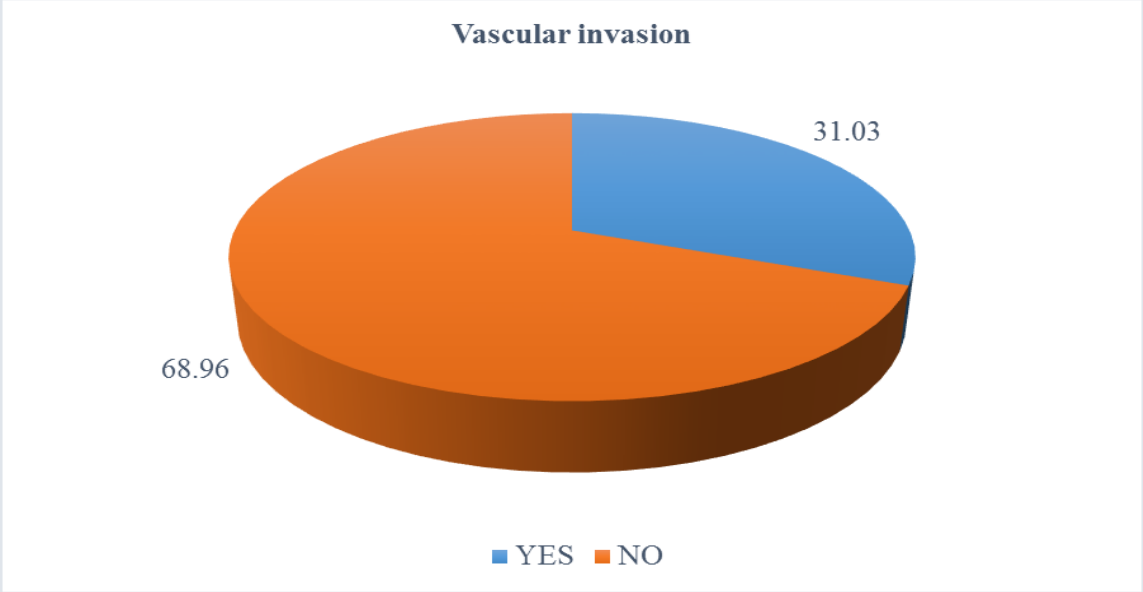
Vascular Invasion:

In present study vascular invasion was seen in 18 (31.03%) of the cases. Majority cases with vascular invasion was seen in IDC, constituting 14 cases, followed by 4 cases in medullary carcinoma. None of the ILC showed vascular invasion.

Table 16: Distribution of cases based on vascular invasion

Vascular invasion	No. of cases	Percentage (%)
Yes	18	31.03
No	40	68.96
Total	**58**	**100**

Chart 11: Vascular invasion.



Vimentin expression

Vimentin expression was noticed in 18 cases (31.03%) of the total 58 breast cancers. A score of more than 30 was considered as positive.

Table 17: Vimentin expression in breast cancer.

Vimentin expression	No of cases	Percentage
Positive (score= >30)	18	31.03
Negative	40	68.96
Total	**58**	**100**

Chart 12: Vimentin expression in breast cancer.

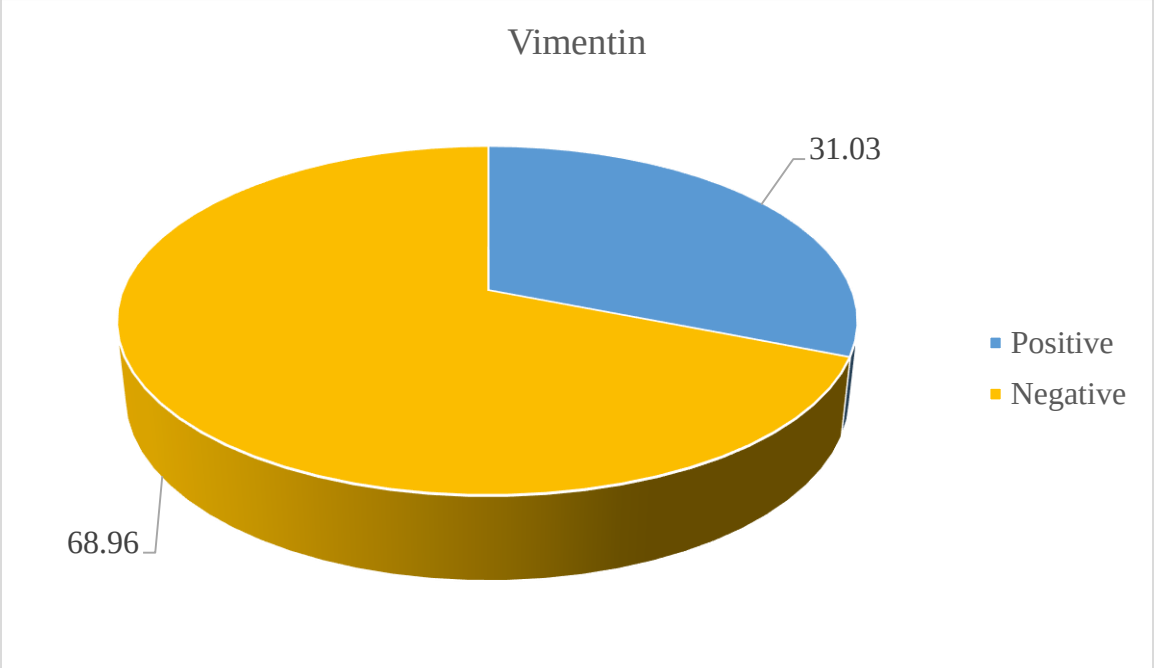


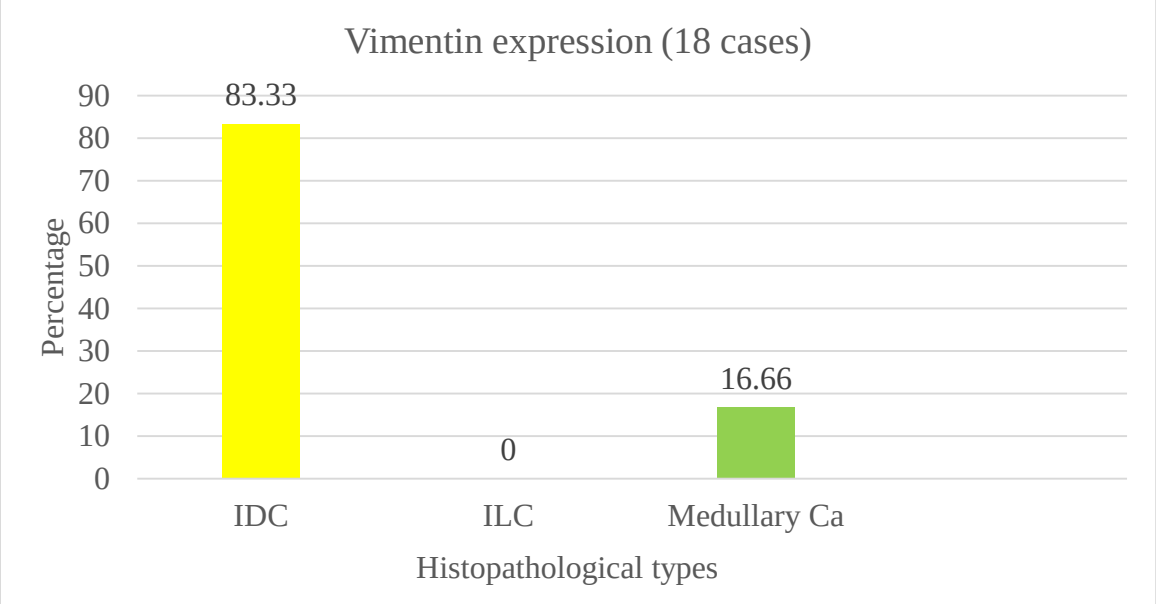
Table 18: Vimentin expression in histopathological types.

Histopathological types	No of cases	Percentage
IDC	15	83.33
ILC	0	-
Medullary carcinoma	03	16.66
Total	**18**	**100**

Majority of vimentin positivity was seen in IDC, constituting 15/18 cases (83.33%). Out of 6 cases of medullary carcinoma, 3/18 cases (16.66%) showed positive vimentin expression. None of the ILC cases (0/6) showed vimentin expression.

Out of 46 cases of IDC, 15 (32.6%) cases showed vimentin positivity. 50% (3/6) cases of medullary carcinoma showed vimentin expression.

Chart 13: Vimentin expression in histopathological types.



E cadherin expression

Among the total 58 cases studied, which included IDC, ILC and medullary carcinoma, 36 cases (62.06%) showed moderate or strong (score 2, 3 respectively) E cadherin expression. Remaining 22 cases (37.93%) showed weak to absent (score 1, 0 respectively) E cadherin expression.

Table 19: E cadherin expression in breast cancer.

E cadherin expression	No of cases	Percentage
Positive (Score= 2 ,3)	36	62.06
Negative Score= 0,1)	22	37.93
Total	**58**	**100**

Chart 14: E cadherin expression in breast cancer.

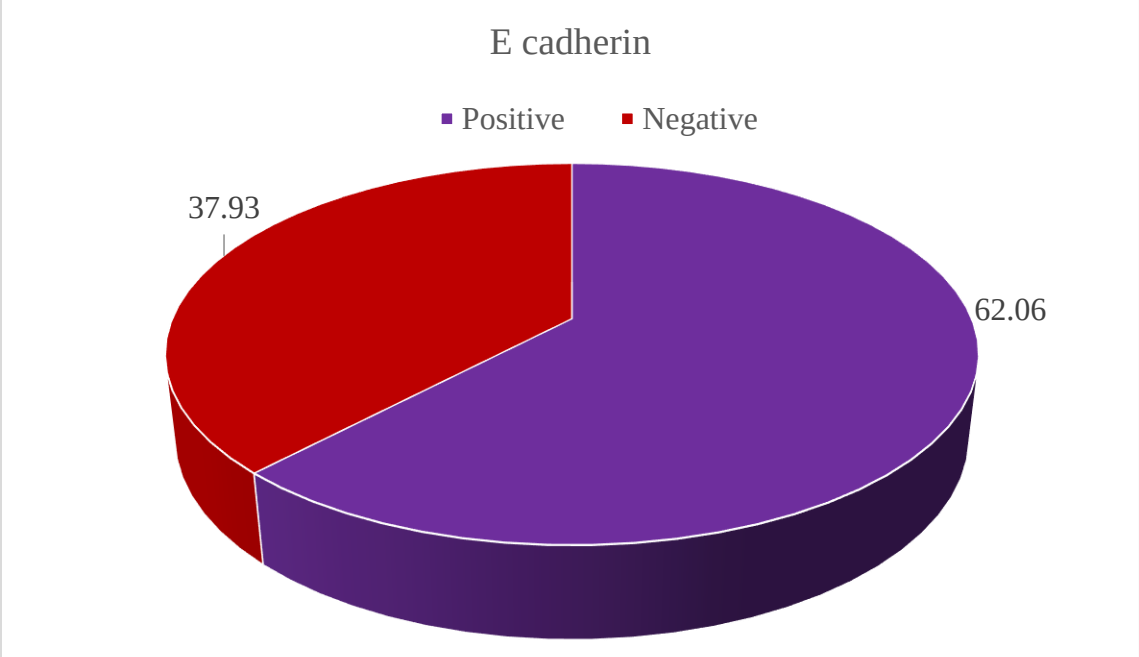
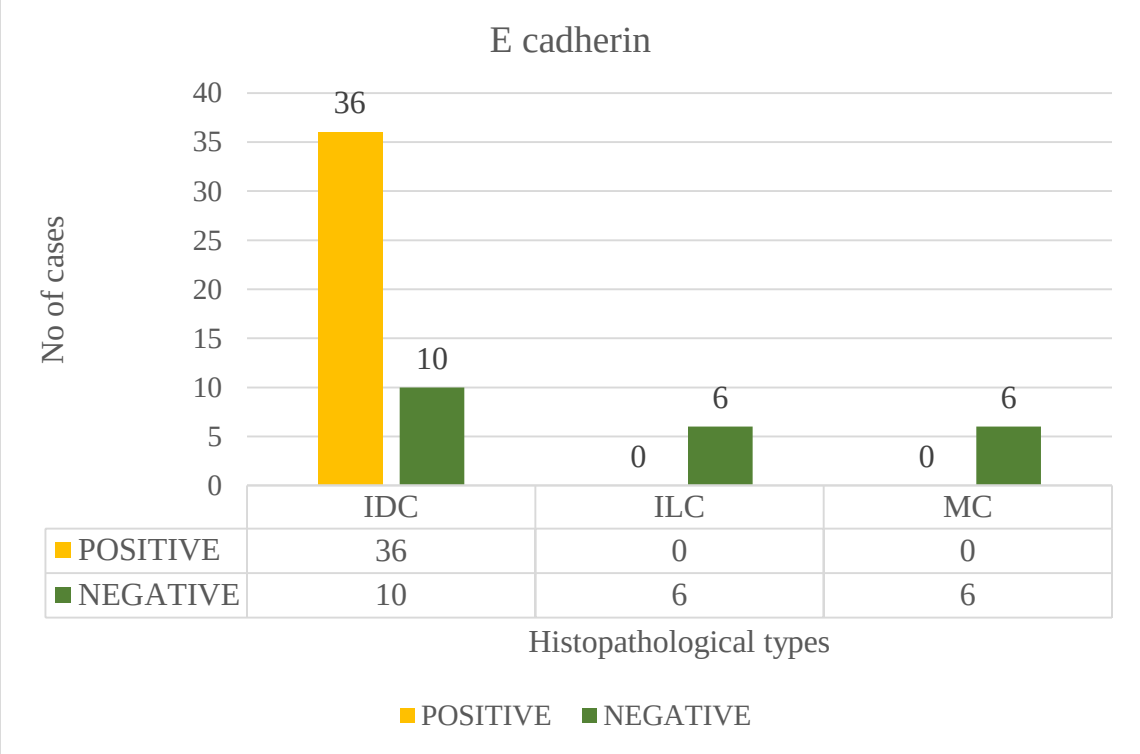


Table 20: E cadherin expression in histopathological types

Histopathological types	No of cases	E cadherin	
		Positive (score 2 or 3)	Negative (score 0 or 1)
IDC	46	36	10
ILC	06	0	06
Medullary carcinoma	06	0	06
Total	**58**	**36**	**22**

Out of 46 cases of IDC, 36 cases (78.26%) showed positive E cadherin expression (score of 2 or 3). Remaining 10 cases of IDC (21.73%) showed weak (score of 1) E cadherin expression. Both ILC and medullary carcinoma cases showed score of 0.

Chart 15: E cadherin in histopathological types.



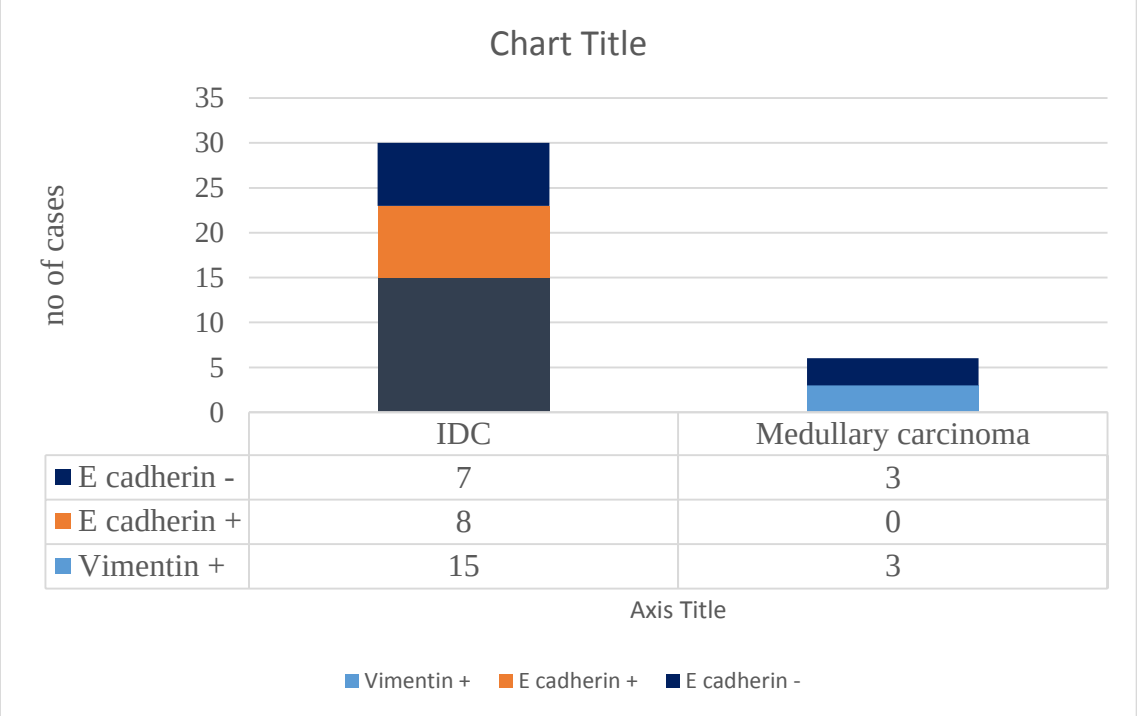
Comparison of vimentin positive cases and E cadherin in histopathological types:

Among the 15 vimentin positive IDC cases, 8 cases showed moderate to strong E cadherin expression and 7 cases showed weak E cadherin expression**.** In medullary carcinoma cases, 0/3 vimentin positive cases showed no E cadherin expression. ILC is not included here, as none of the cases (0/6) showed any vimentin expression.

Table 21: Distribution of vimentin positivity and E cadherin expression.

Histopathological type	Vimentin Positive	E cadherin	
		Positive	Negative
IDC	15	8	7
Medullary carcinoma	3	0	3

Chart 16: Histopathological types and IHC markers.



RESULTS OF INDIVIDUAL TUMOR TYPES:

1. Infiltrating ductal carcinoma:

a. Tumor grade in IDC

46 cases of IDC were included in this study. Majority of the IDC cases belonged to grade II, constituting 24 cases (52.17%), followed by 16 cases (34.78%) which belonged to grade I and 6 cases (13.04%) belonged to grade III.

Table 22: Distribution of tumor grade in IDC cases.

Tumor grade	No of cases (46)	Percentage
I	16	34.78
II	24	52.17
III	06	13.04
Total	**46**	**100**

Out of 46 cases of IDC, E cadherin was positive in 36 cases (78.27%) (score 2,3) and negative in 10 cases (21.73%) (score 1). Among the 36 E cadherin positive cases, 14 cases belonged to grade I, 18 cases belonged to grade II, and only 4 cases belonged to grade III. Majority of tumors belonging to low grade showed E cadherin expression.

Among the 10 E cadherin negative cases, 6 cases belonged to grade II, 2 cases each to grade III and grade I, thus E cadherin expression diminishes as the grade increases.

Among the 46 IDC cases, 31 cases (67.4%) were negative for vimentin positivity. Among the negative cases, 15 cases belonged to grade I, 15 cases belonged to grade II and only 1

case belonged to grade III. This showing that vimentin is usually not expressed in low grade tumors.

Out of 15/46 cases (32.6%) which showed positive vimentin expression (score >30), majority of the tumors belonged to grade II, constituting 9 cases (60%), followed by grade III, constituting 5 cases (33.33%) and only 1 case (6.66%) belonged to grade I. Therefore vimentin expression increases with the increase in grade of tumors.

Comparison of vimentin and E cadherin expression in IDC with regards to grade:

Table 23: Vimentin positive cases and E cadherin status with regards to grade.

Tumor grade	**Vimentin Positive**	**E cadherin**	
		Positive	**Negative**
	15	8	7
I	1	1	0
II	9	4	5
III	5	3	2

Grade I tumors constituted 1 case which was Vim +/ Ec +, grade II constituted 9 cases, which showed 4 Vim+/Ec + , 5 Vim+/Ec – and grade III constituted 5 cases, which showed 3 Vim+/Ec+ , 2 Vim+/Ec- .

b. Tumor size in IDC

Majority of the IDC tumor size ranged between 2 and 5 cm, constituting 32 cases (69.57%), 12 cases (26.08%) with tumor size above 5 cm and 2 cases (4.35%) with size < 2 cm.

Table 24: Distribution of tumor size in IDC cases.

Tumor size	No of cases (46)	Percentage
<2	02	4.35
2- 5	32	69.57
>5	12	26.08
Total	**46**	**100**

Comparison of vimentin and E cadherin in IDC with regards to tumor size:

Table 25: Vimentin and E cadherin with regards to size.

Tumor size (cm)	Vimentin Positive (n)	E cadherin	
		Positive(n)	Negative(n)
	15	8	7
<2	2	1	1
2- 5	9	5	4
>5	4	2	2

9 tumors had size between 2-5 cm which showed 5 Vm +/ Ec + and 4 Vm+/Ec- , tumors with size >5 cm constituted 4 cases, which showed 2 Vm+/Ec + , 2 Vm+/Ec – , and finally tumors with size <2cm constituted 2 cases, which showed 1 Vm+/Ec+ , 1 Vm+/Ec-.

c. Lymph node status in IDC

41/46 cases of IDC underwent MRM with axillary clearance. 26 cases of IDC showed axillary lymph node metastasis, with majority showing metastasis in 1- 3 lymph nodes, constituting 17 cases (41.46%), 9 cases (21.95%) in >4 lymph nodes. However, 15 cases (36.59%) did not have lymph node metastasis.

Table 26: Distribution of lymph node status in IDC cases.

Lymph node	No of cases (41)	Percentage
0	15	36.59
1-3	17	41.46
>4	09	21.95
Total	**41**	**100**

Comparison of vimentin and E cadherin in IDC with regards to lymph node:

14 cases were considered which underwent MRM. 5 tumors had axillary lymph node metastasis in 1-3 lymph nodes, which were 3 Vm +/ Ec + and 2 Vm+/Ec- , 2 cases had >4 lymph node metastasis, which were 2 Vm+/Ec-, and 7 cases did not have lymph node metastasis, which were 5 Vm+/ Ec +, 2 Vm+/Ec-.

Table 27: Vimentin and E cadherin with regards to lymph node status.

Lymph node status	Vimentin Positive (n)	E cadherin	
		Positive(n)	Negative(n)
	14	8	6
0	7	5	2
1-3	5	3	2
>4	2	0	2

d. NPI score

NPI score was calculated in 41/46 cases of IDC. Majority of the IDC cases had a NPI score of 3.4-5.4, accounting to 17 cases (41.47%), 14 cases (34.15%) had a NPI score of >5.4

Table 28: Distribution of NPI score in IDC cases.

NPI	No of cases (41)	Percentage
<3.4	10	24.4
3.4- 5.4	17	41.47
>5.4	14	34.15
Total	**41**	**100**

Comparison of vimentin and E cadherin expression in IDC with regards to NPI score.

6 cases (42.85%) with vimentin positivity had a NPI score between 3.4-5.4, which were 4 Vm +/ Ec + and 2 Vm+/Ec- , 5 cases (35.71%) had NPI score >5.4, 2 Vm+/Ec+, 3 Vm+/Ec- and 3 cases (21.42%) had a NPI score of <3.4, 2 Vm+/ Ec +, 1 Vm+/Ec-.

Table 29: Vimentin and E cadherin with regards to NPI.

NPI	Vimentin Positive (n)	E cadherin	
		Positive(n)	Negative(n)
	14	8	6
<3.4	3	2	1
3.4-5.4	6	4	2
>5.4	5	2	3

e. Tumor stage

Majority of the IDC cases belonged to stage IIA, (15 cases, 32.6%), followed by IIB (13 cases, 28.27%), stage III (10 cases, 21.73%), stage IIIB (7 cases, 15.21%).

Table 30: Distribution of tumor stage in IDC cases.

Tumor Stage	No of cases (46)	Percentage
I	01	2.17
IIA	15	32.6
IIB	13	28.27
III	10	21.73
IIIB	07	15.21
IV	0	-
Total	**46**	**100**

Majority of vimentin positivity was seen in stage IIA tumors- 7 cases (46.66%), 4 cases (26.66%) in stage IIIB, 3 cases (20%) in stage IIB,1 case (6.66%) in stage I.

Comparison of vimentin and E cadherin in IDC with regards to tumor stage.

Table 31: Vimentin and E cadherin with regards to stage.

Tumor Stage	No of cases (15)	E cadherin	
		Positive	Negative
I	1	0	1
IIA	7	5	2
IIB	3	1	2
III	0	0	0
IIIB	4	2	2
IV	0	0	0
Total	**15**	**8**	**7**

7 vimentin positive cases belonged to stage IIA which were 5 Vm+/Ec+, 2Vm+/Ec-, 4 cases belonged to stage IIIB which were 2 Vm+/Ec+ , 2 Vm+/Ec-, 3 cases belonged to stage IIB which were 1Vm+/Ec+, 2Vm+/Ec- and 1 case belonged to stage I which showed Vm+/Ec-

f. Vascular invasion

Vascular invasion was noted in 18/ 58 cases. Among these, vimentin was positive in 7 cases and negative in 11 cases. E cadherin expression was seen in 11 cases and loss in 7 cases.

In IDC, 14/ 46 cases (30.43%) showed vascular invasion. Remaining 4 cases of medullary carcinoma showed vascular invasion.

Among the vimentin positive IDC cases, vascular invasion was noted in total 4/15 cases (26.66%) and were 2 Vm+/Ec + and 2 Vm+/Ec-.

Table 32: Distribution of vimentin positive tumors with vascular invasion.

Vascular invasion	Vimentin + (15)	Percentage
Yes	4	26.66
No	11	73.33

g. Skin involvement

7/46 cases of IDC showed skin involvement.

Skin involvement was noted in total 4 cases (26.66%) of 15 IDC vimentin positive cases. Among these, 2 cases expressed Vm+/Ec+ and 2 cases expressed Vm+/ Ec-.

Table 33: Distribution of vimentin positive tumors with skin involvement.

Skin involvement	Vimentin + (15)	Percentage
Yes	4	26.66
No	11	73.33

II. Invasive lobular carcinoma

Table 34: Distribution of clinicopathological parameters, vimentin, E cadherin in ILC

Parameters	No of cases	Percentage	Vimentin + (n)	E cadherin	
				Positive(n)	Negative(n)
Tumor size(n=6)					
<2	0	0	-	-	0
2-5	6	100	-	-	6
>5	0	0	-	-	0
Tumor grade(n=6)					
I	6	100	-	-	6
II	0	0	-	-	0
III	0	0	-	-	0
Lymph node(n=5)					
0	2	40	-	-	2
1-3	2	40	-	-	2
>4	1	20	-	-	1
NPI (n=5)					
<3.4	2	40	-	-	2
3.4-5.4	3	60	-	-	3
>5.4	0	0	-	-	0
Tumor stage (n=6)					
IIA	3	50	-	-	3
IIB	2	33.3	-	-	2
III	1	16.67	-	-	1
Vascular involvement					
Yes	0	0	-	-	0
No	6	100	-	-	6
Skin involvement					
Yes	0	0	-	-	0
No	6	100	-	-	6

In this study, we did not get any vimentin positivity cases (0/6) in invasive lobular carcinomas. E cadherin expression was also negative in all the six cases.

III. Medullary carcinoma

Table 35: Distribution of clinicopathological parameters, vimentin, E cadherin in MC

Parameters	No of cases	Percentage	Vimentin + (n=3)	E cadherin (n=6)	
				Positive(n)	Negative(n)
Tumor size(n=6)					
<2	0	-	0	-	-
2-5	4	66.67	2	-	4
>5	2	33.3	1	-	2
Tumor grade(n=6)					
I	0	0	0	-	0
II	0	0	0	-	0
III	6	100	3	-	6
Lymph node(n=5)					
0	4	80	2	-	4
1-3	1	20	1	-	1
>4	0	-	0	-	0
NPI (n=5)					
<3.4	0	0	0	-	0
3.4-5.4	3	60	1	-	3
>5.4	2	40	2	-	2
Tumor stage (n=6)					
IIA	2	33.3	0	-	2
IIB	2	33.3	1	-	2
IIIB	2	33.3	2	-	2
Vascular involvement					
Yes	4	66.67	3	-	4
No	2	33.3	0	-	2
Skin involvement					
Yes	2	33.3	2	-	2
No	4	66.67	1	-	4

6 cases of medullary carcinoma were included in the study. Out of total 6 cases, 3 cases showed positive vimentin expression and E cadherin was negative in all the cases.

Association between vimentin and E cadherin expression with the clinico-histopathological parameters:

1. Tumor grade:

Table 36: Association between tumor grade and vimentin. (Chi square test)

		Vimentin				p value
		< 30 Negative (n=40)		> 30 Positive (n=18)		
		n	%	n	%	
Tumor grade	I	21	52.5%	1	5.6%	0.001*
	II	15	37.5%	9	50.0%	
	III	4	10.0%	8	44.4%	

In this study, p value significantly correlated (p=0.001) between tumor grade and vimentin expression, indicating that vimentin expression was more in tumors with higher grade.

Table 37: Association between tumor grade and E cadherin. (Chi square test)

		E cadherin				P value
		Negative(n=22)		Positive(n=36)		
		n	%	n	%	
Tumor grade	I	8	36.4%	14	38.9%	0.051*
	II	6	27.3%	18	50.0%	
	III	8	36.4%	4	11.1%	

In this study, p value calculated significantly correlated (p=0.001) between tumor grade and E cadherin expression, indicating that E cadherin expression was lost in tumors with higher grade.

2. Histopathological type

Table 38: Association between histopathological type and vimentin. (Chi square test)

Histopathological type		Vimentin				p value
		< 30 Negative (n=40)		> 30 Positive (n=18)		
		n	%	n	%	
	IDC	31	77.5%	15	83.3%	0.152
	ILC	6	15.0%	0	0%	
	MC	3	7.5%	3	16.7%	

In this study, Grade II and III IDC (15 cases) showed positive vimentin expression, and 3 cases of medullary carcinoma were vimentin positive. However, none of the ILC showed vimentin positivity. P value calculated had no significant association (p=0.152) between histopathological type and vimentin expression.

Table 39: Association between histopathological type and E cadherin. (Chi square test)

		E cadherin				P value
		Negative (n=22)		Positive(n=36)		
		n	%	n	%	
Histopathological Type	IDC	10	45.4%	36	100%	<0.0001*
	ILC	6	27.2%	0	0.0%	
	MC	6	27.2%	0	0.0%	

Strong significant association (p=<0.0001) was noted between histopathological type and E cadherin expression. In this study, E cadherin was not expressed in any of the ILC, indicating that it is specific for ILC diagnosis, and aids in differentiating uncertain histology of IDC from ILC. 10 cases of IDC also showed E cadherin loss, indicating that E cadherin is not always expressed in IDC. We also observed E cadherin loss in medullary carcinoma.

3. Tumor size:

Table 40: Association between tumor size and vimentin. (Chi square test)

		Vimentin				p value
		< 30 Negative (n=40)		> 30 Positive (n=18)		
		n	%	n	%	
Tumor size	<2	0	0.0%	2	11.1%	0.080
	2-5	31	77.5%	11	61.1%	
	>5	9	22.5%	5	27.8%	

In this study majority of the tumors had a tumor size range between 2- 5 cm. No significant correlation was seen (p=0.080) tumor size and vimentin expression.

Table 41: Association between tumor size and E cadherin. (Chi square test)

		E cadherin				P value
		Negative (n=22)		Positive (n=36)		
		N	%	n	%	
Tumor size	<2	1	4.5%	1	2.8%	0.926
	2-5	16	72.7%	26	72.2%	
	>5	5	22.7%	9	25.0%	

In this study, majority of tumors had tumor size between 2- 5 cm. No significant correlation (p=0.926) was seen between tumor size and E cadherin expression.

4. NPI

Table 42: Association between NPI and vimentin. (Chi square test)

		Vimentin				p value
		< 30 Negative (n=34)		> 30 Positive (n=17)		
		n	%	n	%	
NPI	<3.4	9	26.5%	3	17.6%	0.537
	3.4-5.4	16	47%	7	41.2%	
	>5.4	9	25.5%	7	41.2%	

In this study, 7/17 cases (41.2%) had NPI score of >5.4 and 3.4-5.4 each respectively. Vimentin expression is seen more in tumors with high NPI scores. However no significant correlation (p=0.537) was seen between NPI and vimentin expression.

Table 43: Association between NPI and E cadherin. (Chi square test)

		E cadherin				P value
		Negative(n=19)		Positive (n=32)		
		n	%	n	%	
NPI	<3.4	3	15.8%	11	28.1%	0.579
	3.4-5.4	9	47.4%	12	43.8%	
	>5.4	7	36.8%	9	28.1%	

In this study, 7/19 cases (36.8%) had NPI >5.4 which showed loss of E cadherin expression, 9/19 cases (47.4%) had NPI score between 3.4- 5.4. Loss of E cadherin expression is seen

more in tumors with high NPI score. However, no significant correlation (p=0.579) was seen between NPI and E cadherin expression.

5. Lymph node status

Table 44: Association between lymph node and vimentin. (Chi square test)

		Vimentin				p value
		< 30 Negative (n=34)		> 30 Positive (n=17)		
		n	%	n	%	
Lymph node status	0	12	35.3%	9	52.9%	0.415
	1-3	14	41.2%	6	35.3%	
	>4	8	23.5%	2	11.8%	

In this study, 8/15 cases (47.1%) with positive vimentin expression had lymph node metastasis. No significance (p=0.415) was seen between lymph node status and vimentin.

Table 45: Association between lymph node and E cadherin. (Chi square test)

		E cadherin				P value
		Negative (n=19)		Positive (n=32)		
		n	%	n	%	
Lymph node status	0	8	42.1%	13	40.6%	0.562
	1-3	6	31.6%	14	43.8%	
	>4	5	26.3%	5	15.6%	

In this study, 11/19 cases (57.9%) with E cadherin negative status, had lymph node metastasis. More the loss of E cadherin expression, more the lymph node metastasis. However no significant correlation (p=0.562) was seen between lymph node status and E cadherin expression.

6. Tumor stage

Table 46: Association between tumor stage and vimentin. (Chi square test)

		Vimentin				p value
		< 30 Negative (n=40)		> 30 Positive (n=18)		
		n	%	n	%	
Tumor stage	I	0	0.0%	1	5.6%	0.011*
	IIA	13	32.5%	7	38.9%	
	IIB	13	32.5%	4	22.2%	
	III	11	27.5	0	0.0%	
	IIIB	3	7.5	6	33.3	

In this study, 6/18 cases (33.3%) with vimentin expression belonged to stage IIIB, 7 cases (38.9%) belonged to stage IIA, 4 cases (22.2%) belonged to stage IIB, and 1 case (5.6%) belonged to stage I. Therefore positive vimentin expression is seen with increasing tumor stage as its expression was seen in tumors which high tumor stage. A significant correlation (p=0.011) was seen between tumor stage and vimentin expression.

Table 47: Association between tumor stage and E cadherin. (Chi square test)

		E cadherin				P value
		Negative (n=22)		Positive (n=36)		
		n	%	n	%	
Tumor stage	I	1	4.5%	0	0.0%	0.488
	IIA	7	31.8%	13	36.1%	
	IIB	5	22.7%	12	33.3%	
	III	4	18.2%	7	19.4%	
	IIIB	5	22.7%	4	11.1%	

In this study 5/22 cases (22.2%) belonged to stage IIIB, followed by 4/ 22 cases (18.2%) belonged to stage III, 7/22 cases (31.8%) belonged to stage IIA, 5/22 cases belonged to stage IIB, and only 1/22 case belonged to stage I. Therefore loss of E cadherin is seen with increasing stage of tumor. However, no significant correlation (P=0.488) was seen between tumor stage and E cadherin expression.

7. Vascular invasion

Table 48: Association between vascular invasion and vimentin. (Chi square test)

		Vimentin				p value
		< 30 Negative (n=40)		> 30 Positive (n=18)		
		n	%	n	%	
Vascular invasion	N	29	72.5%	11	61.1%	0.386
	Y	11	27.5%	7	38.9%	

In this study, 7/18 (38.9%) cases showed vascular invasion with positive vimentin expression. However no significant correlation (p=0.386) was seen between vascular invasion and vimentin expression.

Table 49: Association between vascular invasion and E cadherin. (Chi square test)

Association		E cadherin				P value
		Negative (n=22)		Positive (n=36)		
		n	%	n	%	
Vascular invasion	NO	14	63.6%	26	72.2%	0.493
	YES	8	36.4%	10	27.8%	

In this study, 8 cases (36.4%) with loss of E cadherin expression had vascular invasion. However no significant correlation (p=0.493) was noted between vascular invasion and E cadherin expression.

8. Skin involvement

Table 50: Association between skin involvement and vimentin. (Chi square test)

Skin involvement		Vimentin				p value
		< 30 Negative (n=40)		> 30 Positive (n=18)		
		n	%	n	%	
	N	37	92.5%	12	66.7%	0.012*
	Y	3	7.5%	6	33.3%	

In this study, 6/18 cases (33.3%) with positive vimentin expression had involvement of skin. A positive correlation (p=0.012) was seen between skin involvement and vimentin expression.

Table 51: Association between skin involvement and E cadherin. (Chi square test)

		E cadherin				P value
		Negative (n=22)		Positive (n=36)		
		n	%	n	%	
Skin involvement	NO	17	77.3%	32	88.9%	0.236
	YES	5	22.7%	4	11.1%	

In this study, no significant association (p=0.236) was seen between skin involvement and E cadherin expression.

9. Age

Table 52: Association between age and vimentin expression.

	Vimentin		P value
AGE	**< 30 Negative (n=40)**	**> 30 Positive (n=18)**	
(average)	54.10+/- 12.510	51.39+/- 9.95	0.421

No association was seen between age and vimentin expression. (p=0.421)

Table 53: Association between age and E cadherin expression.

	E cadherin		P value
AGE	**Negative**	**Positive**	
(average)	54.77+/- 10.61	52.33+/- 12.45	0.448

No association was seen between age and E cadherin expression. (p=0.448)

Co- expression of vimentin and E cadherin and correlation with clinicpathological parameters.

Correlation		Vimentin								P value
		< 30 Negative				> 30 Positive				
		E cadherin				E cadherin				
		Negative		Positive		Negative		Positive		
		n	%	n	%	n	%	n	%	
Tumor grade	I	8	66.7%	13	46.4%	0	0.0%	1	12.5%	0.495
	II	1	8.3%	14	50.0%	5	50.0%	4	50.0%	
	III	3	25.0%	1	3.6%	5	50.0%	3	37.5%	
NPI	< 3.4	3	30.0%	7	29.2%	1	11.1%	4	50.0%	0.214
	3.4-5.4	4	40.0%	10	41.7%	4	44.4%	2	25.0%	
	> 5.4	3	30.0%	7	29.2%	4	44.4%	2	25.0%	
Lymph node status	0	4	40.0%	8	33.3%	4	44.4%	5	62.5%	0.357
	1- 3	3	30.0%	11	45.8%	3	33.3%	3	37.5%	
	> 4	3	30.0%	5	20.8%	2	22.2%	0	0.0%	
Vascular invasion	N	9	75.0%	20	71.4%	5	50.0%	6	75.0%	0.280
	Y	3	25.0%	8	28.6%	5	50.0%	2	25.0%	
Skin involvement	N	11	91.7%	26	92.9%	6	60.0%	6	75.0%	0.502
	Y	1	8.3%	2	7.1%	4	40.0%	2	25.0%	
HPE	IDC	3	25%	28	100%	7	70.0%	8	100.0%	0.090
	ILC	6	50.0%	0	3.6%	0	0.0%	0	0.0%	
	MED	3	25.0%	0	0.0%	3	30.0%	0	0.0%	

Tumor size	<2	0	0.0%	0	0.0%	1	10.0%	1	12.5%	0.966
	2- 5	10	83.3%	21	75.0%	6	60.0%	5	62.5%	
	> 5	2	16.7%	7	25.0%	3	30.0%	2	25.0%	
Tumor stage	I	0	0.0%	0	0.0%	1	10.0%	0	0.0%	0.287
	IIA	5	41.7%	8	28.6%	2	20.0%	5	62.5%	
	IIB	2	16.7%	11	39.3%	3	30.0%	1	12.5%	
	III	4	33.3%	7	25.0%	0	0.0%	0	0.0%	
	IIIB	1	8.3%	2	7.1%	4	40.0%	2	25.0%	

Table 54: Co- expression and correlation with clinicopathological parameters.

In this study, combination of E cadherin and vimentin expression was found to be closely associated with histopathological type, but was not statistically significant (p=0.090). No other parameters showed statistically significant association between the expressions.

Histopathology study
Specimen of Invasive ductal carcinoma of breast

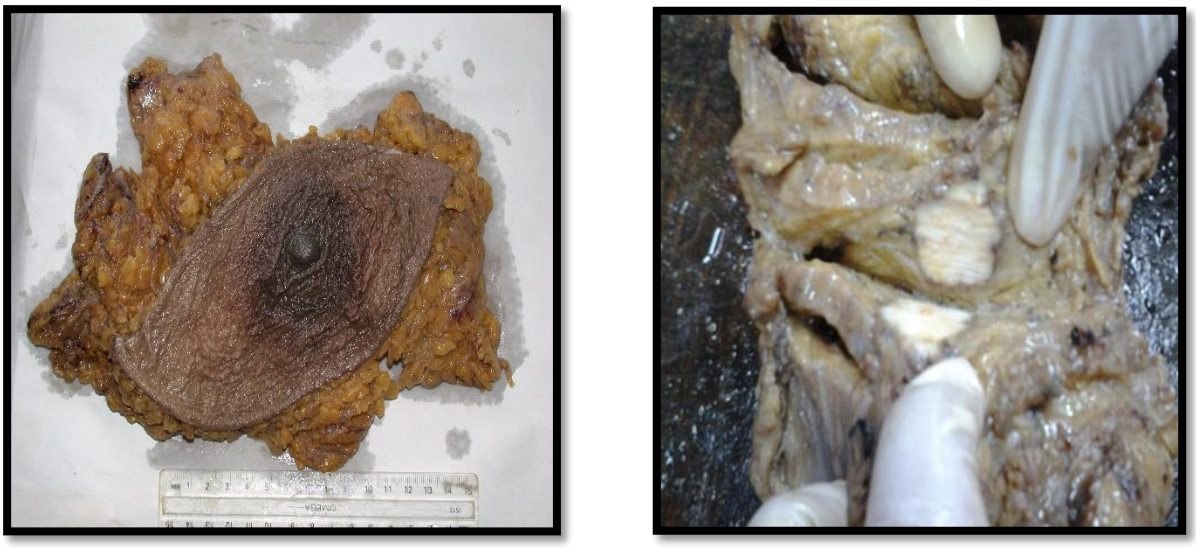


Figure 5 a,b: Gross appearance- Mastectomy specimen (a)- cut section showing grayish white tumor mass, measuring 3x3x2 cm, at the lower inner quadrant. (b)

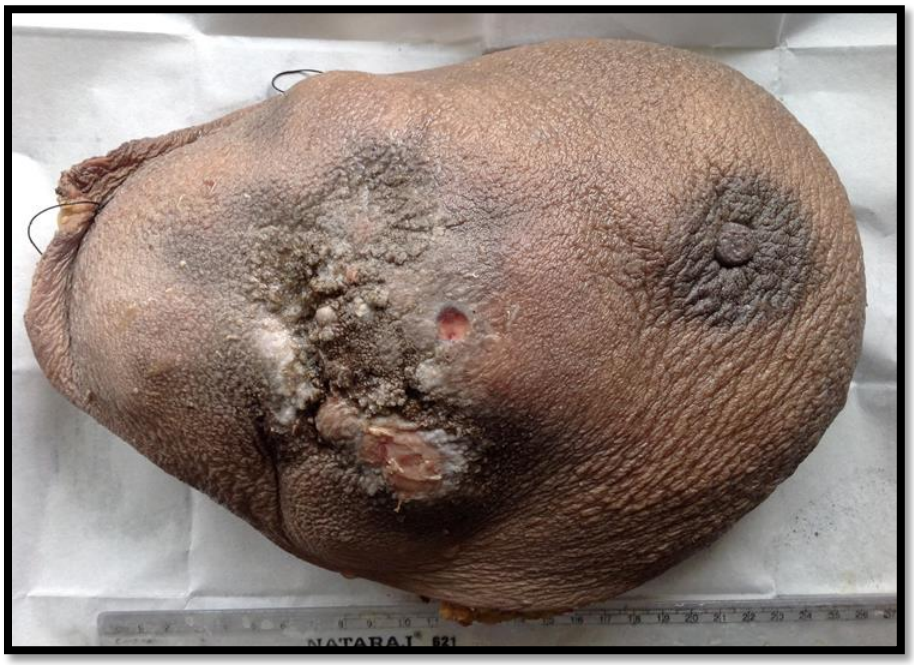


Figure 6: Gross appearance- Mastectomy specimen measuring 20x15x10cm with skin measuring 20x15cm showing ulceration.

Figure 7: **Cut section of tumor showing solid and cystic areas measuring 5x3x2cm**

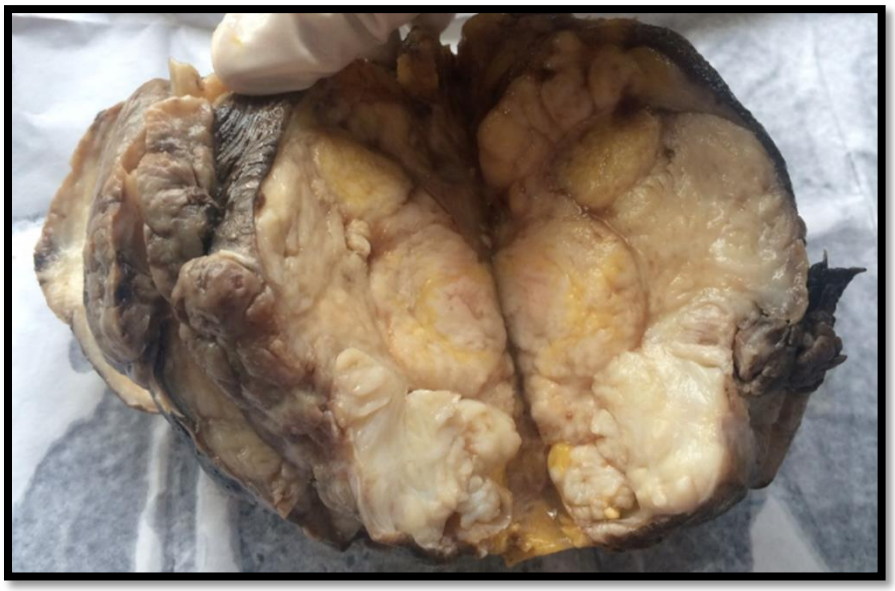


Figure 8: **Cut section of tumor showing large grey white mass with lobulations measuring 10x9x6cm.**

Invasive Ductal Carcinoma of Breast

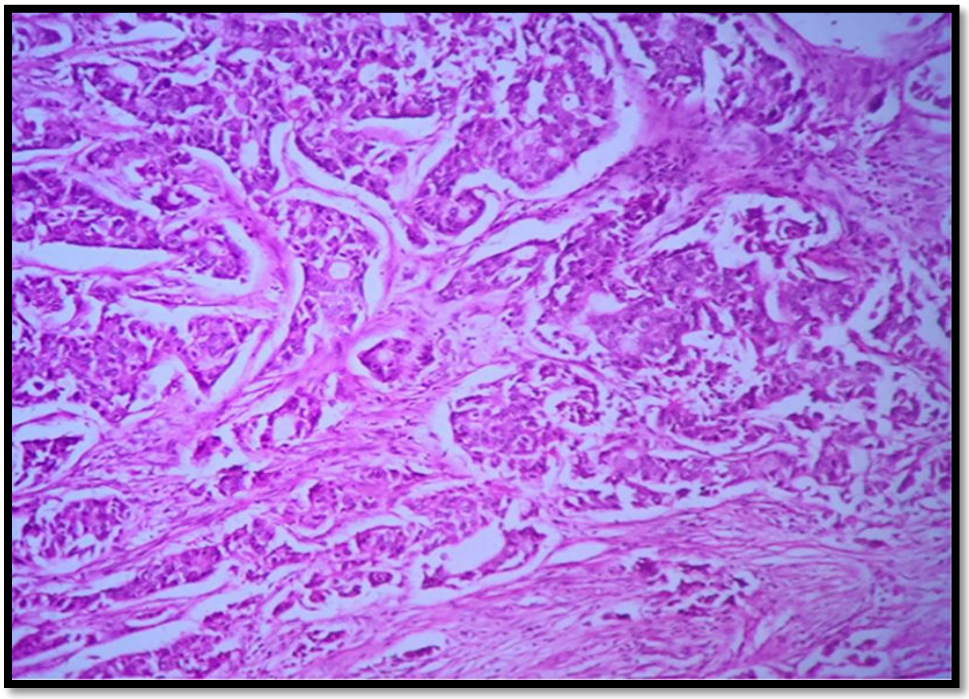


Figure 9: **Microscopic appearance- low power- Large ductal cells with hyperchromatic nuclei, infiltrating the stroma.**

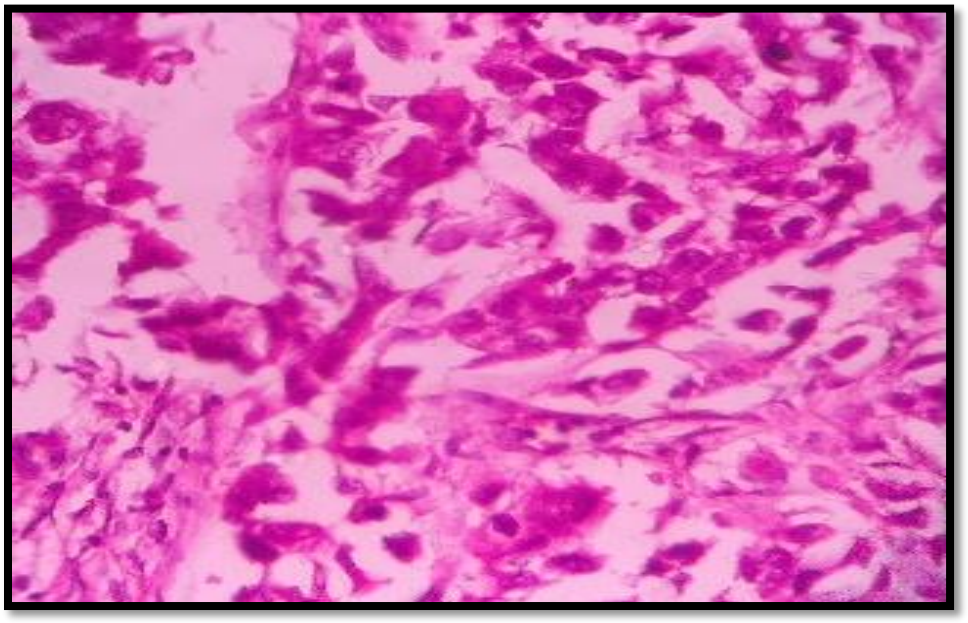


Figure 10: **Microscopic appearance- high power-large ductal cells with hyperchromatic nuclei, irregular nuclear membrane and prominent nucleoli.**

Invasive Lobular Carcinoma

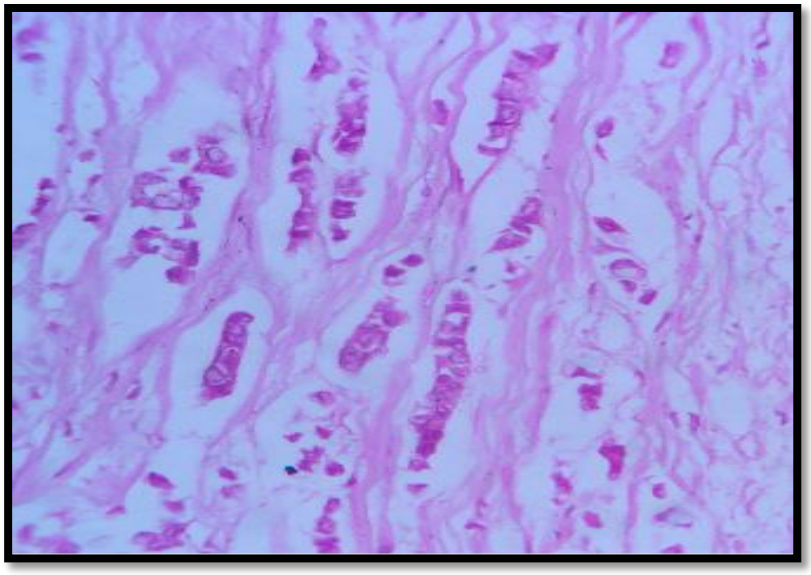


Figure 11: **Microscopic appearance- Low power- showing small to medium sized uniform cells in an Indian file pattern.**

Medullary Carcinoma

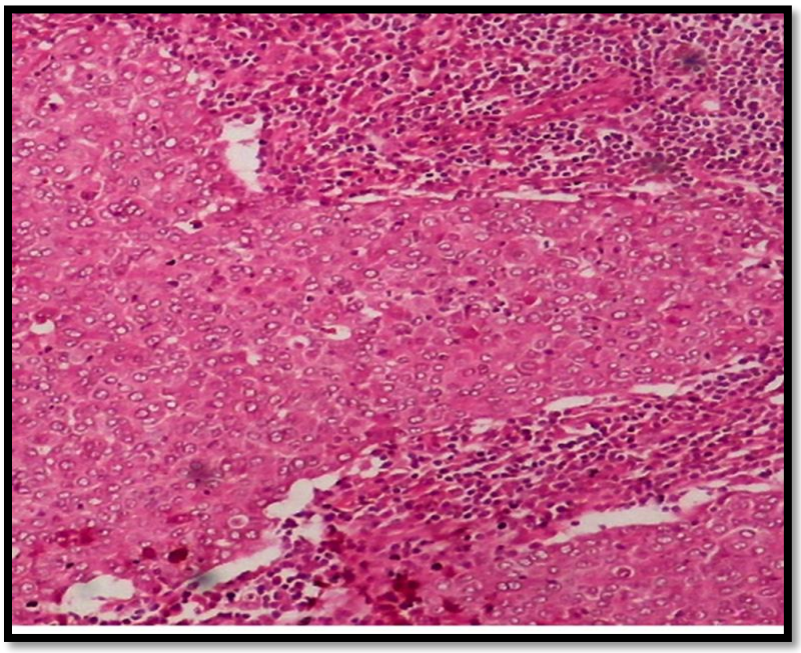


Figure 12: **Microscopic appearance- Low power- Solid, syncytium like sheets of large cells having vesicular, pleomorphic nuclei and prominent lymphocytic infiltration.**

Histopathological grade

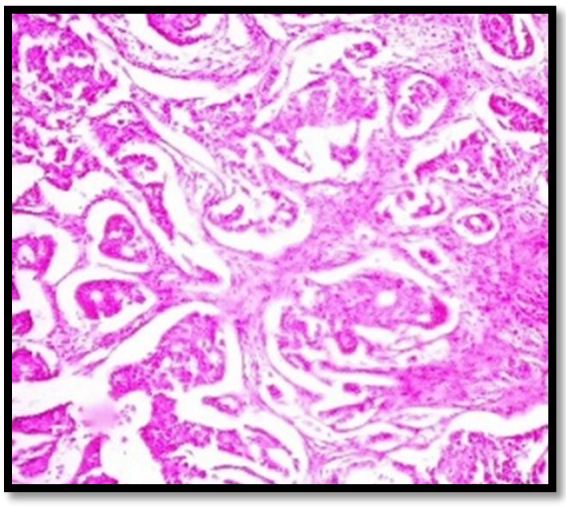


Figure 13a: Grade I

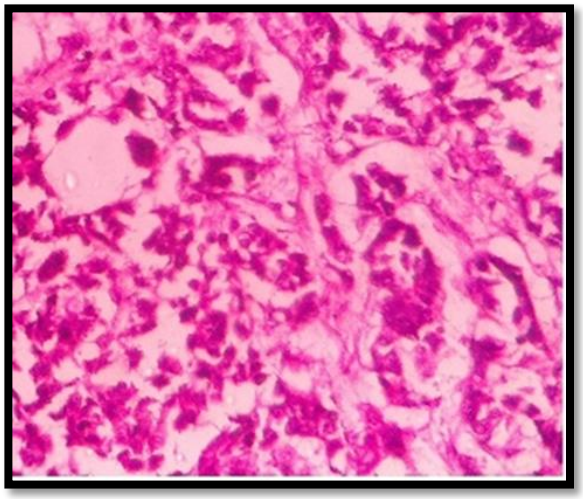


Figure 13b: Grade II

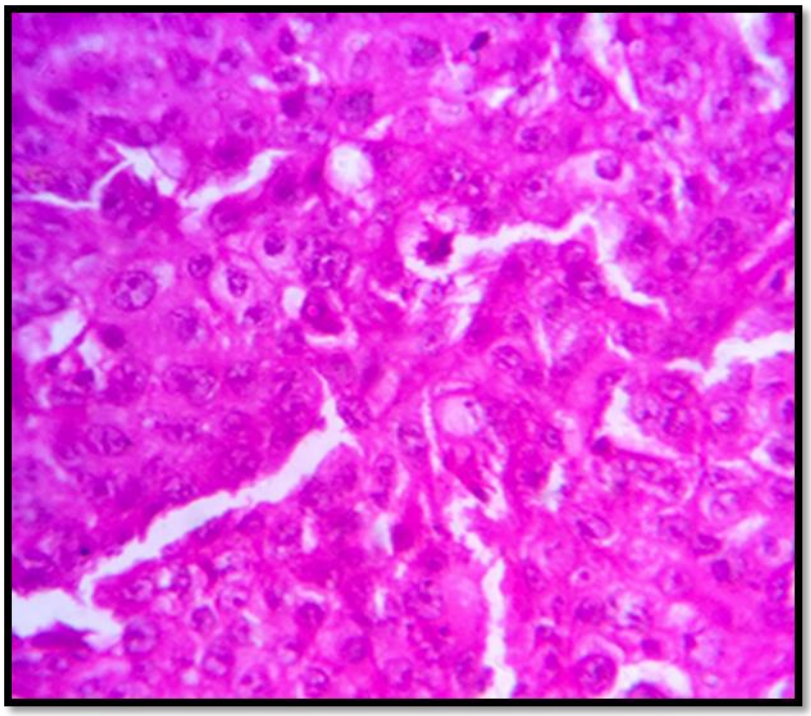


Figure 13c: Grade III

Vimentin Expression in IDC

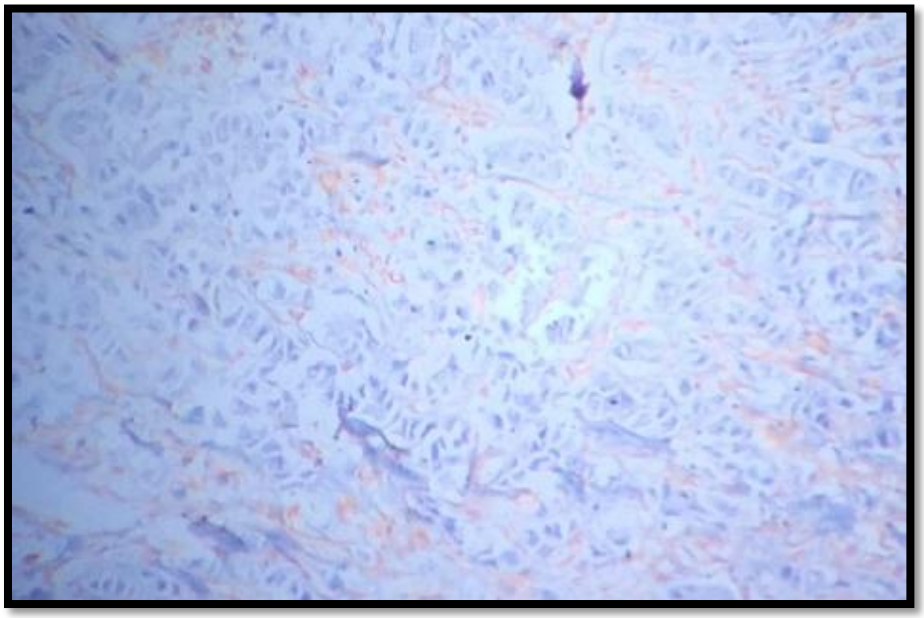


Figure 14 a: **Microscopic appearance of vimentin expression with score <30 in IDC**

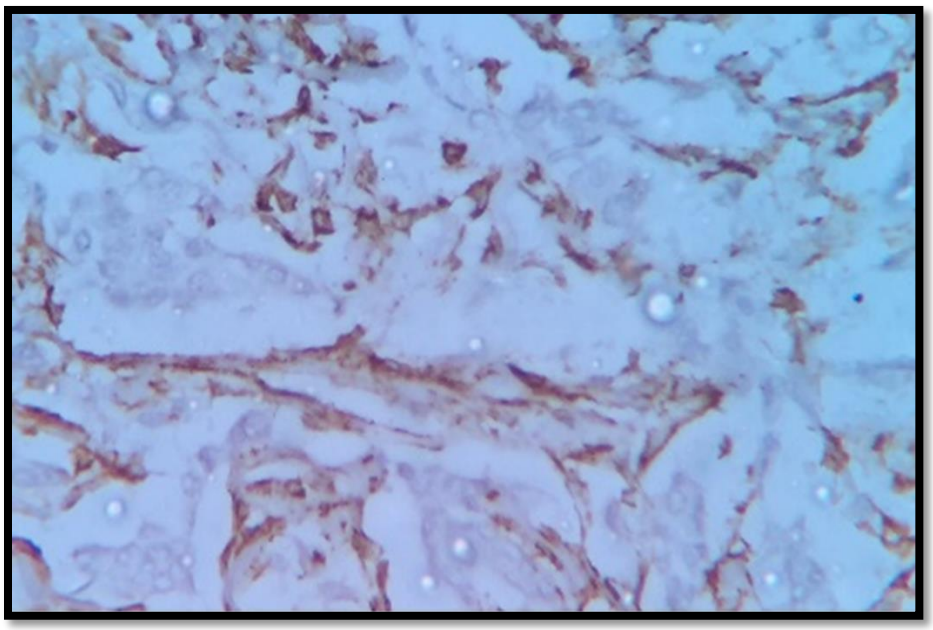


Figure 14b: **Microscopic appearance of vimentin expression showing cytoplasmic staining with score >30 in IDC**

E cadherin expression in IDC

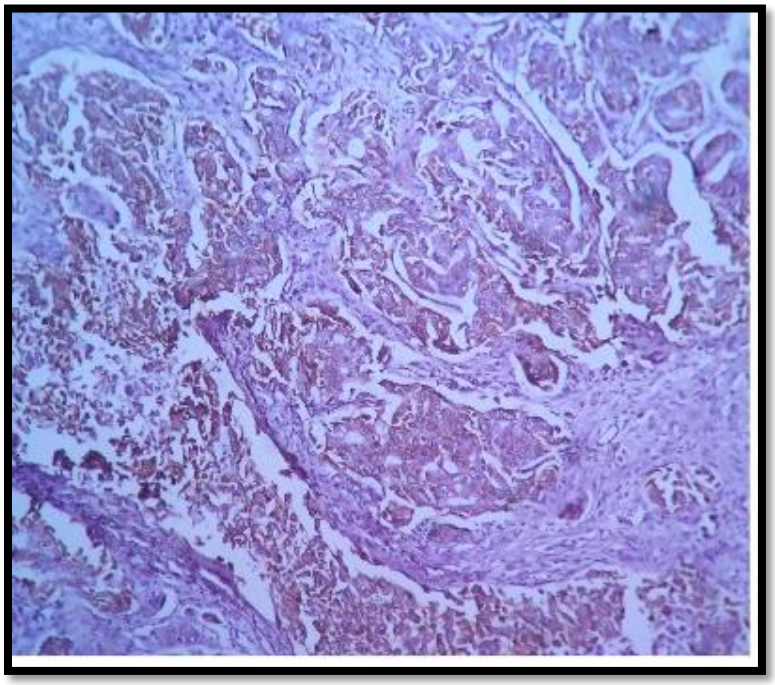


Figure 15a: **Microscopic expression –Low power- E cadherin positivity with score 3**

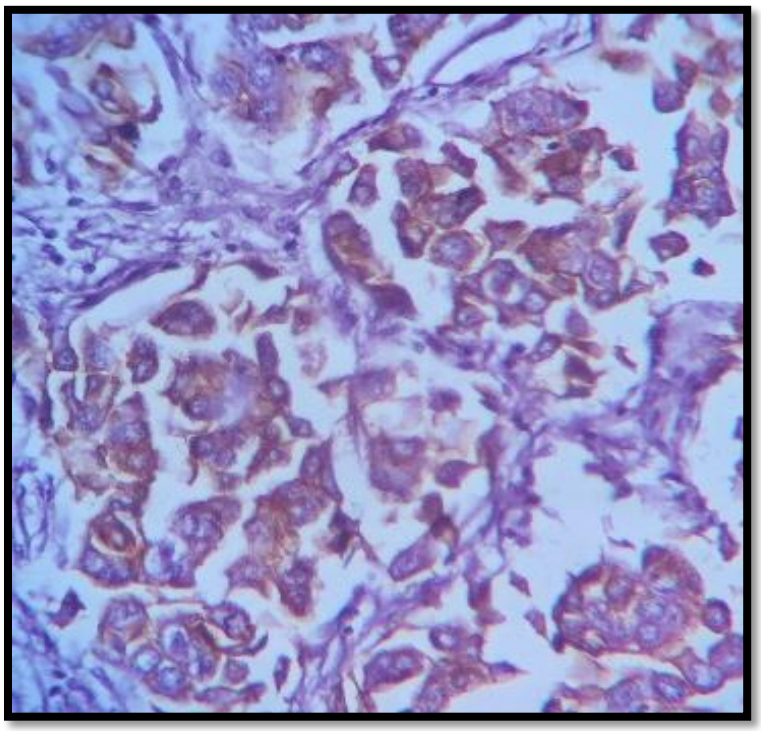
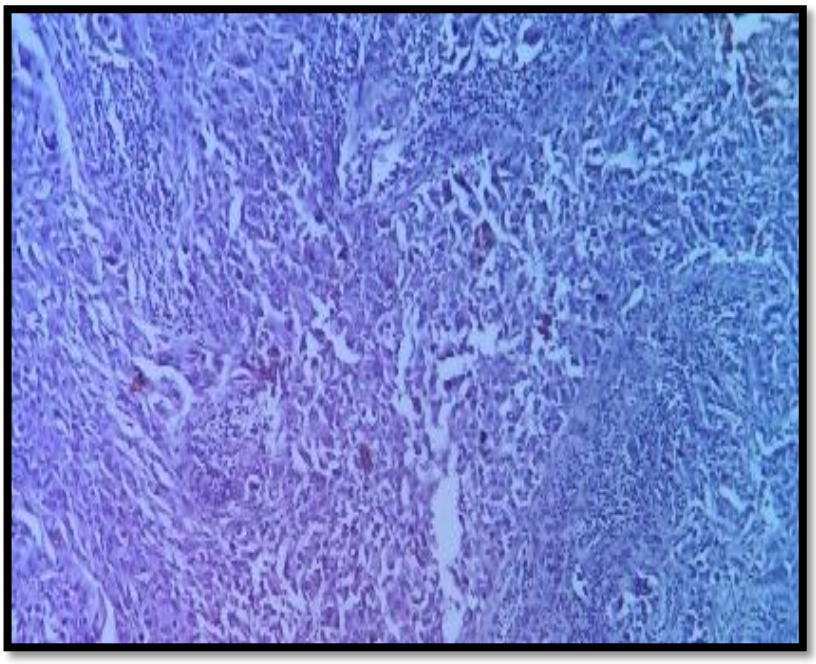
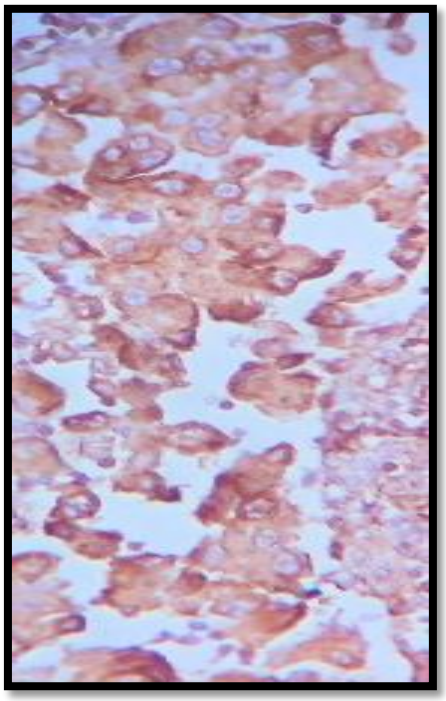


Figure 15b: **Microscopic appearance- High power- Membranous staining.**

Medullary carcinoma



<u>Figure 16a</u>: Microscopic appearance- Low power- E cadherin negative expression (score 0) in medullary carcinoma.



<u>Figure 16b:</u> Microscopic appearance- Strong vimentin positivity in medullary carcinoma.

DISCUSSION

DISCUSSION

1. Vimentin expression

Table 55: Comparison of vimentin positivity with other studies.

Authors	Hema et al[11]		Niveditha et al[51]		Domgala et al[52]		Present study	
	n	Vm +	n	Vm+	n	Vm+	n	Vm+
IDC	50	9(18%)	40	7 (17.5)	68	15 (22%)	46	15 (32%)
ILC	-	-	4	0	15	0	6	0
Medullary Carcinoma	-	-	2	1(50%)	3	1(33%)	6	3 (50%)

In the present study, 32% (15/46) of IDC showed positive vimentin expression, followed by 50% of medullary carcinoma (3/6). None of the ILC (0/6) cases showed vimentin positivity. However findings observed in studies done by Hema et al [11] (18%), Niveditha et al [51] (17.5%), Domgala et al [52] (22%) were lesser compared to our study (32%) as these had included only IDC in their study. But Thomas et al [53] observed vimentin expression in 47.1% of breast cancer cases. In few articles, they have explained this difference in vimentin expression may be due to the type of fixative used for processing. [11,16] Therefore, vimentin traditionally is used in immunohistochemistry to assess the extent of antigen damage occurring in tissues due to fixation and processing.

This expression of vimentin in tumors which is not normally seen, is due to the theory of epithelial mesenchymal transition, giving the tumors enhanced capability of invasiveness. In one study done by Hassan et al concluded that vimentin expression is not useful in determining the aggressive behavior of breast cancers, as it is expressed in both breast cancers and normal breast tissues.[14] But in our study we didn't find vimentin expression in all breast cancers or in the normal breast tissue sections.

Korsching et al, proposed a different theory for the acquisition of vimentin expression apart from EMT. He proposed that vimentin expressing breast carcinoma cells are derived from breast progenitor cells having bilinear differentiation (glandular and myoepithelial) potential and not due to EMT.[54]

E cadherin expression

Table 56: Comparison of E cadherin positivity with other studies.

	Qureshi et al[12]			Kowalski et al[55]			Present study		
	n	**Ecad +**	**Ecad -**	**n**	**Ecad +**	**Ecad -**	**n**	**Ecad +**	**Ecad -**
IDC	204	203(99%)	1(0.5%)	22	12(55%)	10(45%)	46	36(78%)	10(24%)
ILC	49	5(10%)	44(90%)	8	0	8(100%)	6	0	6(100%)
MC	-	-	-	-	-	-	6	0	6(100%)

In this study, out of 46 cases of IDC, 36 cases (78.26%) showed positive E cadherin expression (score of 2 or 3). Remaining 10 cases of IDC (23.92%) showed weak (score of 1) E cadherin expression. Both ILC and medullary carcinoma cases showed score of 0.

Similar findings of positivity were observed by Kowalski et al [55] (55% positivity), Moll et al [56] (79% of E-cadherin positivity in IDC cases), Howard et al [57] (84% positivity), Gamello et al [58] (94% positivity), Younis KL et al [59] (72% positivity). But in a study done by Qureshi et al had E cadherin expression in almost 99.5% of IDC cases [12].

Negative E cadherin expression in lobular carcinoma was similar to studies done by Moll et al [56], Gamello et al [58], Younis KL et al [59], and Cowin et al [60], showing loss of E cadherin expression in lobular carcinomas. E cadherin, normally, is an invasion suppressor gene,

and since there is loss of its expression in lobular carcinomas, due to its mutation, lobular carcinoma is usually associated with diffuse invasion pattern.

Cowin et al in his study categorically stated that there is loss of expression of E cadherin in ILC due to mutation at two places- loss of heterozygosity at chromosome 6q22 of E cadherin gene CDH1 (50%) in combination with mutation or epigenetic silencing of the remaining CDH1 allele. Thus explaining complete loss of E cadherin in ILC. However in IDC, there is no mutation in the remaining CDH1 allele, thus explaining variability in E cadherin expression in IDC [60]. Similar findings of variation in E cadherin expression in IDC was observed in this study with 78% of IDC showing positive E cadherin expression. Lowering of E-cadherin levels are caused by epigenetic silencing via promoter hypermethylation or transcriptional repression.

Age Distribution

Table 57: Comparison of age distribution in different studies.

Age	Hema et al[11]	Niveditha et al[51]	Suciu C et al[61]	Present study
Age range (years)	35-84	19-74	31-90	23-85
Mean Age	51.2	47.54	60.6	52.3

The age group in this study ranged from 23 to 85 years, with mean age of 52.3years, which was similar to the observations made by Hema et al [11] with age ranged from 35-84 years and mean being 51.2 years, Niveditha et al [51] with age ranged from 19-74 years with mean being 47.74 years and Suciu C et al [61] with age ranged from 31-90 years with mean being 6.6 years. Therefore the most common age group in females to develop breast cancer overall being 51-60 years.

In this study, no significant association was seen between age and vimentin, E cadherin expression.

Tumor size

Table 58: Comparison of size of the tumor in different studies.

Tumor size	Qureshi SH et al[12]	Kwon GY et al[62]	Hemalatha et al[11]	Present Study
<2 cm	153(56.5)	11(24.4%)	8(16%)	2(3.44%)
2-5cm	92(33.9)	25(55.5%)	22(44%)	42(72.41%)
>5cm	26(9.6)	9(20%)	20(40%)	14(24.13%)
Total	271	45	50	58

In the present study, majority of the cases (72.41%) had tumor size between 2 and 5 cm, followed by tumor size greater than 5 cm (24.13%).Similar findings were observed in studies by Kwon GY et al [62] and Ogaway et al [63] and with majority of their cases also having tumor size between 2 to 5cm (47 and 55% respectively).

In this study, no significant association was seen between the tumor size and vimentin, E cadherin expression. This was further supported by observations made in studies by Hema et al [11] (p=0.670), Yagasaki et al [16] (p=0.4612). When compared to western countries, breast cancer screening programs are regularly conducted and most of the tumors are detected in early stages, as compared to the scenario in India, due to lack of effective screening initiatives, by the time the patients present, the tumor is of much bigger size and at advanced stages. Therefore effective screening programs are required to detect breast cancer in early stages in our country.

Histopathological Type

Table 59: Comparison of histopathological types with other studies

Types	Medri et al[64]		Qureshi SH et al[12]		Erdogan N et al[65]		Kowalski JP et al[55]		Present study	
	No.	%	No	%	No.	%	No	%	No.	%
IDC	301	82.2	206	73.9	27	89%	22	73	46	79.3
Lobular	30	8.2	59	21.4	4	12.9%	8	27	6	10.3
Others	35	9.6	13	4.7	-	-	-	-	6	10.3 (MC)
Total	366	100	278	100	31	100	30	100	58	100

In the present study, infiltrating ductal carcinoma (IDC) was seen in majority of our cases comprising of 79.3%**,** followed by lobular (10.3%) and medullary carcinoma (10.3%). In a study done by Medri et al [64] similar observations were seen with majority of the cases (82.2 %) being infiltrating ductal carcinoma, followed by lobular carcinoma (8.2%). Another study by Erdogan N et al [65] showed similar observations with majority of the cases (89%) being infiltrating ductal carcinoma and Qureshi et al [12] showed (73.9%) cases of infiltrating ductal carcinoma, 21.4% of lobular carcinomas.

In this study, we found significant association between histopathological type and E cadherin expression. 6/6 cases (100%) of ILC showed loss of E cadherin, thereby being specific for diagnosis of ILC. Similar findings were observed by Qureshi et al, where 90%

of ILC cases showed loss of expression but 10% cases showed preserved E cadherin expression, owing to large size tumor. He concluded that E cadherin correlates with histological type in breast cancer and not with prognostic parameters [12]. Similar findings of loss of E cadherin in ILC has been reported by Wahed et al [66], Acs et al [67], Leeuw De et al [68], Gamallo et al [58]. Study done by Parker et al, found significant associations between E cadherin expression and tumor type ($P \leq 0.001$) and concluded that E-cadherin expression in human breast cancer appears to have minimal prognostic value, but may have a role as a phenotypic marker.[69]

In this study, 18 cases were positive for vimentin expression. 15 IDC and 3 medullary carcinoma. However, we did not find significant association (p=0.152) between histopathological type and vimentin expression. Vimentin does not help in identifying the tumor type as it is usually expressed in breast tumors with higher grade. Similar findings were observed in medullary carcinoma.

Tumor grade

Table 60: Comparison of tumor grade in different studies.

Tumor grade	Qureshi SH et al [12]	Parker et al [69]	Hemalatha et al [11]	Present Study
I	61(22.1%)	31(18.6%)	22(44%)	22(37.93%)
II	115(41.7%)	69(41.5%)	20(40%)	24(41.37%)
III	100(36.2%)	66(39.7%)	8(16%)	12(20.68)
Total	276	166	50	58

In this study, majority of tumors belonged to grade II (41.37%), followed by grade I (37.93%) and grade III (20.68%). Similar findings were observed in studies done by Hemalatha et al [11], Qureshi et al [12] and Parker et al [69] with most of the tumors in grade II.

In this study, a positive association was seen between tumor grade and vimentin expression and E cadherin expression (p=0.001 and p=0.051 respectively). Therefore vimentin expression is positive in tumors with higher histological grade (17/18 tumors belonged to grade II and III which showed positive vimentin expression). This is supported in studies done by Hemalatha et al [11], where in 7/8 tumors showing grade III had positive vimentin expression. Similarly, Korsching et al [54] (19/21 tumors with grade III had positive vimentin expression), Domagala et al [52] (15/28 tumors in grade III had positive vimentin expression) showed similar findings as in our study. Therefore vimentin expression increases as the histological grading of the tumor increases.

We also found significant association between tumor grade and loss of E cadherin expression (p=0.051) which may be due to cells losing its adhesion with increasing grade of tumor. Study done by Parker at al also found similar findings (p=0.03). In low grade tumors, generally the cell adhesive molecules are intact. As the grade of tumor increases, the cell start becoming less adhesive and gain mesenchymal properties of invasiveness supporting the theory of EMT. In this study we further supported the theory of EMT, by showing the gain in vimentin and loss of E cadherin expression in tumor cells of higher grade tumors.

Lymph node status

Table 61: Comparison of lymph node status in different studies.

Lymph node	Qureshi SH et al[12]	Parker et al [69]	Hemalatha et al [11]	Present Study
Positive	82(40.19%)	69(41.31%)	30(81.08%)	30 (58.8%)
Negative	122(59.8%)	98(58.68%)	7(18.91%)	21(41.17%)
Total	204	167	37	51

In this study, 58.8% of tumors were positive for lymph node metastasis. Qureshi et al [12] and Parker et al [69] also had similar findings. Study done by Hemalatha et al [11] had lymph node metastasis in 81.08% of the tumors. This difference may be due to selection of tumors with more lymph node metastasis as most patients by the time they seek medical advice, would have already developed metastasis in at least one lymph node. The other reason may be due to less sample size included in our study.

However we did not find significant association between lymph node status and vimentin, E cadherin expression. Similar observation were noted in studies done by Hemalatha et al [11], Qureshi et al [12], , Niveditha et al [51] and Parker et al [69]. Gamallo et al concluded that E cadherin expression is associated with histological type and grade but not with lymph node status, as E cadherin expression is not related to invasiveness and metastatic potential [58]. But in a study done by Yagasaki et al, they found significant correlation between lymph node status and vimentin and E cadherin expression and suggested that inhibition of E cadherin function increases the release of cancer cells from the primary site [16]. In a study

done by Thompson et al, they concluded that vimentin expression gives useful information of invasion or progression of breast cancer cells, but its exact role in metastasis is unclear[8]. Domagala et al found that vimentin expression is a strong indicator of poor prognosis in node negative invasive ductal carcinoma but not in node positive cancer [52].

Hence node negative lymph node may not always exclude aggressive disease or distant metastasis. Therefore there is a strong association between E cadherin expression and node negative cases. Younis KL et al, found significant correlation between node negative breast tumors and E cadherin expression and concluded that node negative tumors are independent predictors of strong E cadherin expression and node positive tumors predict negative E cadherin expression [59].

NPI

Table 62: Comparison of NPI with other studies.

NPI	Hemalatha et al [11]	Present Study
<3.4	9(24.32%)	12(23.52%)
3.4-5.4	20(54.05%)	23(45.09%)
>5.4	8(21.62%)	16(31.37%)
Total cases	37	51

NPI score is calculated using the formula, (0.2xtumor size) + tumor grade +lymph node status. It helps in determining the prognosis following surgery for breast cancers. Scores <3.4 have 5 year survival rate of 85% and scores above 5, have 5 year survival rate of about 50%.

No significant association was seen between NPI score and vimentin, E cadherin expression. This was supported by study done by Hemalatha et al. [11]

Tumor stage

Table 63: Comparison of tumor stage with other studies.

Tumor stage	Kowalski et al [55]	Yagasaki et al [16]	Present Study
I	03	11	01
II	13	41	37
III	6	25	20
IV	02	3	0
Total	24	80	58

In the present study, majority of cases belonged to stage II. We observed similar findings in studies by Kowalski et al [55] and Yagasaki et al. [16]

In this study, we found significant association between tumor stage and vimentin expression (p=0.011). Therefore vimentin expression is associated with tumors which show higher stage, and therefore its expression can aid in identifying tumors of increasing stage. However in studied done by Yagasaki et al, did not find association between tumor stage and vimentin, E cadherin expression [16].

In this study, we did not find any association between tumor stage and E cadherin expression (p=0.488). Younis KL et al [59] found positive association between tumor stage and E cadherin expression (p=0.014).

It can be hypothesized that E cadherin expression is still maintained in tumors of lower stage and such tumors may lose their expression as the stage increases. Therefore higher the tumor stage, more is the vimentin expression and loss of E cadherin and lower the stage of tumor, E cadherin is preserved.

Correlation between vimentin and E cadherin co-expression with histopathological parameters:

In this study we found close association between histopathological type and vimentin, E cadherin co-expression in breast cancers, but was not statistically significant. We did not find correlation with other parameters like age, tumor size, tumor grade, lymph node status, NPI, vascular invasion and skin involvement. However in a study done by Yagasaki et al [16], they found correlations between histological grade and axillary lymph node status. We did not find such results in our study, may be due to the small sample size of only 58 cases included in our study and the lesser number of higher grade tumors and lymph node status.

CONCLUSION

Majority of patients affected were in golden age group of 41-60 years. Vimentin expression was seen in total 18 cases out of 58 breast cancer cases included in the study. Its expression was associated in tumors with higher grade and stage. Therefore vimentin expression may be added as a new routine prognostic marker, that helps in identifying tumors known to exhibit aggressive behavior independently of lymph node status, tumor grade, thereby benefitting patients from early adjuvant therapy. Discovery of an anti-vimentin drug called Withaferin A, can be given to such patients which aids in prolonging the life of patients with breast cancer.

Loss of E cadherin in breast cancer is associated with increased invasiveness and metastatic potential.

Therefore identifying tumors with vimentin gain and E cadherin loss in breast cancer help in prolonging survival periods as they are associated with bad prognostic markers.

Summary:

1. The present study was conducted in department of pathology, Sri Devaraj Urs Medical College, Kolar from January 2014 to July 2015.
2. A total of 58 cases of breast carcinoma was undertaken, involving only female patients.
3. Majority of patients belonged to 41 – 50years which constituted 31.03%
4. Majority of the lumps were seen in right breast 32 (55.17%) and 26 (44.8%) were in left breast.
5. Majority of the cases had tumor size range from 2 to 5 cm, constituting 42(72.4%).
6. The histological types we studied were infiltrating ductal carcinoma seen in 46 (79.3%) cases, followed by medullary carcinoma 6 (10.34%) and invasive lobular in 6 (10.34%). Out of which IDC was commonest. We did not get other histological types in our department.
7. Majority of the tumors were in histological grade 2 (41.37%).
8. Vascular invasion was seen in 18 (31.03%) of the cases.
9. Skin involvement was seen in 9 (15.51%) of the cases.
10. DCIS component was seen in 13 (22.41%) of the cases.
11. Out of 51 cases, lymph node metastasis was seen in 30 cases.
12. Out of 51 cases, majority of cases had NPI score between 3.4- 5.4 (45.09%).
13. Majority of cases belonged to tumor stage IIA (34.49%).
14. Vimentin expression was seen in 18 cases (31.03%) of the total 58 cases. Out of which 15 cases were of IDC (83.3%) and 3 cases of medullary carcinoma (16.6%).

15. E cadherin expression was positive in 36 cases (62.06%) and 22 cases (37.93%) had weak to negative E cadherin expression.
16. Out of 15 IDC vimentin positive cases, 8 were E cadherin positive and 7 E cadherin negative. 0/3 vimentin positive medullary carcinoma showed negative E cadherin expression. ILC were vimentin and E cadherin negative.
17. We found significant association between vimentin expression and tumor grade, tumor stage, and skin involvement. No significant association was found between vimentin and age, histopathological type, tumor size, lymph node status, NPI, tumor size, vascular invasion.
18. We found significant association between E cadherin expression and histopathological type, tumor grade. No significant association was found between E cadherin expression and age, tumor size, lymph node status, NPI, tumor stage, vascular invasion and skin involvement.
19. Correlation between vimentin and E cadherin co-expression was closely associated with histopathological type but not statistically significant.

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ANNEXURES

Proforma

Case No:

Name: Age:

Ip. No: Biopsy No:

Presenting Complaints:

Past History: Family History:

Menstrual History:

Neoadjuvant therapy received: YES/ NO

Clinical Diagnosis with TNM staging:

Type of surgery: Mastectomy/ MRM

Side: Right/ Left

Gross features

Measurement: Skin:

Cut surface

Size: Appearance:

Consistency: Margins:

Histopathological diagnosis: IDC/ ILC/ MC

Histological Grading: I/ II/ II

Vascular invasion: Y/ N

Skin involvement: Y/ N

Associated with DCIS: Yes/ No:

Closest relevant margin to invasive tumor:

Axillary lymph node

Total number examined: No of LN positive:

pTNM staging:

Nottingham prognostic index: (0.2x size of tumor)+ LN's grade+ tumor grade =

(Nodes graded as no of nodes positive: 0 =1; 1-3= 2; >3= 3)

Immunohistochemistry:

1. E- cadherin:

Immunoscore= ____ % of positive cells x staining intensity.

Staining intensity: no staining (0), weak (1+), moderate (2+), strong (3+).

0= 0; 1+= 1-10%; 2+= 11-50%; 3+= 51-80%; 4+= >80%

Final score: 1,0 – Negative ; score 2,3- Positive.

2. Vimentin:

Immunoscore = ____% of positive cells x staining intensity [no staining (0), weak (1+), moderate (2+), strong (3+)].

A score of more than 30 is considered significant.

KEYS TO MASTER CHART

Case No- Case number

NOS- Not otherwise specified

DCIS- Ductal carcinoma in situ

N- No

Y- Yes

NA- Not attempted

Vimentin

% - % of tumor cells positive (500 cells counted)

Score= % of positivity x staining intensity

Vimentin positive- score >30

E cadherin

Score= % of tumor cells positive x staining intensity.

Final score= 0 = 0; 1- 5= 1; 6-8= 2; 9-12= 3

E cadherin positive tumors- final score- 2,3

E cadherin negative tumors- final score- 0,1

Case No	Hospital number/ biopsy no	Size of the tumor (cm)	Age	Nature of specimen Received	Histopathological Type	DCIS	Histopathological			Final grade	Nottingham prognostic index	skin involvement	Vascular invasion	Total no of lymph nodes	Number of lymph nodes positive	Pathological(TNM) staging	STAGE	Immunohistochemistry(IHC)			
							Tubule formation	Nuclear Atypia	Mitosis/10 hpf									Vimentin		E- cadherin	
																		%	Score (% positivity x intensity)	Score (% positivity x intensity)	Final score
1	830889/ B-1644-12	3.5X2.5X2.5	50	Modified Radical Mastectomy	Left Infiltrating ductal carcinoma- NOS	N	1	2	2	I	2.7	N	N	4	0	T2N0Mx	IIA	28	56	6	2
2	879988/ B-269-13	3.5X3X2	60	Modified Radical Mastectomy	Left Infiltrating ductal carcinoma- NOS	N	2	3	2	II	5.7	N	N	9	7	T2N1Mx	IIB	7.3	14.6	8	2
3	888120/ B-462-13	4X3X3	45	Modified Radical Mastectomy	Left Infiltrating ductal carcinoma- NOS	N	2	3	2	II	5.8	N	Y	7	4	T2N2MX	III	1.8	1.8	8	2
4	888134/ B-552-13	4X2X2	58	Modified Radical Mastectomy	Left Infiltrating ductal carcinoma- NOS	N	2	3	2	II	5.4	N	Y	8	3	T2N2MX	III	2.1	2.1	6	2
5	1015402/ B-989-14	3X2X0.8	62	Mastectomy	Right Infiltrating ductal carcinoma- NOS	N	1	2	2	II	NA	N	N	NIL	NIL	T2NxMX	IIA	11.9	11.9	9	3
6	889235/ B-594-15	1.7X1.3X0.8	49	Mastectomy	Right Infiltrating ductal carcinoma- NOS	N	2	2	2	II	NA	N	N	NIL	NIL	T1NxMx	I	34.2	68.4	3	1
7	109869/ B-1219-15	5X4X3.5	45	Modified Radical Mastectomy	Right Infiltrating ductal carcinoma NOS	N	2	1	2	I	3	N	N	2	0	T3N0Mx	IIB	7.1	14.2	8	2
8	827149/ B-1552-12	5X4X1.5	71	Mastectomy	Left Invasive lobular carcinoma	N	3	1	1	I	NA	N	N	NIL	NIL	T2NxMX	IIA	3.8	3.8	0	0
9	819116/ B-1461-12	4.5X4X3.3	40	Modified Radical Mastectomy	Left Medullary carcinoma	N	3	2	3	III	5.8	Y	Y	4	2	T4bN1Mx	IIIB	42	126	4	1
10	927149/ B-1542-13	5X3X1.8	71	Modified Radical Mastectomy	Left Invasive lobular carcinoma	N	3	1	1	I	3	N	N	7	0	T2N0Mx	IIA	8.9	8.9	0	0
11	771600/ B-436-12	3X2.5X1	51	Modified Radical Mastectomy	Left- Infiltrating ductal carcinoma NOS	N	2	1	2	I	4.6	N	N	10	10	T2N2aMx	III	2.2	4.4	4	1
12	815918/ B-1489-12	4X3X2	37	Modified Radical Mastectomy	Left- Invasive lobular carcinoma	N	3	1	1	I	4.8	N	N	14	14	T2N2aMx	III	4.8	4.8	0	0
13	815307/ B-1363-12	5X4X3	50	Modified Radical Mastectomy	Left Infiltrating ductal carcinoma- NOS	Y	2	2	3	II	6	N	N	11	9	T2N1Mx	IIB	10.4	20.8	6	2
14	827513/ B-1573-12	5x3x1.4	60	Mastectomy	Right Infiltrating ductal carcinoma- NOS	N	2	2	1	I	NA	N	N	NIL	NIL	T2NxMX	IIA	6.7	13.4	12	3
15	784592/ B-486-12	3X2X1	48	Modified Radical Mastectomy	Right Infiltrating ductal carcinoma- NOS	N	3	2	2	II	3.6	N	Y	4	0	T2N0MX	IIA	33.6	100.8	2	1
16	795926/ B-843-12	2X2X1	37	Modified Radical Mastectomy	Left Infiltrating ductal carcinoma- NOS	N	2	3	2	II	2.4	N	N	2	0	T2N0MX	IIA	20.8	62.4	1	1
17	124421 /B-773-15	3.2X1X1	53	Modified Radical Mastectomy	Right Infiltrating ductal carcinoma- NOS	Y	3	2	1	II	3.64	N	N	30	0	T2N0MX	IIA	24.2	48.4	6	2
18	92288/ B-06-15	2.3X1.6X1	37	Modified Radical Mastectomy	Right Infiltrating ductal carcinoma NOS	N	2	1	1	I	2.46	N	N	3	0	T2N0Mx	IIA	19.7	19.7	8	2
19	889235 / B- 594-13	1.8X1.5X1	45	Modified Radical Mastectomy	Right Infiltrating ductal carcinoma NOS	Y	2	3	3	III	4.3	N	N	1	0	T2N0Mx	IIA	34	78	6	2
20	62056/ B-2477-14	5.2X3X2	60	Modified Radical Mastectomy	Left Infiltrating ductal carcinoma NOS	N	2	2	1	I	5.04	N	Y	6	3	T3N1aMx	III	18	18	6	2
21	877307/ B-255/13	4X3.5X2.5	35	Modified Radical Mastectomy	Right Invasive lobular carcinoma	N	3	1	1	I	3.8	N	N	4	1	T2N1aMx	IIB	11.2	22.4	0	0
22	954790/ B-2123-13	5X2.2X2	45	Modified Radical Mastectomy	Right Infiltrating ductal carcinoma NOS	Y	2	1	1	I	7	N	N	17	14	T2N2aMx	III	4	4	6	2
23	911874/ B-997-13	3.5X2X1.5	40	Modified Radical Mastectomy	Right Infiltrating ductal carcinoma NOS	Y	2	2	2	II	2.7	N	N	3	0	T2N0Mx	IIA	48	144	6	2
24	90989/ B-110-15	4X2X1	48	Modified Radical Mastectomy	Left Infiltrating ductal carcinoma NOS	Y	2	3	3	III	5.8	N	N	9	1	T2N1Mx	IIB	38.9	116.7	2	1
25	678793/B-384-11	3x2x1.8	60	Modified Radical Mastectomy	Left Invasive Lobular carcinoma	N	3	1	1	I	2.6	N	N	3	0	T2N0Mx	IIA	5.3	5.3	0	0
26	96768/ B-1374-14	3X1.5X1.2	54	Modified Radical Mastectomy	Left Infiltrating ductal carcinoma NOS	Y	2	2	1	I	3.6	Y	Y	3	1	T4aNIaMx	IIIB	6	6	3	1
27	973001/ B-2391-13	4.5X2X1	60	Modified Radical Mastectomy	Right Infiltrating ductal carcinoma NOS	Y	3	2	3	III	5.9	N	Y	2	2	T2N1Mx	IIB	8.6	8.6	9	3
28	971659/ B-2359-13	7X4.5X2.5	45	Modified Radical Mastectomy	Right Infiltrating ductal carcinoma NOS	Y	2	1	2	I	4.4	N	Y	11	3	T3N1aMx	III	2.8	5.6	6	2
29	921133/ B-2063-13	4.5X2.8X2.5	52	Modified Radical Mastectomy	Right Infiltrating ductal carcinoma NOS	N	2	1	2	I	3.9	N	N	3	1	T2N1aMx	IIB	9.3	18.6	8	2
30	819116/ B-1461-12	4.5X4X3.4	63	Modified Radical Mastectomy	Right Medullary carcinoma	N	3	2	3	III	4.9	Y	Y	13	0	T4bN0Mx	IIIB	40	120	3	1
31	576341/ B-411-10	2X2X1	50	Modified Radical Mastectomy	Right Medullary carcinoma	N	3	3	3	III	4.4	N	N	4	0	T2N0Mx	IIA	23	23	0	0
32	163650/ B-1861-15	4X3X0.8	58	Modified Radical Mastectomy	Left Infiltrating ductal carcinoma NOS	N	2	1	2	I	2.8	N	N	7	0	T2N0Mx	IIA	4.7	9.4	9	3
33	104850/ B-311-15	4X3.5X2	50	Modified Radical Mastectomy	Left Infiltrating ductal carcinoma NOS	N	2	2	2	II	4.8	N	Y	6	1	T2N1Mx	IIB	11.9	23.8	12	3
34	14843/ B-1419-14	2X1.5X1.2	65	Modified Radical Mastectomy	Right Infiltrating ductal carcinoma NOS	N	2	2	2	II	4.4	N	N	13	2	T2N1aMx	IIB	3.8	7.6	6	2
35	991899/ B-430/14	2X1X1	35	Medial quadrentectomy	Right Infiltrating ductal carcinoma NOS	N	2	2	1	I	NA	N	N	NIL	NIL	T2NXMX	IIA	8.6	8.6	9	3
36	722385/B-1601-11	5.2X3X1.5	67	Modified Radical Mastectomy	Left Medullary carcinoma	N	3	3	2	III	5.04	N	Y	5	0	T3N0Mx	IIB	9.1	9.1	4	1
37	684860/ B-484-11	5.5X4.5X3	55	Modified Radical Mastectomy	Right Infiltrating ductal carcinoma NOS	N	2	2	3	II	6.1	Y	N	7	6	T4bN2aMx	IIIB	11.8	11.8	6	2
38	109750/ B-851-15	18x17.5x7	55	Modified Radical Mastectomy	Right Infiltrating ductal carcinoma NOS	N	3	3	2	III	9.6	Y	Y	16	16	T4bN2aMx	IIIB	28.3	84.9	2	1
39	148469/B-1403-15	11X9X5	45	Modified Radical Mastectomy	Right Infiltrating ductal carcinoma NOS	N	2	2	3	II	6.2	N	Y	23	3	T3N1aMx	III	6.5	13	3	1
40	660835/ B-2748-12	6X6X3	50	Modified Radical Mastectomy	Right Infiltrating ductal carcinoma NOS	N	2	1	1	I	3.2	N	N	5	0	T3N0Mx	IIB	5.9	5.9	6	2
41	566573/ B-80/11	5X5X4	85	Mastectomy	Right Infiltrating ductal carcinoma NOS	N	2	2	2	II	NA	Y	N	NIL	NIL	T4aNxMx	IIIB	8.3	8.3	9	3
42	726686/ B-1727-11	3X3X3	67	Modified Radical Mastectomy	Right Invasive lobular carcinoma	N		1	1	I	3.6	N	N	6	2	T2N1aMx	IIB	2.8	2.8	0	0
43	90989/ B-110-15	4X2X1	65	Modified Radical Mastectomy	Left Infiltrating ductal carcinoma NOS	Y	2	2	2	II	4.8	N	N	9	1	T2N1Mx	IIB	30.4	91.2	1	1
44	649523/ B-2553-10	3x1.5x1	60	Mastectomy	Right Medullary carcinoma	N	3	2	3	III	NA	N	N	NIL	NIL	T2NxMx	IIA	11.2	22.4	3	1
45	755965/ B-2487-11	7X4X3	74	Modified Radical Mastectomy	Right Infiltrating ductal carcinoma NOS	N	3	1	1	II	5.4	N	Y	8	1	T3N1Mx	III	8.8	21.3	6	2
46	62641/ B-2873-14	8X8X3	38	Modified Radical Mastectomy	Right Infiltrating ductal carcinoma NOS	Y	3	3	2	III	6.6	Y	N	4	3	T4aNIaMx	IIIB	90	270	6	2
47	868865/ B-84-13	4X3X2.3	70	Modified Radical Mastectomy	Left Infiltrating ductal carcinoma NOS	N	3	1	2	II	3.8	N	N	3	0	T2N0Mx	IIB	5.6	16.8	9	3
48	168678/B-2088-15	5.6X2.5X2	65	Modified Radical Mastectomy	Left Infiltrating ductal carcinoma NOS	N	2	3	2	II	6.12	Y	N	11	4	T4cN2aMx	IIIB	21.6	64.8	4	1
49	925479/ B-1355-13	4X3.3X2.5	43	Modified Radical Mastectomy	Right Infiltrating ductal carcinoma NOS	N	2	3	1	II	4.8	N	Y	7	2	T2Nl1aMx	IIB	17.6	52.8	6	2
50	169220/B-2040-15	4.8X3.6X2.3	40	Modified Radical Mastectomy	Left Infiltrating ductal carcinoma NOS	Y	2	2	2	II	3.96	N	N	8	0	T2N0Mx	IIA	1.8	1.8	12	3
51	176606/B-2072-15	2.5X1.5X1.2	65	Modified Radical Mastectomy	Left Infiltrating ductal carcinoma NOS	N	2	3	3	III	4.5	N	N	15	0	T2N0Mx	IIA	29.6	59.3	6	2
52	175651/ B-2071-15	4X2.5X2	48	Modified Radical Mastectomy	Left Infiltrating ductal carcinoma NOS	N	2	1	1	I	2.6	N	N	3	1	T2N1aMx	IIB	11.3	11.3	9	3
53	659784/ B-57-11	10X10X5	64	Modified Radical Mastectomy	Right Infiltrating ductal carcinoma NOS	Y	3	2	1	II	6	Y	Y	2	2	T4bN1Mx	IIIB	17.5	35	6	2
54	90989/B-223-15	3X2.5X2	65	Modified Radical Mastectomy	Left Infiltrating ductal carcinoma NOS	N	2	1	2	I	2.6	N	N	11	0	T2N0Mx	IIA	7.3	7.3	9	3
55	167964/B-2018-15	2.6X2.1X1	50	Modified Radical Mastectomy	Right Infiltrating ductal carcinoma NOS	N	2	1	2	I	2.52	N	N	7	0	T2N0Mx	IIA	4.5	9	12	3
56	120768/B-601-15	12X6.5X5.5	23	Modified Radical Mastectomy	Right Infiltrating ductal carcinoma NOS	N	3	2	1	II	6.4	N	Y	1	1	T3N1aMx	III	3.9	7.8	12	3
57	649523/ B-2594-10	10X8X4	57	Modified Radical Mastectomy	Left Medullary carcinoma	N	3	2	3	III	6	N	Y	5	0	T3N0xMx	IIB	24	48	0	0
58	689206/ B-525-11	8X5X4	49	Modified Radical Mastectomy	Right Infiltrating ductal carcinoma NOS	N	2	2	2	II	6.6	N	N	9	7	T3N2aMx	III	7.2	14.4	9	3