A STUDY OF HISTOMORPHOLOGICAL PROFILE OF TRIPLE-NEGATIVE BREAST CANCER

DISSERTATION

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

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In partial fulfillment of
the requirements for the degree of

M.D. (PATHOLOGY)

BRANCH – III

DEPARTMENT OF PATHOLOGY

TIRUNELVELI MEDICAL COLLEGE HOSPITAL

TIRUNELVELI - 627011

MAY-2018

CERTIFICATE

I hereby certify that this dissertation entitled **"A STUDY OF HISTOMORPHOLOGICAL PROFILE OF TRIPLE-NEGATIVE BREAST CANCER"** is a record of work done by **Dr. VIDHYA.M**, in the Department of Pathology, Tirunelveli Medical College, Tirunelveli, during her postgraduate degree course period from 2014- 2018. This work has not formed the basis for previous award of any degree.

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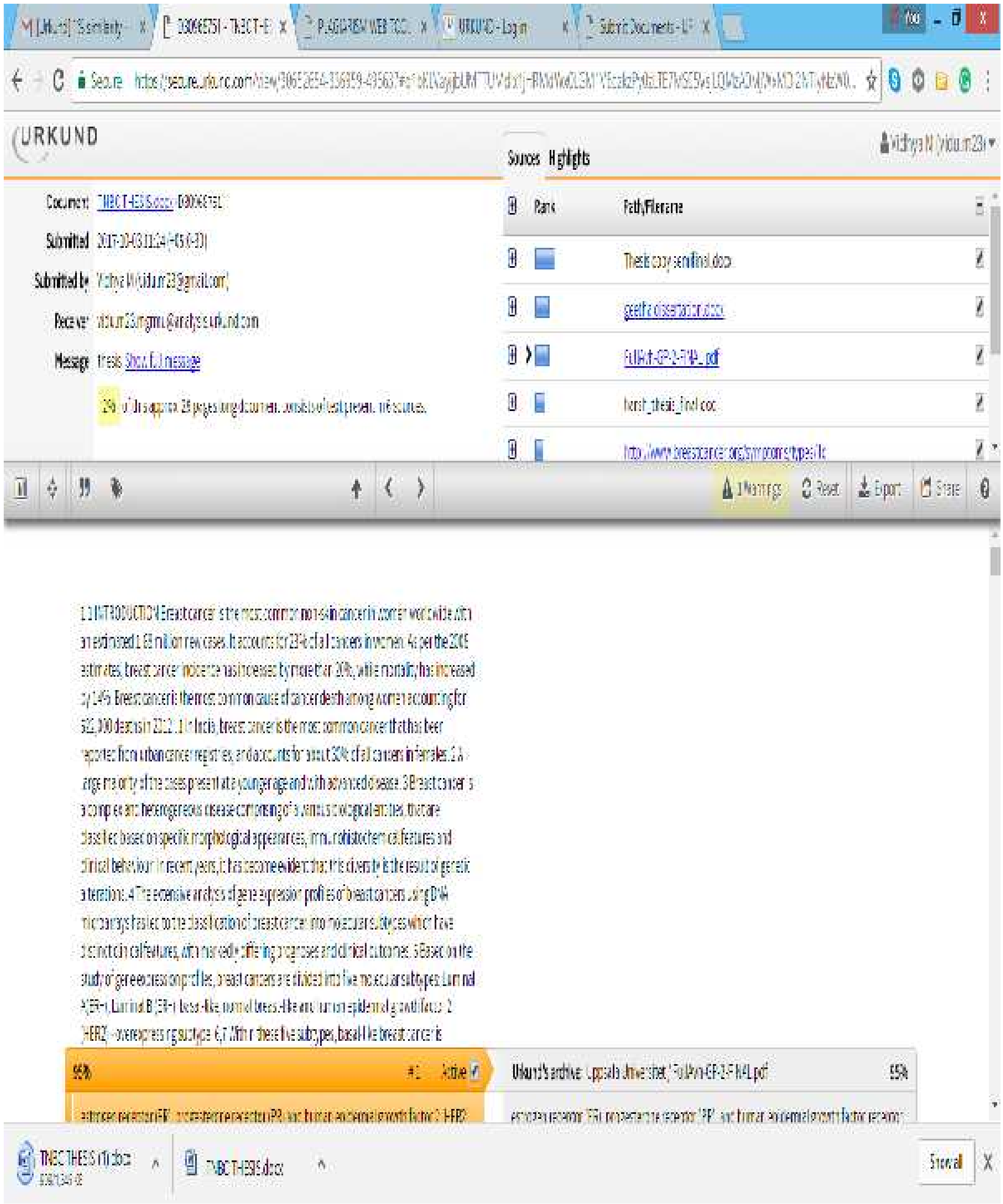
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DECLARATION

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ABBREVIATIONS

1.	ER	Estrogen Receptor
2.	PR	Progesterone Receptor
3.	HER2neu	Human Epidermal Growth Factor
4.	TNBC	Triple negative breast cancer
5.	CK	Cytokeratin
6.	EGFR	Epidermal growth factor receptor
7.	BRCA	Breast cancer, early onset.
8.	GEP	Gene expression profiling
9.	PTEN	Phosphatase and tensin homolog
10	ATM	Ataxia telangiectasia, mutated
11.	IDC, NOS	Invasive Ductal carcinoma, Not otherwise specified
12.	DCIS	Ductal Carcinoma In Situ
13.	MUC	Mucin
14.	PAS	Periodic acid Schiff
15.	GCDFP	Gross cystic disease fluid protein
16.	MIB1	Mindbomb E3 Ubiquitin Protein Ligase 1
17.	EMA	Epithelial membrane antigen
18.	CEA	Carcino embryonic antigen

19.	NPI	Nottingham Prognostic Index
20.	WHO	World Health Organization
21.	AJCC	American joint committee of cancer
22.	LN	Lymph Node
23.	H&E	Haematoxylin and Eosin
24.	CD	Cluster of differentiation
25.	PCR	Polymerase chain reaction
26.	FISH	Fluorescent in situ hybridization
27.	ASCO	American Society of Clinical Oncology
28.	CAP	College of American Pathologists
29.	IHC	Immunohistochemistry
30.	cDNA	Complementary DNA
31.	VEGF	Vascular endothelial growth factor
32.	TRIS - EDTA	Trizma base (Tris – hydroxyl methyl aminomethane) – Ethylene diamine tetra acetic acid
33.	HRP	Horse Radish Polymer
34.	DAB	Diamino-benzidine tetrachloride

CONTENTS

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PROTOCOL TITLE: A STUDY OF HISTOMORPHOLOGICAL PROFILE OF TRIPLE- NEGATIVE BREAST CANCER.

PRINCIPAL INVESTIGATOR: DR.M.VIDHYA.MBBS,

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Dear , Dr. M. Vidhya. MBBS, The Tirunelveli Medical College Institutional Ethics Committee (TIREC) reviewed and discussed your application during the IEC meeting held on 09.02.15.

THE FOLLOWING DOCUMENTS WERE REVIEWED AND APPROVED

1.	TIREC Application Form	☐
2.	Study Protocol	☐
3.	Department Research Committee Approval	☐
4.	Patient Information Document and Consent Form in English and Vernacular Language	☐
5.	Investigator's Brochure	☐
6.	Proposed Methods for Patient Accrual Proposed	☐
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12.	Clinical Trial Agreement (CTA)	☐
13.	Memorandum of Understanding (MOU)/Material Transfer Agreement (MTA)	☐
14.	Clinical Trials Registry-India (CTRI) Registration	☐

THE PROTOCOL IS APPROVED IN ITS PRESENTED FORM ON THE FOLLOWING CONDITIONS

1. The approval is valid for a period of 2 year/s or duration of project whichever is later
2. The date of commencement of study should be informed
3. A written request should be submitted 3weeks before for renewal / extension of the validity
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CERTIFICATE - II

This is certify that this dissertation work title **"A STUDY OF HISTOMORPHOLOGICAL PROFILE OF TRIPLE-NEGATIVE BREAST CANCER"** of the candidate **Dr. VIDHYA.M** with registration Number **201413307** for the award of **M.D.** Degree in the branch of **PATHOLOGY (III).** I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion page and result shows 2 percentage of plagiarism in the dissertation.

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INTRODUCTION

Breast cancer is the most common non-skin cancer in women worldwide with an estimated 1.68 million new cases. It accounts for 23% of all cancers in women. As per the 2008 estimates, breast cancer incidence has increased by more than 20%, while mortality has increased by 14%. Breast cancer is the most common cause of cancer death among women accounting for 522,000 deaths in 2012 .[1] In India, breast cancer is the most common cancer that has been reported from urban cancer registries, and accounts for about 30% of all cancers in females.[2] A large majority of the cases present at a younger age and with advanced disease.[3]

Breast cancer is a complex and heterogeneous disease comprising of a various biological entities, that are classified based on specific morphological appearances, immunohistochemical features and clinical behaviour. In recent years, it has become evident that this diversity is the result of genetic alterations.[4] The extensive analysis of gene expression profiles of breast cancers using DNA microarrays has led to the classification of breast cancer into molecular subtypes which have distinct clinical features, with markedly differing prognoses and clinical outcomes.[5]

Based on the study of gene expression profiles, breast cancers are divided into five molecular subtypes: Luminal A(ER+), Luminal B (ER+),basal-like, normal breast-like and human epidermal growth factor 2 (HER2) overexpressing subtype.[6,7] Within these five subtypes, basal like

breast cancer is characterized by absence of expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor 2 (HER2). It is associated with aggressive histomorphology, poor prognosis and unresponsiveness to the hormonal chemotherapy, shorter survival and BRCA1 association. Although triple negative breast cancer (TNBC) is universally used as a surrogate marker, triple negative and basal like are not synonymous.[8]

Triple-negative breast cancer (TNBC) is defined by the lack of expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor 2 (Her2*neu*) expression and associated with aggressive clinical course and poor prognosis. Two subtypes of triple-negative breast cancers have been described: basal and nonbasal.[5] Triple-negative breast cancer(TNBC) accounts for 10-17% of all breast cancers with increased incidence in premenopausal women.[9]

Basal type TNBC characteristically exhibits high histological grade, p53 mutation, in most instances expresses basal cytokeratin (CK5/6, CK14 and CK17), epidermal growth factor receptor (EGFR) overexpression, is significantly associated with Ki-67 labelling index, p53 expression, and BRCA1 expression, and show a shorter survival than nonbasal type. Gene expression profiling is the gold standard for the identification of basal type TNBC. Since GEP is costly, cumbersome to perform and requires fresh or frozen samples, it is still not feasible to perform GEP in low resource settings. Hence Rakha et al. suggested the use of immunohistochemical surrogates panel for the identification of basal-like subtype of TNBC. The panel includes four markers – ER, her2,

cytokeratin 5/6 and epidermal growth factor receptor EGFR which identifies basal-like TNBC with 100% specificity and 76% sensitivity.[10] This study is to apply the immunohistochemical panel to identify basal-like triple negative breast cancer in our setting.

AIM

To study the clinical, histomorphological characteristics of triple negative breast cancer and to identify basal-like triple negative cancer subtype using immunohistochemical panel of markers including CK5/6 and EGFR.

OBJECTIVES

1. To assess the clinicopathologic features of triple negative breast cancers.
2. To study the detailed histomorphological appearances of triple negative breast cancers using various parameters.
3. To apply an immunohistochemical markers panel using CK5/6 and EGFR for the identification of basal subtypes of triple negative breast cancers.
4. To assess the correlation between the basal markers CK 5/6 and EGFR expression and clinicopathologic parameters.

REVIEW OF LITERATURE

HISTORY

The paleopathologic findings from ancient mummies have indicated the existence of cancers in prehistoric times, proving the old saying that cancer is as old as the human race. The earliest written description of breast cancer is found in the Edwin Smith Papyrus which dates back to 3000 BC. Edwin Smith Papyrus describes breast cancer as bulging tumour of the breast which correlates with the present scientific descriptions of breast cancer. The Egyptians were the first who attempted to treat cancers. Later, Hippocrates (460 –375 BC) from Greece described that the growing tumours were caused by humoral imbalance and named them as carcinos meaning a tumour from the Greek word crab.[11]

CARCINOMA OF BREAST

Breast cancer is the most common cancer among women worldwide[12].The increased use of mammography has resulted in an increase in the early detection of breast cancers in the western countries. Although there is a reasonable reduction in the breast cancer mortality in the developed countries due to the early diagnosis and advances in the treatment, it is still on the rise in most of the Asian countries.[13]

EPIDEMIOLOGY

Breast cancer is the most common malignant tumour and it is the leading cause of cancer deaths across the world with more than one million cases occurring annually.[13] It is now regarded as the most common cancer both in developed and

developing regions with 690,000 new cases estimated in each region of the world. In India, among the cancers occurred in Chennai, breast cancer is the most common cancer with an age-standardized incidence rate of 30.8 per 100000 population.[14]

CLINICAL PRESENTATION

A large number of breast cancer cases present symptomatically. Recently because of the widely used screening methods including mammography, the incidence of asymptomatic cases has come to a rise. The symptomatic women present most commonly with breast lumps (60-70%), followed by pain (14-18%), nipple abnormalities like retraction and discharge (7-9%). Family history is seen in about 3-14% of the cases. The least common presenting symptom being inflammation (1%). The breast tumours are most commonly found in upper outer quadrant of the breast (40-50%), followed by central, upper inner, lower outer to lower inner quadrant.[15]

All the symptomatic breast diseases should be assessed using triple assessment that includes clinical examination, imaging studies(mammography, ultrasound) and tissue sampling (fine needle aspiration cytology or core needle biopsy).[15]

RISK FACTORS FOR BREAST CANCER

AGE

Breast cancer is extremely rare in young women aged below 25 years excepting some familial cases. Although breast cancer is rare in young age group

females, half of those affected are either ER negative or HER2/*neu* positive in the Western population. About 77% of breast cancers occur in women over 50 years of age with average age at diagnosis being 64 years. The incidence rises throughout the lifetime of a woman. [16]

AGE AT MENARCHE AND MENOPAUSE

Early age at menarche has been consistently associated with an increased risk of breast cancer[17]. The estimated decrease in risk per five year delay in menarche is 22%[18]. Late menopause occurring after the age of 55 years doubles the risk of breast cancer than those who attain menopause before the age of 45 years[19].

AGE AT FIRST CHILDBIRTH AND PARITY

Childbearing at a younger age lowers the risk of breast cancer. First childbirth at a younger age of <30 years and multiple pregnancies reduces the risk of breast cancer. The relative risk of developing breast cancer is estimated to increase by 3% for each year of delay. The risk of breast cancer is reduced by 7% with each full term delivery and overall multiparous women have a 30% lower risk of developing breast cancers than nulliparous women.[20]

RACE AND ETHNICITY

The incidence of mortality due to breast malignancies is more in African – American women who present at a more advanced stage at diagnosis when compared to white women[21]. A large number of breast cancer cases are diagnosed in black women younger than 40 years of age, and the breast tumours

occurring in black women exhibit higher nuclear grade, frequently do not express hormone receptors, and have sporadic mutations in p53 gene[22].

BREAST FEEDING

Breast feeding has consistently been associated with reduction in the risk of breast cancer. The longer duration a woman breastfeeds, the greater is the protection against breast cancer, and the risk is reduced by 4% for every 12 months of breastfeeding. In addition, the risk of breast cancer in women who had breastfed for more than two years was 33% lower than those who had never breastfed[23]. On the other hand, the higher incidence of breast cancer in developed countries has been attributed to the lack of breast feeding or short lifetime duration of breast feeding in these countries[19].

PROLONGED EXPOSURE TO EXOGENOUS ESTROGEN

According to recent studies, women undergoing hormone replacement therapy with combined estrogen and progestin have an increased risk of developing breast cancer[16].

RADIATION THERAPY

Ionized radiation forms an established risk factor for breast cancer. The risk of developing breast cancer was the highest in women who had increased number of exposures and at a younger age[24].

PREVIOUS BREAST DISEASE

Women with history of benign breast diseases have increased risk of developing breast cancer. Women who had proliferative disease without atypia on biopsy specimens have a two-fold increase and proliferative disease with atypia have a five-fold increase in risk of breast cancer development[25].

GENETIC AND FAMILY HISTORY

Women who have two or more first-degree relatives affected at an early age have four-fold increase in the risk of development of breast cancer. Several studies have shown consistent link between risk of breast cancer development and inherited mutations in many genes like BRCA1, BRCA2, p53, PTEN and ATM. The breast cancer genes, BRCA1, located on the long arm of chromosome 17 and BRCA2, which is located on the long arm of chromosome 13, have been identified and mutations in these genes are established risk factors of breast cancer. Inherited mutations in p53 and PTEN genes are associated with familial syndromes like Li-Fraumeni and Cowden syndrome, and are at high risk of developing breast cancer[26].

OBESITY

Overweight and obesity, as defined by the measurement of body mass index (BMI), are associated with increased incidence of breast cancers. The risk is greater in postmenopausal women than in premenopausal women[27].

DIET

Several epidemiological studies have suggested that an increased consumption of dietary fat is associated with an increase in breast cancer risk[28].

SMOKING AND ALCOHOL CONSUMPTION

A meta-analysis study has found that the risk is higher in women who start smoking at younger age, before the age of 20 or before the birth of their first child[29]. Alcohol intake is consistently associated with increased risk of breast cancer and the risk is dose-dependent regardless of the type of alcohol consumed[30].

PHYSICAL ACTIVITY

Several studies have found a strong association between physical activity and breast cancer, with about 15-20% reduction of risk of developing breast cancer in the most active women, with the strongest association shown for post-menopausal women[31].

INVASIVE CARCINOMA OF BREAST

Microscopically, invasive carcinoma of breast is classified into various special types depending on the particular pattern they exhibit. Approximately 75% of the carcinomas do not represent any special types and they are labelled as invasive ductal carcinoma, not-otherwise-specified (NOS) type.[13]

WHO CLASSIFICATION OF INVASIVE CARCINOMA OF BREAST

1. Invasive ductal carcinoma, not otherwise specified
    a. Pleomorphic carcinoma
    b. Carcinoma with melanotic features
    c. Carcinoma with choriocarcinomatous features
    d. Carcinoma with osteoclast like giant cells
2. Invasive lobular carcinoma
    a. Classic lobular carcinoma
    b. Solid lobular carcinoma
    c. Alveolar lobular carcinoma
    d. Pleomorphic lobular carcinoma
    e. Tubulolobular lobular carcinoma
    f. Mixed lobular carcinoma
3. Tubular carcinoma
4. Cribriform carcinoma
5. Carcinoma with medullary features
    a. Medullary carcinoma
    b. Atypical medullary carcinoma
    c. Invasive carcinoma NST with medullary features
6. Metaplastic carcinoma of no special type
    a. Low-grade adenosquamous carcinoma
    b. Fibromatosis-like metaplastic carcinoma
    c. Squamous cell carcinoma

d. Spindle cell carcinoma

e. Metaplastic carcinoma with mesenchymal differentiation

i. Chondroid differentiation

ii. Osseous differentiation

iii. Other types of mesenchymal differentiation

f. Mixed metaplastic carcinoma

g. Myoepithelial carcinoma

7. Mucinous carcinoma
8. Carcinoma with signet-ring-cell differentiation
9. Carcinoma with neuroendocrine features
10. Carcinoma with apocrine differentiation
11. Invasive papillary carcinoma
12. Invasive micropapillary carcinoma
13. Adenoid cystic carcinoma
14. Mucoepidermoid carcinoma
15. Salivary gland/skin adnexal type tumours
16. Polymorphous carcinoma
17. Inflammatory carcinoma
18. Bilateral breast carcinoma and non-synchronous breast carcinoma
19. Exceptionally rare types and variants

Secretory carcinoma

Oncocytic carcinoma

Sebaceous carcinoma

Lipid-rich carcinoma

Glycogen-rich clear cell carcinoma

Acinic cell carcinoma [32]

1. INVASIVE DUCTAL CARCINOMA, NOT OTHERWISE SPECIFIED

Invasive ductal carcinoma of NOS type is a heterogeneous group of tumours that do not have characteristics that are specific to any special type of carcinoma to be classified as special types such as tubular, mucinous, medullary or lobular carcinoma. It is the most common type of infiltrating breast cancers which constitute about 47-75% of the cases.[33] It is considered to be the prototype of all breast cancers.[34]

GROSS FEATURES:

Grossly, the tumour varies in size and shape and presents as an ill circumscribed, firm to hard mass. When cut, it gives a gritty feel. The cut surface is greyish- yellow with a stellate outline produced by the trabeculae radiating into the surrounding fat through the parenchyma. In larger tumours, there may be areas of necrosis, cystic degeneration and haemorrhage seen. The cut surface of these tumours, sometimes show a few ‘chalky streaks’, which are due to the duct elastosis rather than necrosis.

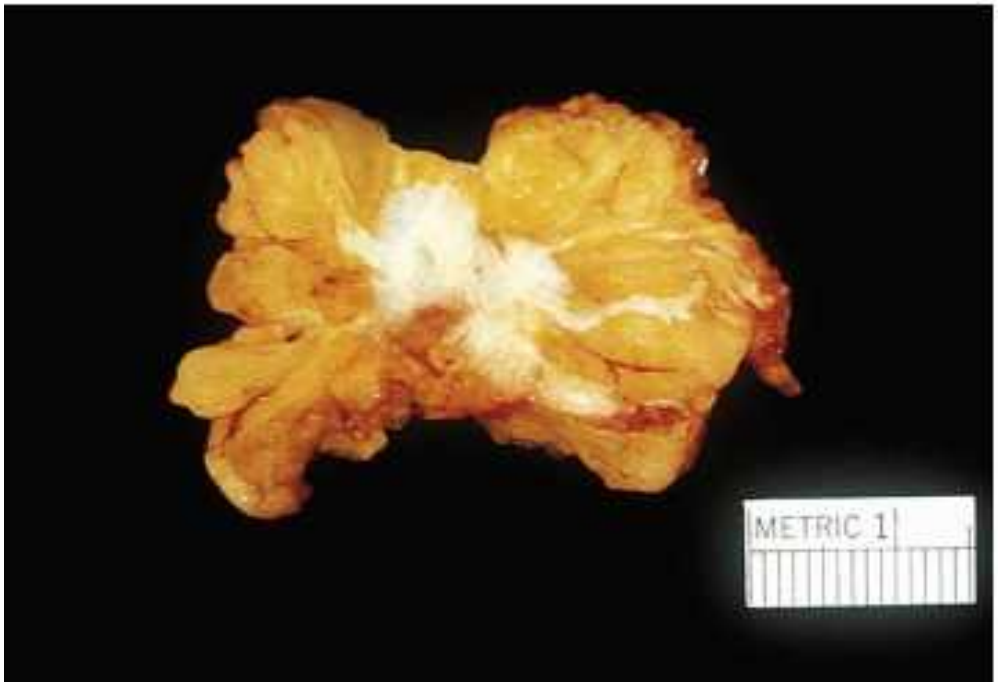


FIG. 1(A) - INVASIVE DUCTAL CARCINOMA- CUT SURFACE SHOWING WHITISH IRREGULAR MASS WITH CHALKY STREAKS.

MICROSCOPY:

Microscopically, the tumour cells grow in varying patterns such as diffuse sheets, cords, nests, trabeculae and even as individual cells. Glandular differentiation may be marked, focally present or totally absent. The tumour cells are large, pleomorphic, sometimes with multinucleated tumour giant cells. They show characteristic features of malignancy like prominent nuclei and nucleoli with numerous mitotic figures. About 60% of the cases show areas of necrosis. Focal areas of squamous, apocrine metaplasia or clear cell change may be present. As in any other carcinomas, the amount of stroma varies from nil to abundant. The stroma ranges from fibrotic to desmoplastic in appearance, with

desmoplastic stroma being found in most of the cases. Stromal elastosis and foci of periductal and perivenous elastosis may also be present. Calcifications in the form of both coarse or fine granules and rarely psammoma bodies are seen in about 60% of the cases. The interphase between tumour and stroma, often shows lymphoplasmacytic inflammatory infiltrate. Fisher et al., found angioinvasion, perineural invasion and lymphatic invasion in 33%, 28% and 5% respectively.[13]

In up to 80% of cases, foci of in situ component may be present, however, the relative proportion of invasive carcinoma and in situ component may largely vary in individual cases.[33]

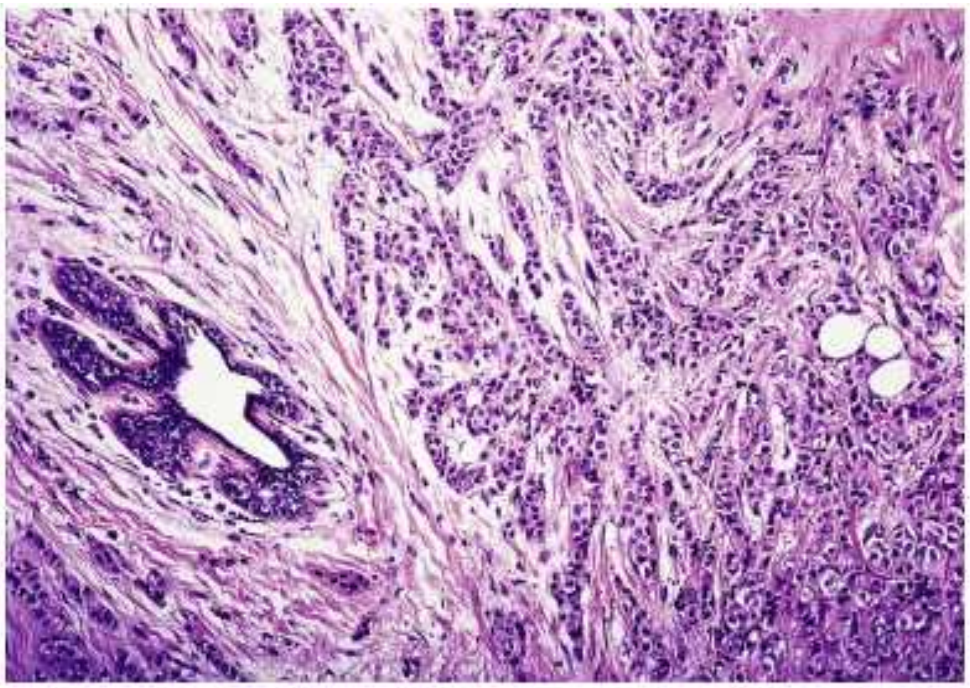


FIG. 1(B): PROTOTYPICAL INVASIVE DUCTAL CARCINOMA

2. INFILTRATING LOBULAR CARCINOMA:

Infiltrating lobular carcinoma is the second most common type of breast cancer constituting to about 5-15% of the cases.[33]

HISTOLOGIC VARIANTS:

CLASSIC FORM:

The malignant cells are small, less pleomorphic and are arranged singly, in a linear single file pattern, narrow trabeculae within the stroma. It may also show a 'pagetoid'/ concentric growth pattern around the lobules exhibiting lobular neoplasia in situ. Often, the stroma appears dense and fibrous with periductal and perivenous elastosis.[13]

PLEOMORPHIC VARIANT:

The growth pattern of this variant is similar to that of classic form with lack of cohesion. The tumour cells exhibit nuclei that are highly pleomorphic, anaplastic and show high mitotic rate. It sometimes, shows apocrine differentiation and signet ring morphology.[13]

SOLID VARIANT:

It accounts for about 10% of lobular carcinoma cases. The tumour cells are similar to classic type, but are arranged in diffuse sheets rather than single files with very less amount of intervening stroma.[33]

ALVEOLAR VARIANT:

It is an uncommon variant of lobular carcinoma in which the tumour cells with typical lobular features are arranged in globular clusters of 20 or more cells in each cluster.[33]

TUBULOLOBULAR VARIANT:

In this variant, the overall infiltrative pattern of lobular carcinoma cells is admixed with small tubules showing minute or undetectable lumen. Often, there is presence of in situ component, which may be of ductal, lobular or mixed type.[13]

HISTIOCYTOID CARCINOMA:

It is composed of tumour cells growing in a diffuse pattern with individual cells showing abundant, granular and foamy cytoplasm. It is currently considered a variant of invasive lobular carcinoma with apocrine differentiation.[13]

IHC:

About 80-95% of invasive lobular carcinomas are positive for ER and 65-75% is positive for PR. 70-75% of the tumours are positive for both ER and PR. About 85-90% of invasive lobular carcinoma cases show loss of E-cadherin expression.[35]

3. TUBULAR CARCINOMA:

Tubular carcinoma is an uncommon histologic type of breast carcinoma. It accounts for about 1-4% of all breast malignancies.[32]

GROSS:

Macroscopically, tubular carcinoma is indistinguishable from the invasive ductal carcinoma. It has poorly circumscribed margins and hard in consistency. Characteristically, the size of the tumour is small ranging from 2mm to 1.5 cm. Most tumours are less than 1 cm in size.

MICROSCOPY:

Microscopically, it is difficult to differentiate from benign conditions like radial scar and microglandular adenosis due to the well differentiated appearance of tubular glands, scarcity/absence of pleomorphism, necrosis and mitoses. The tubules are arranged haphazardly in the stroma and are often angulated. The stroma is often cellular and desmoplastic in nature. The tubules may show intraluminal basophilic secretions, apocrine snouts in the apical cytoplasm. The tumour may infiltrate into the adjacent adipose tissue, at the periphery.

The term ‘tubular carcinoma’ is best reserved for the tumours showing at least 90% of tubular pattern.[36] These tumours show a favourable prognosis. DCIS is frequently present in association with tubular carcinoma. The in situ component is usually of low grade showing cribriform or papillary pattern.[37] The tubular carcinoma can be seen associated with invasive ductal carcinoma, NOS type or sometimes with invasive lobular carcinoma, which leads to dilemma in making a diagnosis. In such instances, the term tubular NOS and tubular mixed can be employed. Mixed tubular carcinomas are associated with increased tumour size, increased nodal metastases and worse clinical outcome.[38]

Immunohistochemically, these tumours are 90% of these tumours are positive for ER, 70-80% are positive for PR and rarely they show HER2 overexpression.

4. CRIBRIFORM CARCINOMA:

Invasive cribriform carcinoma is a relatively rare neoplasm of breast which is similar to tubular carcinoma and shares a favourable prognosis with it. This tumour shows a cribriform appearance of the invasive component, reminiscent of the in situ counterpart, which is frequently associated with it. Cribriform pattern is often associated with tubular formations, but the relative proportion of both determines the terminology of the tumour, as proposed by Page et al.,[13]

5. MUCINOUS CARCINOMA:

Mammary mucinous carcinomas are also described as colloid, mucoid, or gelatinous carcinomas. It is characterized by the production of abundant mucin, both intracellular and extracellular.

Macroscopically, these tumours are characteristically sharply circumscribed, soft tumours with gelatinous and glistening cut surface.

Microscopically, the tumour cells are arranged in clusters and islands composed of 10-20 cells in lakes of extracellular mucin. The mucin stains positive with mucicarmine, MUC2 and MUC6. The term mucinous carcinoma is employed only when at least 90% of the tumour shows mucinous appearance.[39]

Immunohistochemically, these tumours are always positive for hormone receptors whereas HER2neu is almost always negative.[40]

6. CARCINOMA WITH SIGNET-RING CELL DIFFERENTIATION:

Signet-ring cell carcinoma is a type of malignancy in which the cells show abundant intracellular mucin which pushes the nucleus to one side of the cell, resembling the characteristic signet-ring appearance. It is important that primary invasive breast carcinomas with signet-ring cell morphology to be differentiated from metastases from other organs.[39]

7. CARCINOMA WITH MEDULLARY FEATURES:

`The tumours of the breast which show medullary features are typically associated with triple negative phenotype i.e., lack of expression of ER, PR and lack of her2 amplification. These tumours have been found to be associated with carriers of BRCA1 germ line mutation, which shows worse prognosis. The medullary-like carcinoma is associated with expression of basal cytokeratins, which leads to them being classified as basal-like breast cancers.[41]

Grossly, these tumours are well circumscribed and have soft and uniform consistency.

Microscopically, there are three major morphologic criteria, for a tumour to be classified as medullary-like carcinoma. They are, more than 75% of the tumour cells to be arranged in syncytial network, absence of fibrosis, prominent stromal lymphocytic infiltrates, lack of gland formation, and well circumscribed tumour with pushing margins rather than infiltrative margins. The cells have

abundant cytoplasm, pleomorphic vesicular nuclei with one or more prominent nucleoli. Mitotic rate is high with numerous giant mitoses.[33]

8. METAPLASTIC CARCINOMA:

Metaplastic carcinoma represents a group of rare primary breast malignancies, which exhibit either an admixture of epithelial and mesenchymal components including chondroid, osseous, spindle cell and rhabdomyoid cells, or purely the mesenchymal elements. Immunohistochemically, metaplastic carcinomas have been found to be negative for ER, PR, her2neu expression and they express basal cytokeratins like CK5/6 and EGFR.[39]

9. INVASIVE PAPILLARY CARCINOMA:

Invasive papillary carcinoma of the breast is a very rare neoplasm, constituting about <1% of all breast malignancies.[36] The major differential diagnosis include papillary carcinoma in situ, encysted papillary carcinoma and benign papilloma with ductal carcinoma in situ.[33]

The diagnostic criteria include the presence of papillary pattern of tumour cells constituting more than 90% of the tumour area. Microscopically, the invasive component of the tumour shows papillary architecture with fibrovascular cores lined by malignant epithelial cells.[39]

10. INVASIVE MICROPAPILLARY CARCINOMA:

Pure micropapillary carcinoma is an extremely rare tumour, constituting about <1% of all the tumours of breast. The histopathological appearance is distinctive, with formation of pseudo papillary structures without the

fibrovascular cores and tubular structures floating in clear empty spaces. The tumour cell clusters exhibit a distinctive ‘inside out’ appearance due to the inversion of polarity which can be evidenced by the pattern of MUC staining. These tumours have increased propensity for lymphatic invasion with nodal metastases and high local recurrence and are associated with a poorer prognosis.[42]

Immunohistochemistry and genomic studies have identified that majority of invasive micropapillary carcinomas express ER and have led to the classification of these tumours as luminal A or B subtype.

11. CARCINOMA WITH APOCRINE DIFFERENTIATION:

It is a very rare tumour that constitutes about 0.5% of invasive breast carcinomas. Apocrine differentiation has been found in association with invasive carcinoma NST and various special type carcinomas including lobular, tubular, papillary and medullary carcinomas. [32]

Microscopically, it is characterized by cells with abundant granular eosinophilic cytoplasm which is positive for PAS and diastase resistant or cells with foamy cytoplasm due to intracellular lipid or both. The nuclei are enlarged with prominent nucleoli.

Immunohistochemical staining shows characteristic ER and PR negativity and HER2 positivity. They are positive for androgen receptor and GCDFP-15.[43,44]

12. SECRETORY CARCINOMA:

Secretory carcinomas are exceptionally rare in the breast and they account for less than 0.15% of all breast cancers. It is primarily found in children and young adults.[32] Grossly, the tumour is usually small and well-circumscribed. Microscopically, it shows three patterns including solid, tubular and microcystic. The presence of both intracellular and extracellular rounded vacuoles of varying sizes containing secretory material which stains positive with alcian blue and PAS with diastase digestion is diagnostic of secretory carcinoma.

Immunohistochemically, secretory carcinomas are usually triple negative that is ER, PR and HER2 negative along with p63 negativity.[45]

13. CARCINOMA WITH NEUROENDOCRINE FEATURES:

Primary neuroendocrine tumours of the breast constitute for about 1-4% of all invasive breast malignancies. They are designated as neuroendocrine carcinomas when the tumours express neuroendocrine markers in more than 50% of its cell population.[32]

Histopathologically, these tumours are classified as well-differentiated neuroendocrine carcinomas, poorly differentiated/ small cell carcinoma, invasive breast carcinoma with neuroendocrine differentiation.

Immunohistochemical staining of these tumours shows expression of chromogranin, synatophysin and neuron specific enolase. Electron microscopy demonstrates the presence of dense core granules.[33]

14. SPREAD RELATED VARIANTS:

INFLAMMATORY CARCINOMA:

The term inflammatory carcinoma is entirely based on the characteristic clinical features in which the breast appears warm, red and shows edema of the overlying skin, reminiscent of mastitis. The appearance of inflammatory carcinoma is essentially due to the carcinomatous involvement of dermal lymphatic channels. The occurrence of clinical inflammatory appearance may or may not show microscopic dermal lymphatic invasion by tumour cells and its reverse is also possible, wherein the tumour showing histopathologic permeation by tumour cells, of the dermal lymphatic vessels may or may not have a clinical inflammatory carcinoma.

The presence of microscopic dermal lymphatic invasion is a sign of poor prognosis, irrespective of whether the patient has clinical features of inflammatory carcinoma or not.[13]

According to a study by Charafe-Jauffret et al., inflammatory carcinoma is found to be ER negative and positive for E-cadherin, MIB1, MUC1 and HER2neu.

PAGETS DISEASE:

Paget disease of the nipple is a crusted lesion of the nipple that is almost always associated with high grade ductal carcinoma in situ, with or without stromal invasion. It occurs in about 2 % of all breast cancers, clinically presenting as an eczematous or erythematous ulcerating rash of the nipple.

Clinically, it is indistinguishable from benign eczematous dermatitis. Hence, these patients should be thoroughly examined for breast malignancies.

Microscopically, the epidermis shows single or small clusters of large pleomorphic cells with abundant, often clear cytoplasm, usually in the basal layer. These cells stain positive for PAS positive diastase- resistant mucin.

Immunohistochemistry shows EMA, CEA, MUC1, CK7 and HER2 positivity, whereas the tumour cells are negative for high molecular weight cytokeratins and melanoma specific markers such as S-100, HMB45 and MELAN-A.[13]

PROGNOSTIC AND PREDICTIVE FACTORS IN BREAST CANCER

1. SIZE OF THE TUMOR:

The gross tumour size is one of the most significant independent prognostic factors in breast cancers. With increasing size of the tumour, the incidence of axillary nodal metastases increases and the survival decreases.

T1 Tumour≤20 mm in greatest dimension

T1mi Tumour≤1 mm in greatest dimension

T1a Tumour>1 mm but ≤5 mm in greatest dimension

T1b Tumour>5 mm but ≤10 mm in greatest dimension

T1c Tumour>10 mm but ≤20 mm in greatest dimension

T2 Tumour>20 mm but ≤50 mm in greatest dimension

T3 Tumour>50 mm in greatest dimension

T4b Tumours that are of any size and show direct extension to the skin over the breast resulting in ulceration.

T4d includes tumours of any size with permeation into dermal lymphatic channels, which is an ominous prognostic sign and inflammatory carcinoma.

According to the Nottingham/Tenovus Primary Breast Cancer Study (NTPBCS), the tumour size is considered an important independent variable and hence it forms an integral component of the Nottingham Prognostic Index (NPI). NPI is an important prognostic factor for management purposes, which incorporates tumour size, lymph node status and histologic grade as a continuous variable.[36]

2. LYMPH NODE METASTASIS

Lymph node status is one of the most significant independent prognostic factors in breast carcinomas and it should be assessed on histopathological examination rather than clinical examination.

Many studies have confirmed that the patients who have microscopic involvement of regional lymph nodes have a poorer prognosis than those without regional node involvement.[46] Furthermore, the prognosis depends on the number of nodes involved, the presence or absence of extra nodal involvement and the size of the nodal metastasis. [33]

Lymph node staging is divided into three categories: stage 1 in which no node is involved; stage 2 which shows involvement in up to 3 axillary lymph

nodes or a single internal mammary node involvement; stage 3 tumours which show 4 or more positive lymph nodes.

3. HISTOLOGIC TYPE

The tumours that belong to special types like tubular carcinoma,[37] mucinous carcinoma,[40] tubulolobular carcinoma,[47] invasive cribriform carcinoma,[36] medullary carcinoma, classic lobular carcinomas are identified to have better prognosis than invasive carcinoma, NST type.[48] The variants like pleomorphic lobular carcinoma, basal-like carcinomas and signet ring cell type carcinomas, usually show a poorer prognosis.

4. HISTOLOGIC GRADE

The first description of histologic grading of ductal carcinoma was by Greenhough in the year 1925.

The most commonly used grading system in the Scarff-Bloom-Richardson grading system. It is based on three morphologic features namely, tubule formation, nuclear grade and mitotic rate. It classifies breast carcinomas into two major groups, which includes low grade and high grade breast carcinomas.

Later, the Elston-Ellis modification of the Scarff-Bloom-Richardson system was made and till date, this has been the most preferred grading system all over the world, for the grading of breast cancers. This system uses scoring system for the morphologic features and hence it is a semi quantitative grading system. It uses score 1 to 3 for each of the features. It is also called as Nottingham grading system.[36] It is the recommended system for

grading of breast cancer by various international bodies including world health organization (WHO) and American joint committee of cancer (AJCC).[32]

TABLE 1(A): SCORING OF TUBULE FORMATION

PERCENTAGE OF TUBULES WITHIN TUMOR	SCORE
>75%	1
10-75%	2
<10%	3

TABLE 1(B): SCORING OF NUCLEAR PLEOMORPHISM

NUCLEAR FEATURES	SCORE
Small, uniform nuclei	1
Moderate increase in size/ variation	2
Marked variation	3

TABLE 1(C): SCORING OF MITOTIC COUNT PER 10 HPF

FIELD DIAMETER 0.59MM/0.274mm^2	FIELD DIAMETER 0.44 mm/0.152mm^2	SCORE
0-9	0-5	1
10-19	6-10	2
>20	>11	3

TABLE 1(D): FINAL GRADING OF NOTTINGHAM MODIFICATION OF SCARFF BLOOM RICHARDSON SYSTEM

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

The Nottingham grading of breast cancer is used along with tumour size and lymph node stage in the Nottingham prognostic index, and is a effective tool for stratification of treatment strategies for various prognostic groups of patients.[33]

5. ANGIOLYMPHATIC INVASION:

Lymphovascular invasion is one of the most significant prognostic factor in breast cancers, as it is one of the important step in the pathogenesis of metastatic breast cancer, leading to increased morbidity, mortality and decreased long-term survival of the patient.[49] Histopathologically, the lymphovascular invasion is assessed using Haematoxylin and Eosin stained sections of tumour and peritumoral region. The presence of tumour cells within the lumen of intratumoral and peritumoral lymphatics and blood vessels which are lined by endothelial cells is considered to be positive for lymphovascular invasion. But, whether the emboli are involving a blood vessel or lymphatic channel, cannot be differentiated by routine histopathological examination. The use of

immunohistochemical stains specific for endothelial cells of lymphatics, like D2-40 and anti-podoplanin is applied for differentiating blood vessel and lymphatic emboli[50].

Lymphatic invasion has been observed in 44% of LN negative and 86% of LN positive accounting to 66% of overall breast cancer patients, as demonstrated by Kahn et al.,[51,52]

The presence of blood vessel invasion is associated with an increased risk of early recurrence in operated lymph node negative breast cancer patients. Thus, lymphovascular invasion forms an important predictive factor for local recurrence and nodal metastasis after conservative surgeries in early breast cancers and flap recurrence in mastectomised patients.[53]

6. TUMOR NECROSIS

Spontaneous tumour necrosis is associated with high histologic grade and earlier lymph node metastasis. It is also associated with decreased long term survival.

Tumour necrosis is commonly seen in tumour of ductal type with high histologic grade and basal phenotype.[13]

7. STROMAL FEATURES

Tumours which show absence or decreased inflammatory infiltrates at the invasive front are associated with better prognosis, lesser incidence of lymph node metastasis and survival.[13]

Stromal fibrosis has been associated with variable prognosis, according to various semi-quantitative studies, from favourable outcome to poor disease-free

survival. Stromal elastosis is yet another variable stromal elastic fibres in cases of breast cancer. Elastosis can be periductal or diffuse in nature. Stromal elastosis, by itself is not an independent prognostic factor, though some studies have shown good prognosis in cases with elastosis.

8. INSITU COMPONENT

Some studies have shown that the presence of prominent in situ component associated with invasive carcinomas bodes better prognosis and decreased lymph node metastases.

9. OTHER HISTOLOGIC PROGNOSTIC FACTORS

INFLAMMATION

Prominent lymphocytic or lymphoplasmacytic infiltration has been associated with good prognosis. Grade 3 ductal carcinoma NST with prominent inflammation has been found to have better prognosis than grade 3 ductal carcinoma NST without prominent inflammation. [54]

MICROVESSEL DENSITY

Increased microvascular density is found to be associated with earlier lymph node metastasis and decreased long-term survival in node-negative carcinomas.[55]

APOPTOTIC INDEX

Apoptosis is characterized by shrinkage of cells and pyknotic and karyorrhectic nuclei with cytoplasmic blebbing. Apoptotic index is counted on H&E stained sections similar to mitotic index. Immunohistochemical stains can also be used to count mitotic index, like caspase 1 and 3 annexin V cleaved

CK18 and CD95, and terminal deoxynucleotidyl transferase–mediateddigoxigenin-11–deoxyuridine triphosphate nick end labeling (TUNEL) method. Although High apoptotic index is associated with higher grade of tumour, higher proliferative activity and lack of expression of hormone receptors,[56] it does not qualify to be an independent prognostic factor for breast cancers. [57]

PERINEURAL INVASION

Perineural invasion is found in about 10-25% of breast carcinomas and is associated with high grade tumours and lymphovascular invasion. But it is not proved to be independently associated with prognosis.

IMMUNOHISTOCHEMICAL TUMOR MARKERS:

HORMONE RECEPTORS

Estrogen and progesterone are steroid hormones that play an important role in the normal glandular development and in breast cancer progression. The presence of hormone receptors namely estrogen and progesterone receptors, in the tumour tissue of breast correlates with the response to hormonal and chemotherapy. Estrogen and progesterone mediate their effects on breast tissue by binding to specific nuclear receptors, leading to transcriptional regulation (activation or repression) of target genes[58]. Estrogen receptor occurs in two different forms namely α and β. While ERα plays an important role in ductal elongation during puberty, PR and ERβ are important for lactational development of lobules[59].

Studies have found that ER-negative breast malignancies are associated with histologic grade 3, pushing margins, comedo-necrosis, central fibrosis and lymphocyte-rich stroma[60]. Mucinous carcinoma, tubular and lobular carcinomas are associated with increased ER-positivity whereas most of the cases of medullary, apocrine and metaplastic carcinomas show ER-negativity.[32]

ER is positive in 70% - 95% of invasive lobular carcinomas and 70% - 80% of invasive ductal carcinomas, whereas PR is positive in 60%- 70% of invasive breast carcinomas[61]. Although the presence of ER and PR in an invasive breast carcinoma is considered as an independent prognostic and predictive factor[48,62] ; Liu et al., 2010), both lose their prognostic value after long-term follow-up.[63–65]

Hormone receptors are evaluated in formalin fixed paraffin-embedded breast tissues using various methods including immunohistochemistry, fluorescent in situ hybridization and PCR,[13] of which immunohistochemistry is the most widely used technique.

ASSESSMENT OF HORMONE RECEPTORS:

Immunohistochemical assessment of hormone receptors is performed by using two parameters, namely the number of positively stained tumour cell nuclei and the intensity of staining. Many scoring systems have been proposed for assessing both the parameters, of which Allred scoring system is the simpler and most widely accepted and recommended method. The number of stained tumour cell nuclei is expressed as percentage of total tumour cell population.

TABLE 2(A): SCORING OF PROPORTION OF STAINED NUCLEI

SCORE FOR PROPORTION OF POSITIVE NUCLEI	PERCENTAGE OF STAINED TUMOR CELL NUCLEI
0	No staining
1	<1%
2	1-10%
3	11-33%
4	34-66%
5	67-100%

TABLE 2(B): SCORING OF INTENSITY OF STAINING

SCORE FOR INTENSITY	INTENSITY OF STAINING
0	No staining
1	Weak staining
2	Moderate staining
3	Strong staining

Both the score for proportion of stained cell nuclei and score for intensity of staining are summed up to give a maximum score of 8.[66]

The presence of these steroid receptors has been found to be very powerful predictor of response to breast cancer hormonal therapy and has

significantly improved the long term clinical outcomes of patients with ER/PR-positive tumours. The response rate to cancer hormonal therapy for ER/PR positive tumours is approximately 80%[67,68]. ER- positive tumours show better prognosis and show good response to adjuvant chemotherapy with tamoxifen[13].

HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR TYPE 2 (Her2)

The human epidermal growth factor receptor 2 (HER2) is a transmembrane tyrosine kinase receptor, homologous to the epidermal growth factor receptor and is encoded by the gene ERBB2 on chromosome 17q12.[69] Her-2 amplification and/or overexpression is observed in about 15-30% of human breast cancers leading to increased activity of tyrosine kinase and downstream pathways stimulating tumour growth.[70] It is associated with clinically aggressive nature of tumour with morphologically high histologic grade, negative ER status, increased rates of recurrence and mortality. The prognosis of patients with her-2 positive tumours has been found to be significantly worse than those with her-2 negative tumours[71,72].

The development of targeted chemotherapy using anti-Her2 monoclonal antibody Trastuzumab (Herceptin) is a major breakthrough in the management of breast cancers. Trastuzumab is useful in the treatment of early stage breast cancers and has been found to reduce the risk of recurrence after surgery[73]. Various studies have shown that the use of Trastuzumab with chemotherapy in advanced breast cancers and metastatic disease has shown increase in overall response rates, decrease in risk of death, longer disease free survival and longer duration of disease progression[72,74–76].

The Her-2 status of the patient is essential for the treatment of Trastuzumab.

The assessment of Her-2 status in breast cancers can be done by using immunohistochemistry and fluorescent *in situ* hybridization (FISH). IHC identifies Her-2 receptor on the cell membrane whereas the Her-2 gene is quantified in FISH[77,78]. On immunohistochemistry, the results for Her-2 testing are classified as positive, equivocal or negative. The most recent guidelines for Her-2 testing and scoring from American Society of Clinical Oncology and College of American Pathologists (ASCO/CAP) have recommended the following interpretation:

A score of 3+ on IHC staining (intense, uniform circumferential staining) in ≥30 % of tumour cells or FISH indicating more than six gene copies per nucleus, or a FISH gene ratio (ratio of Her-2 gene signals to chromosome 17 signals) equal to or greater than 2.2 indicates a positive result. A score of 0 or 1+ on IHC staining (no staining or weak, incomplete membrane staining in any proportion of tumour cells, a FISH result of less than 4 Her-2 gene copies per nucleus, or a FISH gene ratio of less than 1.8 indicates a negative result. Equivocal result is indicated by a score of 2+ on IHC staining (complete membrane staining that is either nonuniform or weak in intensity but with obvious circumferential distribution in at least 10% of cells). Equivocal IHC samples must be referred to further FISH testing for confirmation.[79]

PROLIFERATION MARKERS

Tumour proliferation is one of the hallmarks of cancer, which indicates the cell cycle disruption and imbalance between cell proliferation and cell death. Various markers have been identified to evaluate tumour proliferation including S-phase fraction, thymidine labeling index, mitotic count, and immunohistochemical staining for Ki67, cyclin, topoisomerase IIα, proliferating cell nuclear antigen (PCNA) and geminin. High S-phase fraction is strongly associated with high grade of tumour, ER negativity and Her-2 overexpression, which also contribute to worse prognosis. Mitotic count, which is the number of mitoses in a given area of tumour, has been included in all grading systems of breast cancer and is considered the simplest tool to evaluate proliferation on H&E stained sections [67,80–82].

Ki-67 is the most widely accepted and preferred proliferation marker, which is assessed immunohistochemically using antibodies directed against it. It is present in actively proliferating cells[83]. Ki-67 is evaluated on paraffin-embedded tissue sections using monoclonal antibody MIB-1 and it is expressed as the percentage of stained tumour cell nuclei[84]. The grading protocol for KI-67 has not yet been standardized and various studies have used different cut-off percentages for high Ki-67 index[85,86]. St. Gallen International Expert Consensus has advised to classify Ki-67 index into 3 categories as follows: high Ki-67 index when ≥30%, low when immunopositivity <15% and intermediate when immunopositivity is between 16 to 30%[87,88]. Although high Ki-67 is associated

with worse clinical outcome, shorter overall and disease free survival, it is still not recommended as prognostic marker[89].

MOLECULAR CLASSIFICATION OF BREAST CANCER

Conventionally, breast cancers have been classified based on morphological characteristics until the year 2000, when Perou and colleagues first described the genomic and molecular nature of breast cancers based on cDNA microarray and gene expression profiling. They demonstrated that the expression pattern of a set of genes within the tumour determine the molecular signature of breast cancer and that the intrinsic molecular signatures predict the clinical outcome of the disease[6,7]. Breast cancers can be broadly classified into ER – positive and ER- negative which are further divided into five molecular subtypes:

1. Luminal A
2. Luminal B
3. Her-2 overexpressing
4. Basal-like
5. Normal breast-like.[6,7]

Luminal A tumours have the best prognosis and basal and Her-2 positive tumours have the worst prognosis[7,90]. The luminal A subtype is the most frequent type constituting about 24%-39% of breast cancers, followed by basal-like (17%-37%), luminal B (10%- 18%), HER2 (4-10%) and normal-like (0-5%)[91,92].

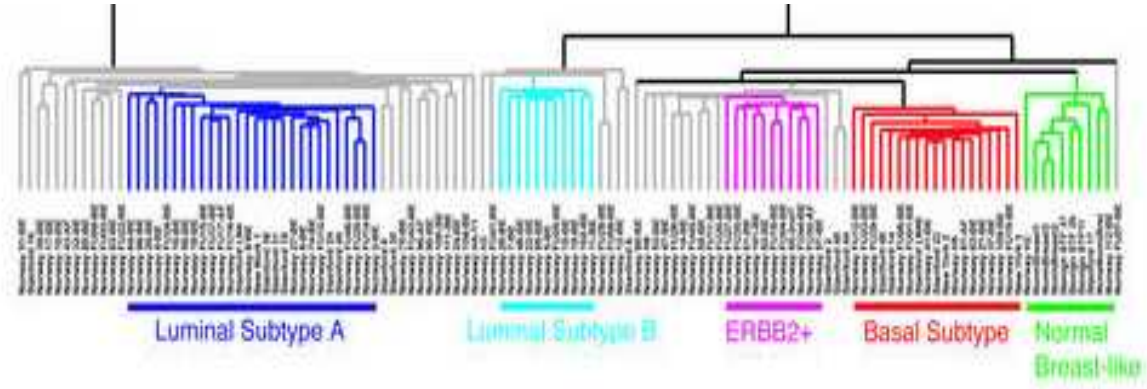


FIGURE 2: HIERARCHICAL CLUSTERING OF 115 TUMOUR TISSUES AND 7 NON-MALIGNANT TISSUES USING THE "INTRINSIC" GENE SET. EXPERIMENTAL DENDROGRAM SHOWS THE CLUSTERING OF THE TUMOURS INTO FIVE SUBGROUPS. BRANCHES CORRESPONDING TO TUMOURS WITH LOW CORRELATION TO ANY OF THE SUBTYPE ARE SHOWN IN GREY. [91]

TABLE 3: RECEPTOR STATUS OF MOLECULAR SUBTYPES OF BREAST CANCERS

MOLECULAR SUBTYPE	RECEPTOR STATUS
Luminal A	ER+/PR+/HER2-
Luminal B	ER+/PR+/HER2+
HER2 overexpressing	ER-/PR-/HER2+
Basal-like	ER-/PR-/HER2-

TABLE 4: FEATURES OF THE MOLECULAR SUBTYPES OF BREAST CANCER [93]

MOLECULAR SUBTYPE	FREQUENCY	CELL OF ORIGIN	ER/PR/ HER2	PROLIFE-RATION RATE	HISTOLOGIC GRADE	PROGNOSIS
Luminal A	50-60%	Luminal epithelial cell	ER and/or PR +ve, Her-2 –ve	Low	Low	Excellent
Luminal B	10-20%	Luminal epithelial cell	a)Her-2-ve, ER and/or PR +ve b) Her-2+ve, ER and/or PR +ve	a) High b) Any	Intermediate/ High	Intermediate/ Bad
HER-2 overexpressing	10-15%	Late luminal progenitor cell	Her-2 +ve ER -ve and PR –ve	High	High	Bad
Normal breast-like	5-10%	Luminal epithelial cell	ER -ve/+ve Her-2 -ve	Low	Low	Intermediate/ good
Basal-like	10-20%	Basal/ myoepithelial cell/ bipotent progenitor	ER -ve, PR -ve, and Her-2 -ve	High	High	Bad

Luminal A tumours are characterized by higher levels of ER and PR expression, negative expression of HER2, low Ki67 staining, and expression of luminal epithelial cytokeratins CK8 and 18 by IHC[94]. Luminal A tumours frequently exhibit low histological grade, and good prognosis[95].

Luminal B tumours are also ER-positive and express CK8 and 18. They are frequently associated with a more aggressive phenotype and worse prognosis,

higher histological grade, lower PR expression, and exhibit higher Ki67 staining[95,96]

HER2+ overexpressing tumours are found to be associated with high tumour proliferation, high histological grade, and frequent p53 mutations[97].

Triple negative breast cancer (TNBC) subtype is one of the breast cancer subtype which constitutes about 10 to 20% of tumors[95]. These tumours show lack of expression of ER, PR, or HER2. Basal-like breast tumours show expression of basal/ myoepithelial markers, such as CK 5/6, 14, and 17[98]. TNBCs account for about 75% of all BRCA1-deficient tumours, frequently associated with p53 mutations, exhibit genomic instability, show high histological grade, and high Ki67 positivity[95].

Thus, molecular classification using gene expression profiling has produced a paradigm shift in the understanding of the pathology, clinical outcome and prognosis of breast cancers and has been applied in the development of targeted therapy for use as part of personalised medicine.

MULTIGENE PROGNOSTIC AND PREDICTIVE TESTS

Many studies have led to the development of commercialized multigene assays for the prognostication and selection of treatment for individual patients[99]. These include Oncotype DX and MammaPrint genomic tests.

The Oncotype DX is a reverse transcription polymerase chain reaction (RTPCR)-based assay that analyses a panel of 21genes expression in formalin fixed paraffin-embedded tumour tissues. It was formulated to predict the likelihood of disease recurrence in patients with node-negative, ER+ breast

cancer who undergo treatment with Tamoxifen. The 21 genes are associated with ER pathway, proliferation, HER2 and invasion, and determines a risk of recurrence (ROR) score ranging from 0 to 100, which is an independent prognostic factor.[100] The Oncotype DX is the most widely used molecular test in the clinical setting for making treatment decisions and is recommended by the St. Gallen Consensus[101].

Mamma-Print® is one another commercially available 70-gene test that provides prognostic information for patients with stage 1 or 2, node-negative invasive breast cancer of tumour size < 5 cm. It quantifies the risk of metastasis in early breast cancers and divides patients into two groups- low and high risk of recurrence irrespective of ER status and prior chemotherapy[102].

Other commercially available prognostic signatures are 76-gene signature named as Veridex, breast cancer index and PAM50 signature.

TRIPLE NEGATIVE BREAST CANCER

Triple negative breast cancers are defined by the negative expression of estrogen receptor, progesterone receptor and lack of overexpression and amplification of HER2 gene. They account for about 10-20% of all breast cancers and also account for most of the deaths due to breast cancer[8]. These tumours have been found to show poor prognosis despite good response to neoadjuvant chemotherapeutic agents and lack of targeted therapy[103].

EPIDEMIOLOGY

TNBC more frequently affects younger patients(<50 years),[104–106] more prevalent in African-American women, increased incidence of distant visceral

metastases and a unique mechanism of haematogenous spread, more frequently presents as interval cancers between consecutive mammograms and are more aggressive than tumours of other molecular subgroups[104,106]. TNBC accounts for 39% of all breast cancers that occur in pre-menopausal African-American women under the age of 50 years, whereas only 16% of breast cancers that are diagnosed in Caucasian women of the same age group. It has been observed that about 14% of breast cancers occurring in post-menopausal African-American women are found to be TNBC[107]. More than 75% of breast cancers arising from a mutation in BRCA1 gene belong to triple-negative phenotype, basal-like subtype or both[8,108].

PATHOLOGIC FEATURES

The prototypical features of triple negative breast cancers are summarised in the table 5.

TABLE 5: PROTOTYPICAL FEATURES OF TRIPLE NEGATIVE BREAST CANCER[109]

Morphological features	High histologic grade Lack of tubule formation Prominent nuclear abnormalities High mitotic count Broad pushing borders Necrotic and fibrotic areas Prominent lymphoplasmacytic infiltrate

Biological features	Lack of expression of ER and PR HER2 negative High Ki-67 index P53 mutation Positive immunostaining for basal cytokeratins, vimentin, p-cadherin, EGFR, PDGFR, IGF-IR and c-kit
Molecular classification	Most commonly basal-like

Histologically, TNBC are often high grade tumours with lack of tubule formation, high mitotic count and high nuclear atypia[48,104]. They show broad pushing margins and more commonly have large areas of necrosis, fibrosis and prominent lymphocytic infiltrate. Most of the TNBC are high grade invasive ductal carcinoma, no special type, metaplastic carcinoma and medullary carcinomas.[110,111].Methylated BRCA1 promoter region has been found in medullary and metaplastic types of breast cancer[112]. There is no convincing association between the prevalence of lymph node involvement at the time of diagnosis and triple negative breast cancers, as various studies show varying results[8]. Only a weak correlation has been observed between tumour size and lymph node metastasis in triple negative breast tumours in a study conducted by Dent et al.[104].

MOLECULAR SUBTYPES OF TNBC

Lehmann et al have identified 6 different molecular subtypes of triple-negative breast cancer based on gene expression profiling of TNBC including

Two basal-like (BL1 and BL2),

Immunomodulatory (IM),

Mesenchymal (M),

Mesenchymal stem-like (MSL), and

Luminal androgen receptor (LAR) subtypes.

These subtypes are thought to be driven by various distinct pathways which can be effectively targeted by specific drugs[113].

CLINICAL COURSE AND OUTCOME

Triple-negative breast cancers are generally aggressive with shorter disease-free interval with adjuvant chemotherapy and aggressive clinical course with earlier nodal and distant metastases with significant shortening of overall survival of the patients, despite their sensitivity and response to initial adjuvant and neoadjuvant chemotherapy with taxanes and anthracyclines[103].

BASAL-LIKE BREAST CANCER

Basal-like breast cancer have been identified by the hierarchical clustering of the variations in the gene expression profile of 496 genes, which is called the "intrinsic subset"[6]. Approximately 80% of triple negative breast cancers are basal-like breast cancers[114] and they account for about 15% of all breast cancers[6]. It is a unique and aggressive breast cancer subtype[7]. Basal-like breast cancers arise from epithelial/ myoepithelial progenitor stem cell of breast[115].

Although most of the triple negative breast cancers and basal-like carcinomas are of high histologic grade, a small subset of them belong to low histologic grade like secretory carcinoma, acinic cell, adenoid cystic and apocrine carcinomas.

EPIDEMIOLOGY AND CLINICAL FEATURES

Basal-like breast cancer is found to be seen more frequently in women who attain early menarche, women who are overweight and obese, high parity and women who had breastfed for a shorter duration during their lifetime[108].

IMMUNOHISTOCHEMICAL FEATURES

The knowledge of basal cytokeratins CK5/6, CK14 and CK17 expression in basal-like tumours have been acquired only recently[7], although the immunohistochemical expression of basal cytokeratins in the breast was demonstrated as early as 1982[116]. They express basal cytokeratins such as CK5, CK6, CK14, CK8/CK18,p63, EGFR, P-cadherin, vimentin, aB crystallin, fascin and caveolin1 and 2[7,41]. These tumours more commonly affect younger patients, about 82% of basal-like cancers show either immunohistochemical expression of p53 or TP53 mutation as compared to only 13% of luminal-A tumours show p53 expression[7]and high proliferation index. About 60% of basal-like tumours express EGFR on the cell surface[41,110].

Many studies have found an association between vimentin expression and aggressive phenotype of breast malignancies. Livasy et al observed a strong expression of vimentin in about 94% of basal-like tumours. The expression of vimentin is attributed to the origin of tumours from myoepithelial cells,

epithelial-mesenchymal transition due to dedifferentiation of tumours and origin of tumours from breast cancer stem cells[117].

The expression of luminal cytokeratins CK8/18, which are expressed in luminal epithelial cells of the breast and the normal myoepithelial cells do not express these luminal markers. Livasy et al observed a strong expression of luminal cytokeratin CK8/18 in about 83% of basal-like tumours, suggesting an origin from breast cancer stem cells which undergo varying degrees of basal and luminal differentiation. These tumours are called as basoluminal subtypes[117].

Since the basal cytokeratins are normally expressed by the basal cell layer of the breast epithelium, these tumours are called with the suffix “basal”. These tumours are called by various terminologies such as basal-like, basal cell phenotype, basal phenotype, basal epithelial phenotype and basal type in the literature. The term ‘basal-like’ usually refers to the cDNA microarray based classification, whereas the other terms are used in immunohistochemical studies.

GENE EXPRESSION OF BASAL CLUSTER

Basal-like cluster in gene expression profiling of breast cancers have found expression of EGFR, c-Kit, cyclin E, basal cytokeratins CK5/14/17, vimentin, P-cadherin, caveolin 1 and 2, alpha B crystalline, TP53 mutations and genomic instability with inactivation of RB pathway[112,118].

BRCA1 MUTATION AND TNBC

Abnormal BRCA1 function due to epigenetic alterations by methylation of BRCA1 promoter region has been observed in triple-negative basal-like breast cancer. The BRCAness i.e. BRCA-associated breast cancer subtypes are

characterised by higher grade at diagnosis, lack of expression of ER and Her-2, amplification of c-myc, medullary type with pushing margins, less common DCIS, TP53 mutations, lymphocytic infiltrate showing basal phenotype and expression of EGFR[112].

IMMUNOHISTOCHEMICAL MARKERS AS SURROGATES FOR MOLECULAR SUBTYPING

Immunohistochemistry (IHC) is a well-established, powerful diagnostic tool which is suitable for use in archival FFPE tissues. Compared to gene expression profiling, IHC is more feasible logistically for routine diagnostic histology laboratories, and it has been validated in multiple studies. The basal subtype of breast tumours specifically has been the subject of interest in many studies attempting to characterise it by IHC. Nielsen et al. used the markers ER, HER2, the basal cytokeratin 5/6 (CK5/6) and epidermal growth factor receptor (EGFR, also known as HER1) to detect basal-like subtype of breast tumors[41]. Basal tumours were identified immunohistochemically as ER negative, HER2-negative, and CK5/6 and/or EGFR-positive.

CK5/14/17 are currently the most widely accepted basal cytokeratins to identify basal-like tumours by immunohistochemical methods and to distinguish from luminal cytokeratins CK8/18/19 expressing tumors[119–122]. Some studies have been done to assess the usefulness of immunohistochemical expression of other myoepithelial markers such as p63, a p53 homolog and smooth muscle actin on the tumour cells[121,123]. Livasy et al studied the immunohistochemical

detection of various biomarkers and have found that smooth muscle actin, p63 and CD10 are of limited value in the identification of basal-like tumors[117].

FUTURE THERAPEUTIC TARGETS IN TNBC

Although triple-negative breast cancers show initial response to chemotherapy, they exhibit high incidence of relapse and distant visceral and bone metastases with aggressive clinical course. Hence, it is important to identify newer therapeutic targets. Currently, several studies are investigating the novel targets like EGFR, Poly ADP- ribose polymerase (PARP), VEGF and breast cancer stem cells.

Recent studies have shown that the triple-negative breast cancer subtype has higher frequency of EGFR expression than non-TNBC subtypes. The EGFR status negatively correlates with the overall survival in TNBC patients suggesting that EGFR could be a potential target for chemotherapy with EGFR inhibitors[124].

MATERIALS AND METHODS

The study was conducted in the Department of Pathology, Tirunelveli Medical College and Hospital, Tirunelveli, after being approved by the Institutional

Ethics Committee of Tirunelveli Medical College, Tirunelveli prior antecedent to conducting the study. The study was carried out during the period September 2014 to September 2016 for 2 years.

RESOURCES

A total of 50 female patients with histopathologically proven triple-negative primary breast carcinomas were who underwent modified radical mastectomies were selected based on the status of negative immunoreactivity for ER, PR and HER-2. The samples were received in the department of pathology, Tirunelveli medical college, Tirunelveli. The archival tissue blocks of formalin-fixed paraffin embedded tissues were retrieved for the study. Detailed histories and clinical examination findings were obtained from the medical records department. Tissue sections of 4-5μm thickness were stained with haematoxylin and eosin stain. The tumours were classified based on WHO classification of breast tumours and graded using modified Scarff-Bloom-Richardson grading system.

SAMPLE SIZE

A total of 50 female patients with triple-negative primary breast carcinomas were selected.

INCLUSION CRITERIA

All histologically proven cases of primary breast malignancies that had undergone resection surgeries.

EXCLUSION CRITERIA

Cases which show positivity for any of the markers ER, PR and Her-2.

MATERIALS REQUIRED

1. Archival formalin-fixed paraffin-embedded tissue blocks of primary breast carcinomas.
2. Haematoxylin and eosin stained tissue sections made from the tissue blocks.
3. Microtome and incubator for obtaining tissue sections and to dewax the sections
4. Positively charged slides for holding tissue sections for IHC.
5. Reagents for preparing buffer solutions for antigen retrieval and washing during staining procedure.
6. Microwave oven for antigen retrieval.
7. The PathnSitu universal immunohistochemistry kit for performing immunohistochemical staining along with primary antibodies for ER, PR, HER 2, CK5/6, EGFR.
8. Microscope, used for interpretation of histologic parameters and grading of IHC.

METHODOLOGY

1. The detailed clinical history and examination findings were obtained from the medical records department.
2. **SECTION CUTTING**

Seven tissue sections of 4-5µm thickness were obtained from each of the paraffin block, on poly-L-lysine (PLL) coated slides followed by dewaxing by incubation of the slides at 60-70°C for one hour.

3. **HAEMATOXYLIN AND EOSIN (H & E) STAINING**

H and E staining was performed in The Department of Pathology, Tirunelveli medical college, Tirunelveli. The tissue sections were first de-waxed and rehydrated using graded alcohols and brought to water. Staining with Harris's haematoxylin was carried out for 10 minutes to stain all the nuclei. The sections were then washed for 5 minutes under running tap water for blueing, counterstained in 1% aqueous Eosin for 8 dips and then washed in tap water. The sections were dehydrated, cleared and mounted. Results: cytoplasm- pink and nuclei-dark blue.

The haematoxylin and eosin stained sections of breast carcinomas were evaluated in detail, to the histopathological parameters such as histologic type, histologic grade, tumour necrosis, angiolymphatic invasion, perineural invasion, associated in situ component, stromal reaction, status of the margins, skin, nipple and areola, and lymph node involvement. The histologic type was determined according to the WHO classification of breast tumours. The histologic grade was

given according to the Nottingham modification of the Scarff- Bloom-Richardson grading system.

4. IMMUNOHISTOCHEMISTRY

Immunohistochemistry is the technique for localization of antigens or proteins in tissue sections by the use of labelled monoclonal antibodies as specific reagents through antigen-antibody interactions that are visualized by a marker such as fluorescent dye. IHC is used for both diagnostic and biological research purposes.

BUFFER SOLUTIONS

The buffer solutions were used for antigen retrieval and as wash buffer according to the manufacturer's guidelines (PATHNSITU).

1. Tris EDTA at a pH of 9 for all the used IHC markers.
2. Tris wash buffer at pH of 7.6 for both.

One representative tissue section was selected and immunohistochemical staining was performed for ER, PR and Her-2. The staining was performed by DAKO envision method according to the manufacturer's guidelines.

DE-WAXING AND REHYDRATION

The tissues were de-waxed by soaking slides in xylene for 2 changes, 15 minutes each to remove the excess wax from the sections, and rehydrated by placing in descending grades of alcohol- 100% ethanol for 2 changes, 5 minutes each, 90% ethanol for 5 minutes, 70% ethanol for 5 minutes, followed by a wash in distilled water for 2 changes, 2 minutes each.

ANTIGEN RETRIEVAL

Antigen retrieval was performed by heat treatment using microwave method. For heat treatment, a TRIS EDTA buffer solution pH9 (PathNSitu) pre-heated in a glass coplin jar in a microwave on full power following which the slides were immersed into the solution and allowed to boil for 15-20 minutes. Following heat treatment, the slides were cooled in the solution at room temperature for at least 15 minutes and then rinsed in TRIS wash buffer for 2 changes, five minutes each.

PREVENTION OF ENDOGENOUS STAINING

To prevent endogenous peroxidase activity, the slides were treated with a ready- to- use peroxidase-blocking hydrogen peroxide solution (PathNSitu) for 5 minutes and then washed once in TRIS wash buffer for 2 changes, five minutes each.

INCUBATION WITH PRIMARY ANTIBODY

The primary antibody is applied to the tissue sections and incubated in a humidified moist chamber at room temperature for 30 minutes.

TABLE 6: BIOMARKERS USED IN IHC

ANTIBODY	SPECIES	CLONE	DILUTION
ER	Rabbit monoclonal	EP1	Ready to use
PR	Rabbit monoclonal	EP2	Ready to use
Her-2	Rabbit monoclonal	EP3	Ready to use
CK5/6	Rabbit monoclonal	EP24/EP67	Ready to use
EGFR	Rabbit monoclonal	EP22	Ready to use
E-Cadherin	Rabbit monoclonal	EP6	Ready to use

The slides were then washed in TRIS wash buffer for 2 changes, 10 minutes each. An amplifier- Polyexcel target binder reagent was then applied to the sections and kept for 15 minutes followed by washing in TRIS wash buffer for 2 changes, 2 minutes each.

INCUBATION WITH SECONDARY ANTIBODY

The secondary antibody used was Polyexcel Horse Radish Peroxidase Polymer (HRP) as prescribed by the manufacturer, and incubated for 12 minutes, in a humidified chamber at room temperature.

VISUALISATION OF POSITIVITY USING DAB CHROMOGEN

The colorimetric detection of signal to detect antigen-antibody reaction was achieved by the Diamino-benzidine tetrachloride DAB method. The working solution of DAB chromogen from the DAB kit (PathNSitu) was prepared by mixing 1 drop of DAB chromogen in 1 ml of DAB substrate buffer immediately before use. This DAB solution was applied to the tissue sections at room temperature and the development of colour was monitored for 2-5 minutes until a brown staining of the section was visualised. The slides were then washed in distilled water for 2 changes.

COUNTERSTAINING, DEHYDRATION, CLEARING AND MOUNTING OF SLIDES

The slides were counterstained by immersion in Harris's Haematoxylin for 30 seconds. They were then washed thoroughly under running tap water for blueing until clear. The slides were then dehydrated by immersing in ascending grades of alcohol- 70% ethanol for 30 seconds, 95% ethanol for 30 seconds, followed by 100% ethanol for 1 minute. The sections are cleared by soaking in xylene for 2 changes, 2 minutes each. The slides were then mounted with cover slip using DPX mounting solution for viewing under microscope.

STAINING PATTERNS OF VARIOUS ANTIBODIES

The positive immunostaining for ER and PR was observed in the nucleus of the tumour cells, Her-2 and E-Cadherin positivity was observed in the plasma membrane and CK5/6 immunostaining was seen in the cytoplasm while EGFR

positivity was observed in the cytoplasm and membrane of the tumour cells. The colour of staining for all six antibodies was brown.

USE OF CONTROL TISSUE SECTIONS

A positive tissue control was examined to confirm that all reagents were functioning properly. A positive reaction was confirmed by the brown staining due to the DAB chromogen labelling the antigen-antibody complex at the site of the target antigen. If the positive control tissues fail to demonstrate the expected staining pattern, all results with the test specimen were considered invalid.

IMMUNOHISTOCHEMICAL EVALUATION AND SCORING SYSTEMS

SCORING FOR ESTROGEN AND PROGESTERONE RECEPTORS

The scoring of ERα and PR were done according to the Allred scoring system, this system employs the proportion and the intensity of the stained nuclei[66]. The score for the proportion of stained nuclei was given on a scale of 0-5 and the intensity of staining was given a score on a scale of 0-3. The total score was calculated by adding the scores for proportions and intensities to give a total score of 0 or 2 as minimum and 8 as maximum score. A total score of 3-8 was considered as positive immunoreactivity whereas a score of ≤2 was regarded as negative[66].

SCORING FOR HER-2NEU

The scoring for Her-2 was based on the proportion and intensity of staining of the plasma membranes of the tumour cells as per the ASCO/CAP recommendations[79].

SCORING FOR CYTOKERATIN (CK5/6)

Cytokeratin 5/6 was scored as positive if any faint or strong cytoplasmic and/or membranous immunostaining was observed in the neoplastic tumour cells[125].

SCORING FOR EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR)

EGFR was scored as positive if more than 1% of the tumour cells showed membrane immunoreactivity of any intensity[125].

STATISTICAL ANALYSES

In this study, we used descriptive statistical analyses and Chi square test using contingency tables for correlation studies. A *P* value of< 0.05 was considered as significant.

OBSERVATION AND RESULTS

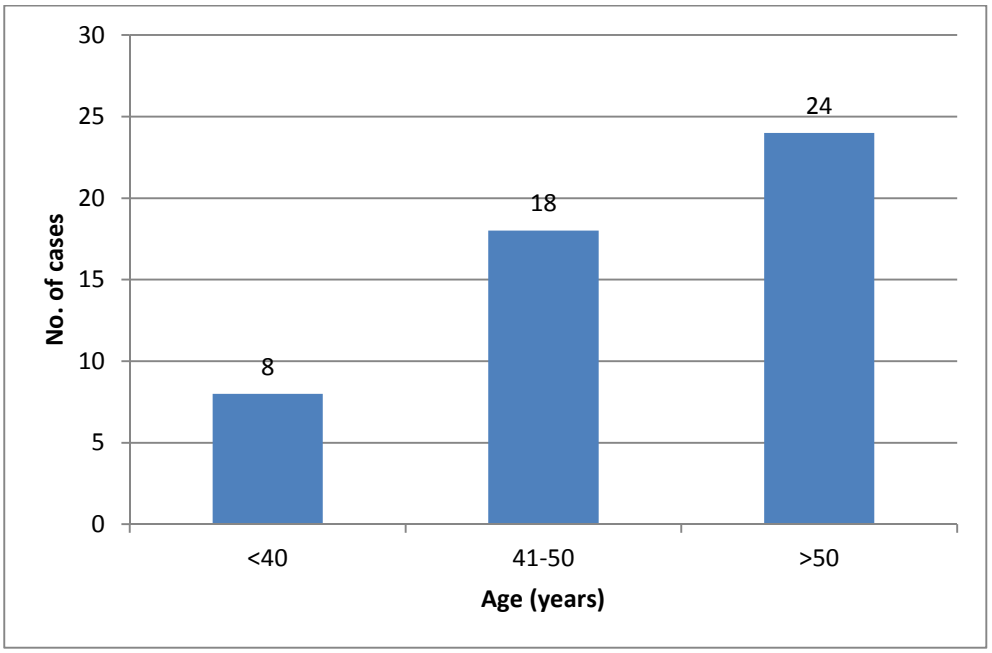
PATIENT CHARACTERISTICS

A total of fifty cases of triple-negative breast carcinoma in south-Indian female patients were studied. The results are as follows:

AGE

Patients' age ranged from 22-70 years with a mean of 50.4±9.7 years. A majority of the patients in the study population were under the age category of ≥ 50 years at the time of diagnosis (fifth decade).

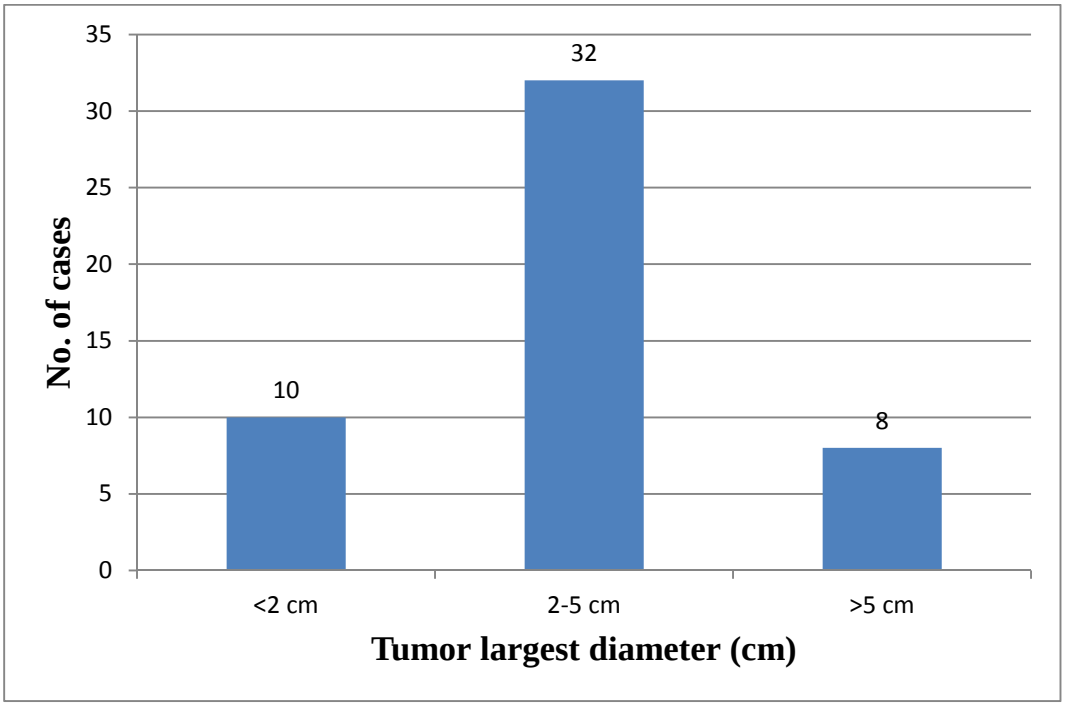
CHART 1: AGE DISTRIBUTION OF THE PATIENTS (n= 50)



LARGEST TUMOR DIAMETER

The tumour size was assessed using the largest diameter of the tumour according to the TNM staging system. The tumours were classified into three groups: ≤2 cm, 2-5 cm and ≥ 5 cm. Among our study population 10 cases (20%) were ≤ 2 cm in diameter, 32 cases (64%) were 2-5 cm in size and 8 cases (16%) were ≥ 5 cm in largest dimension.

CHART 2: DISTRIBUTION OF CASES ACCORDING TO THE TUMOR LARGEST DIAMETER



HISTOLOGICAL PARAMETERS

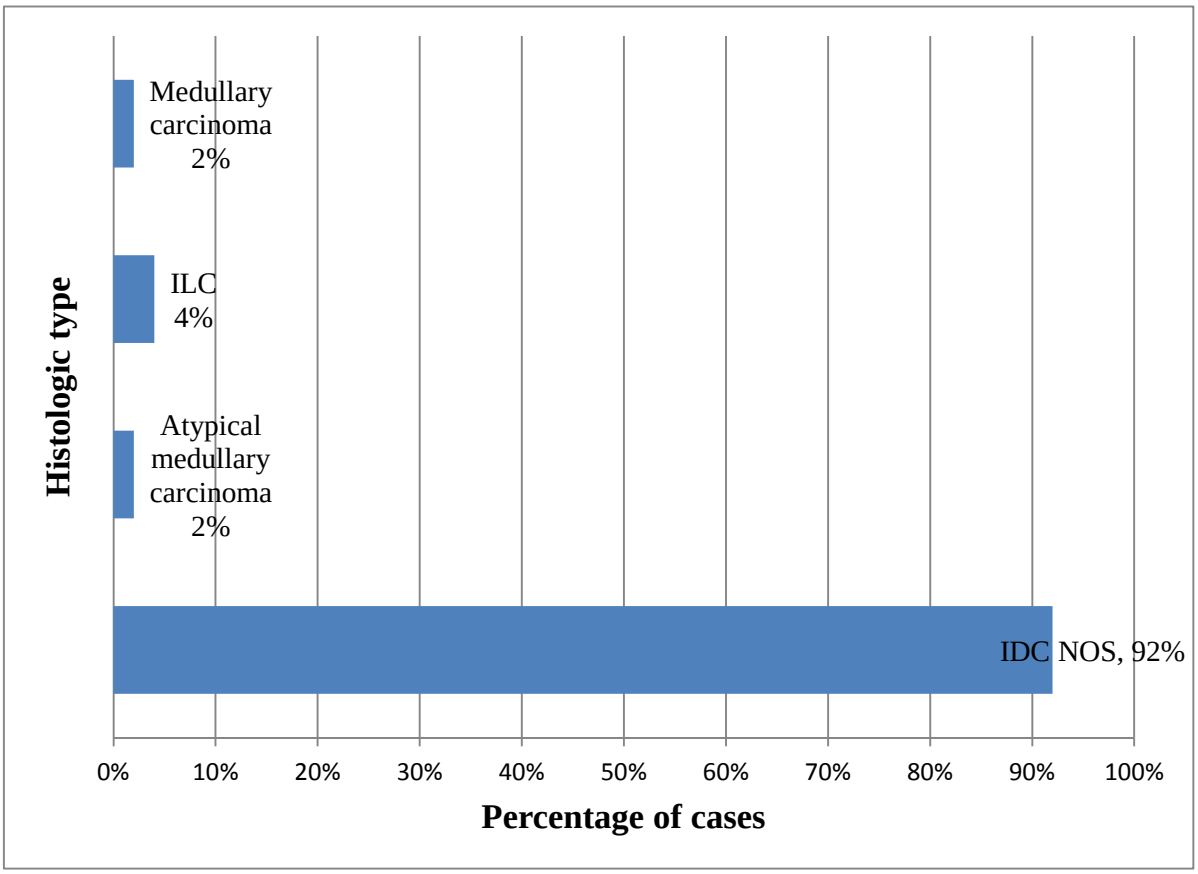
HISTOLOGIC TYPE OF TUMOR

Of the 50 cases studied, the predominant histologic type was infiltrating ductal carcinoma, NOS type in 46 cases (92%). 2 cases (4%) were invasive lobular carcinoma, 1 case (2%) was medullary carcinoma and 1 case (2%) was atypical medullary carcinoma.

TABLE 7: DISTRIBUTION OF SAMPLES BASED ON HISTOLOGIC TYPE

HISTOLOGIC TYPE	NO. OF CASES (n=50)	PERCENTAGE (%)
IDC NOS	46	92
atypical medullary carcinoma	1	2
ILC	2	4
Medullary carcinoma	1	2

CHART 3: DISTRIBUTION OF SAMPLES BASED ON HISTOLOGIC TYPE



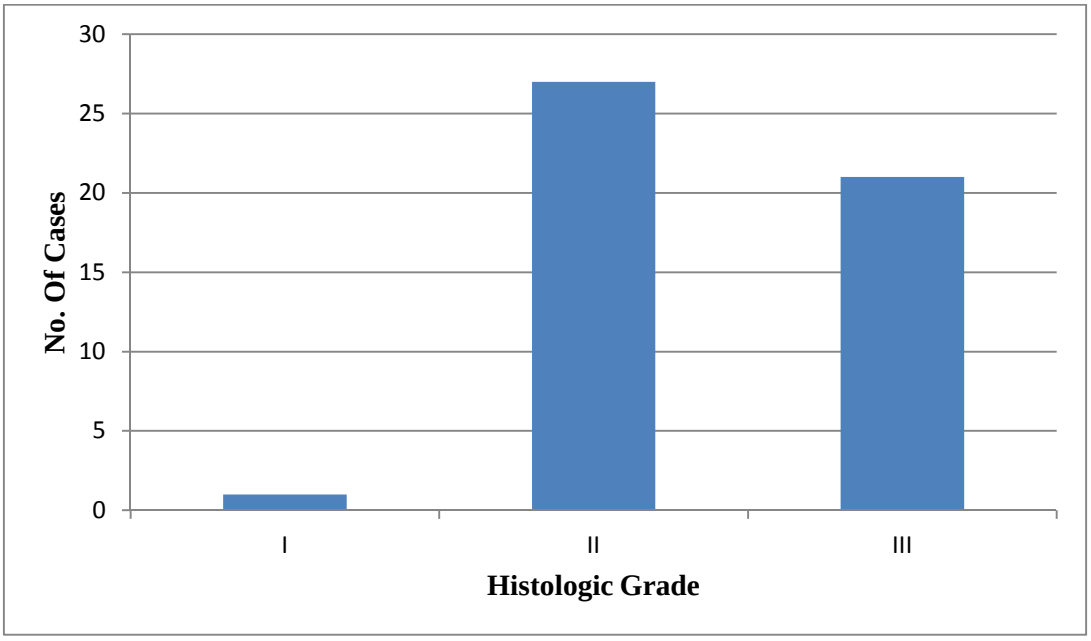
TUMOR GRADE

Grading according to the Nottingham modification of Bloom and Richardson system showed that 2% were grade I; 46% were grade II and 44% were grade III.

TABLE 8: DISTRIBUTION OF CASES ACCORDING TO BLOOM RICHARDSON GRADING SYSTEM

GRADE	No. OF CASES (n=50)	Percentage (%)
I	1	2
II	27	55
III	21	43

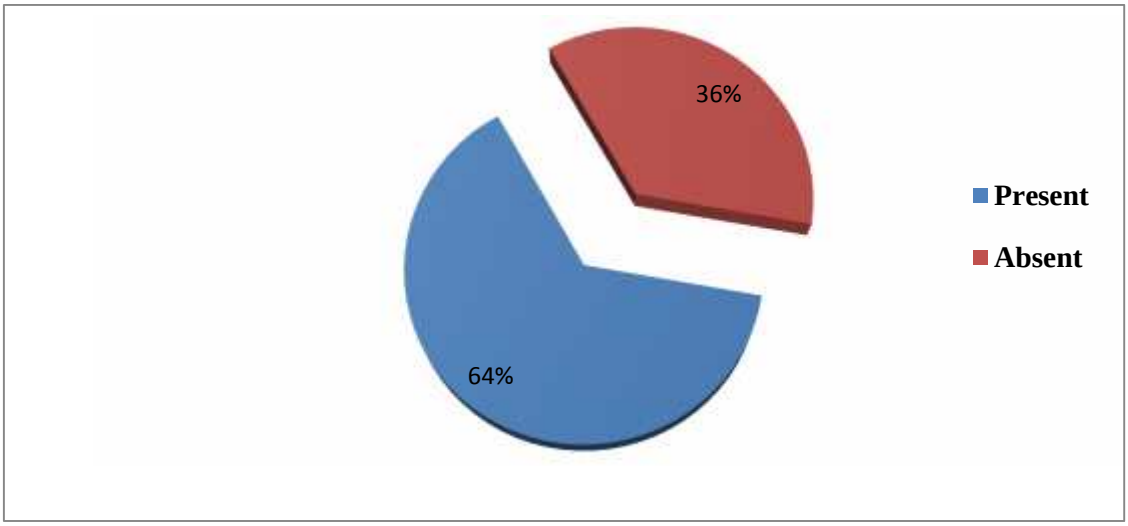
CHART 4: DISTRIBUTION OF CASES ACCORDING TO BLOOM RICHARDSON GRADING SYSTEM



ANGIOLYMPHATIC INVASION

In our study group, lymphovascular invasion on H&E stained sections was observed in 32 cases (64%).

CHART 5: DISTRIBUTION OF CASESWITH LYMPHOVASCULAR INVASION



PERINEURAL INVASION

Of the 50 cases in our study, 2 cases (4%) showed perineural invasion.

TABLE 9: DISTRIBUTION OF CASES WITH PERINEURAL INVASION

PERINEURAL INVASION	NO. OF CASES (N=50)	PERCENTAGE (%)
Present	2	4%
Absent	48	96%

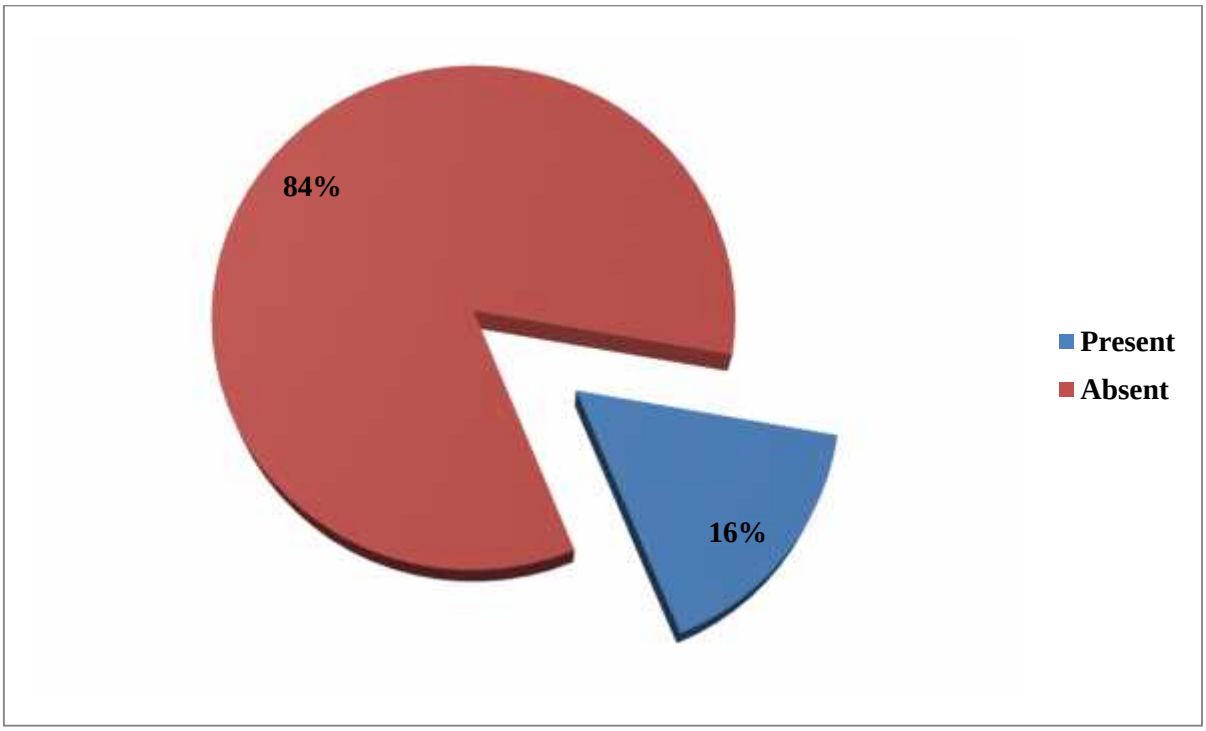
INSITU COMPONENT

Among the 50 cases studied, 8 cases (16%) showed DCIS component in the rest of the parenchyma.

TABLE 10: DISTRIBUTION OF CASES WITH DCIS COMPONENT

DCIS COMPONENT	NO. OF CASES (N=50)	PERCENTAGE (%)
Present	8	16%
Absent	42	84%

CHART 6: DISTRIBUTION OF CASES WITH DCIS COMPONENT



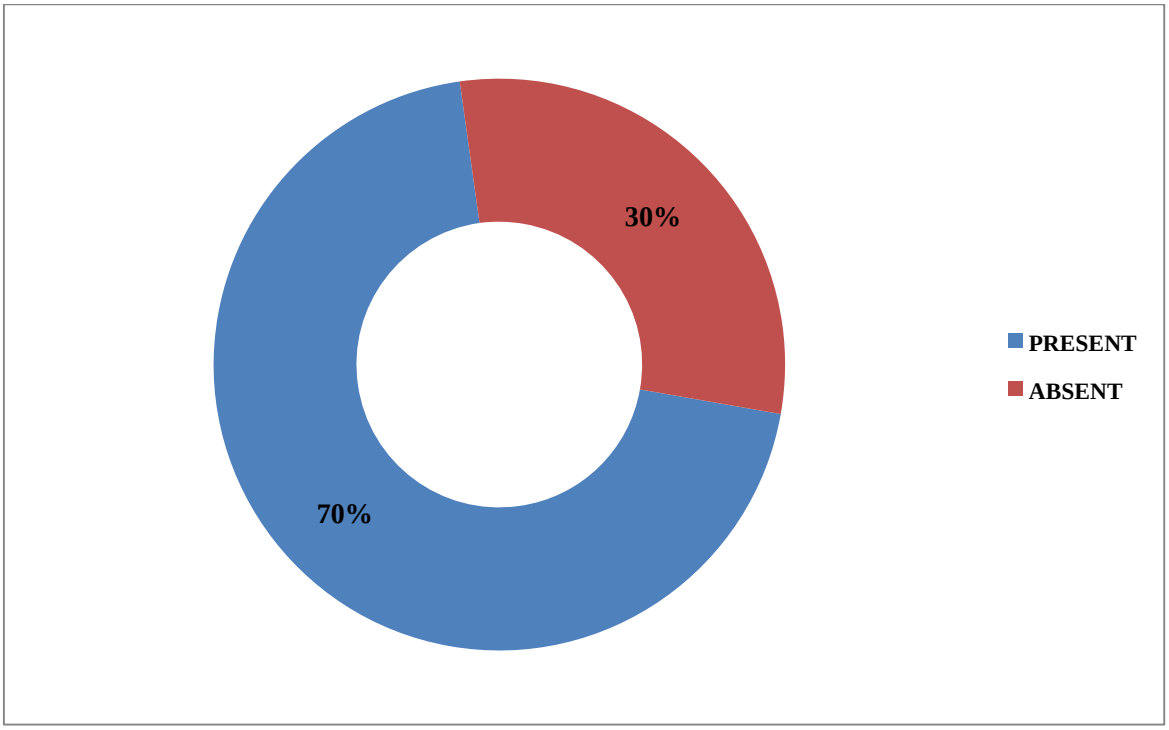
TUMOR NECROSIS

Out of the 50 cases analyzed, 35 cases (70%) showed microscopic tumour necrosis on histopathologic examination.

TABLE 11: DISTRIBUTION OF CASES WITH TUMOR NECROSIS

TUMOR NECROSIS	NO. OF CASES (N=50)	PERCENTAGE (%)
Present	35	70%
Absent	15	30%

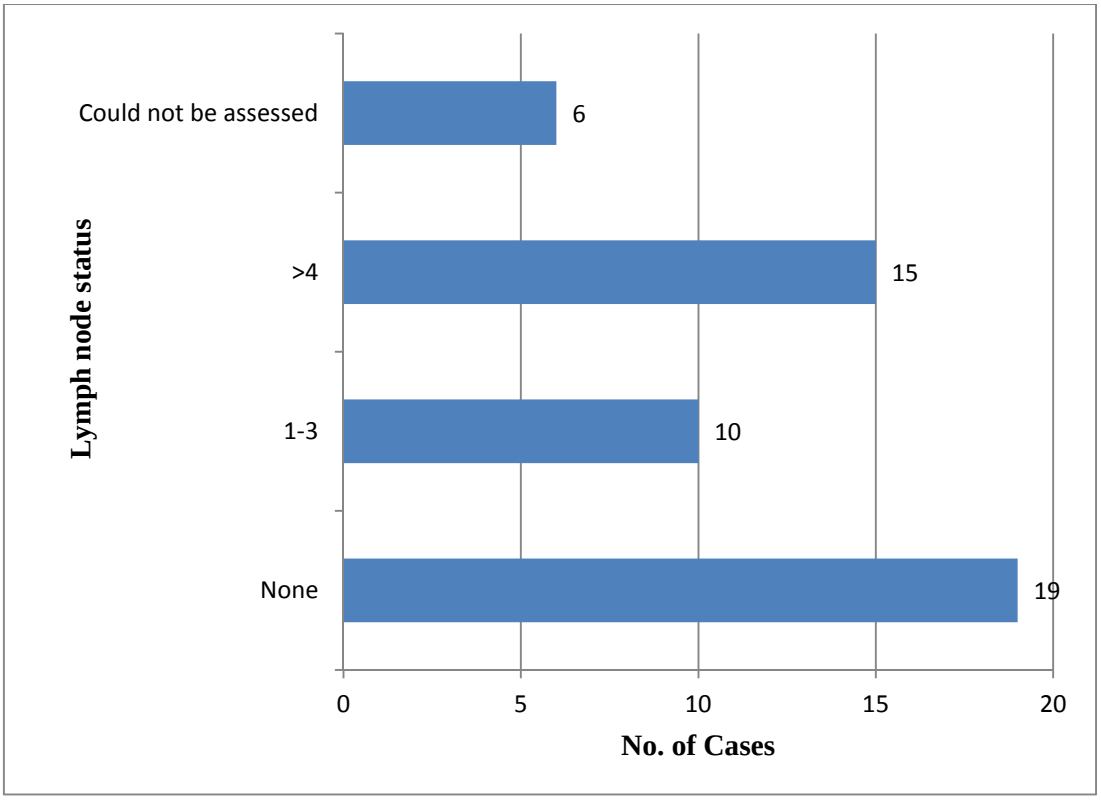
CHART 7: DISTRIBUTION OF CASESWITH TUMOR NECROSIS



LYMPH NODE STATUS

The thorough histological examination of the axillary lymph nodes revealed that 38 % of cases had no lymph node while 20 % had 1-3 positive lymph nodes and 30% had ≥ 4 positive lymph node metastasis. The lymph node status in 12% of the cases could not be assessed.

CHART 8: DISTRIBUTION OF CASES ACCORDING TO LYMPH NODE STATUS



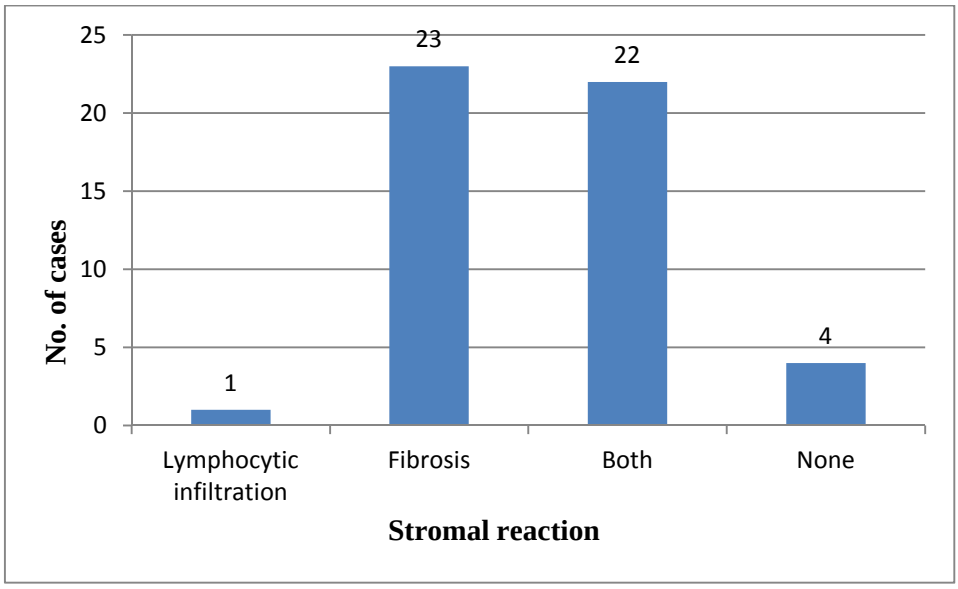
STROMAL REACTION

Out of the 50 cases studies, we observed stromal reaction in about 92% of the cases, out of which 23 cases (46%) showed stromal fibrosis, 1 case (2%) showed lymphocytic infiltration and 22 cases (44%) showed both fibrosis and lymphocytic infiltration.

TABLE 12: DISTRIBUTION OF CASESWITH STROMAL REACTION

STROMAL REACTION	NO. OF CASES (N=50)	PERCENTAGE (%)
Lymphocytic infiltration	1	2%
Fibrosis	23	46%
Both	22	44%
None	4	8%

CHART 9: DISTRIBUTION OF CASES ACCORDING TO LYMPH NODE STATUS



IMMUNOHISTOCHEMICAL STUDY

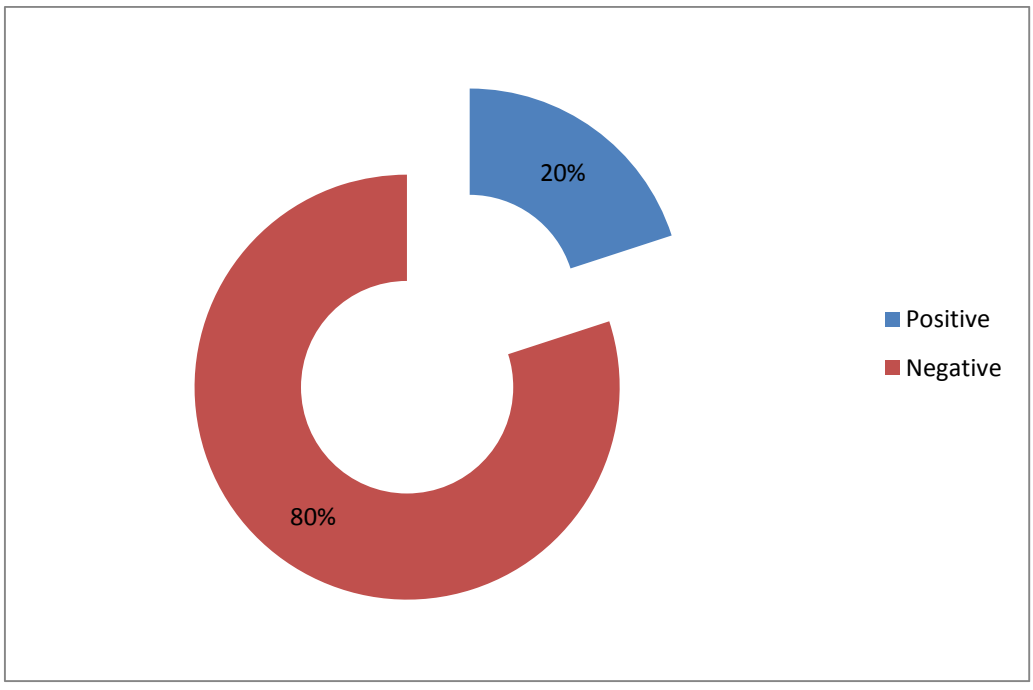
CK 5/6 EXPRESSION

Among the 50 cases in the study population, CK 5/6 expression was observed in only 10 cases (20%) and the remaining 40 cases (80%) showed negative immunoreactivity for CK 5/6.

TABLE 13: DISTRIBUTION OF CASES WITH CK5/6 EXPRESSION

CK5/6 EXPRESSION	NO. OF CASES (N=50)	PERCENTAGE (%)
Positive	10	20%
Negative	40	80%

CHART10: PERCENTAGE OF CASES WITH IMMUNOHISTOCHEMICAL EXPRESSION OF CK5/6 IN TNBC



EGFR EXPRESSION

Out of the 50 cases, EGFR positive immunoreactivity was observed in 34 cases (68%) whereas 16 cases (32%) were negative for EGFR.

TABLE 14: DISTRIBUTION OF CASES WITH EGFR EXPRESSION

EGFR EXPRESSION	NO. OF CASES (N=50)	PERCENTAGE (%)
Positive	34	68%
Negative	16	32%

CHART 11: PERCENTAGE OF CASES WITH IMMUNOHISTOCHEMICAL EXPRESSION OF EGFR IN TNBC

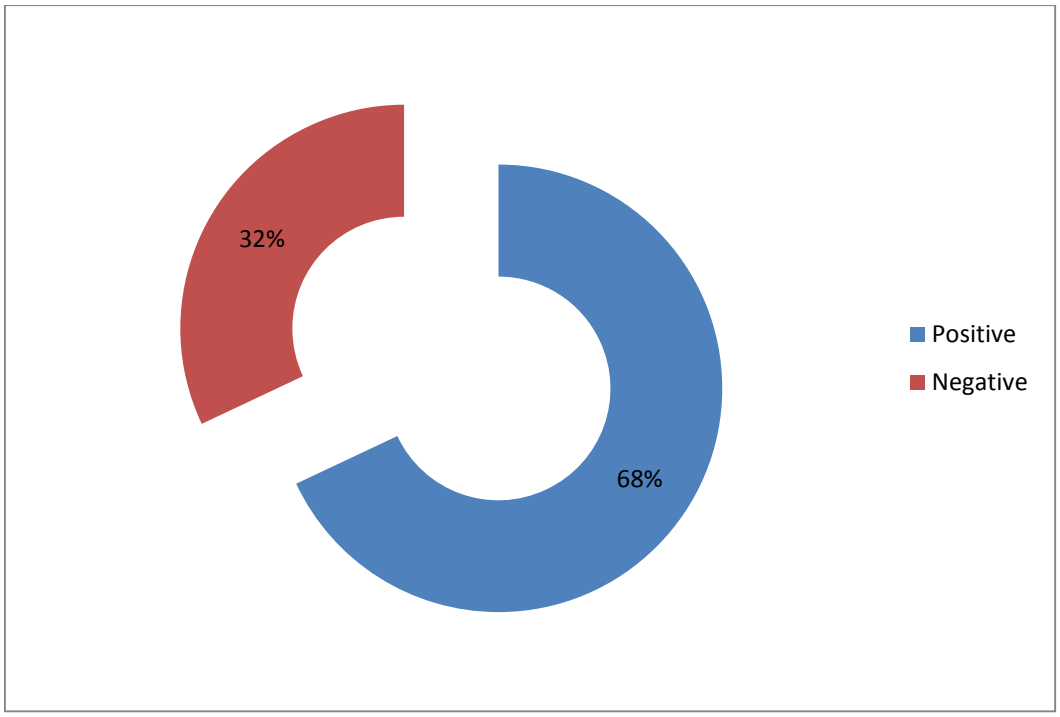


TABLE 15: IMMUNOHISTOCHEMICAL STATUS OF BASAL MARKERS IN TNBC

IMMUNOHISTOCHEMICAL PANEL	NO. OF CASES (N=50)	PERCENTAGE (%)
CK5/6 + / EGFR +	8	16
CK5/6 + / EGFR -	2	4
CK5/6 - / EGFR +	26	52
CK5/6 - / EGFR -	14	28

CORRELATION OF TUMOR SIZE WITH LYMPH NODE STATUS

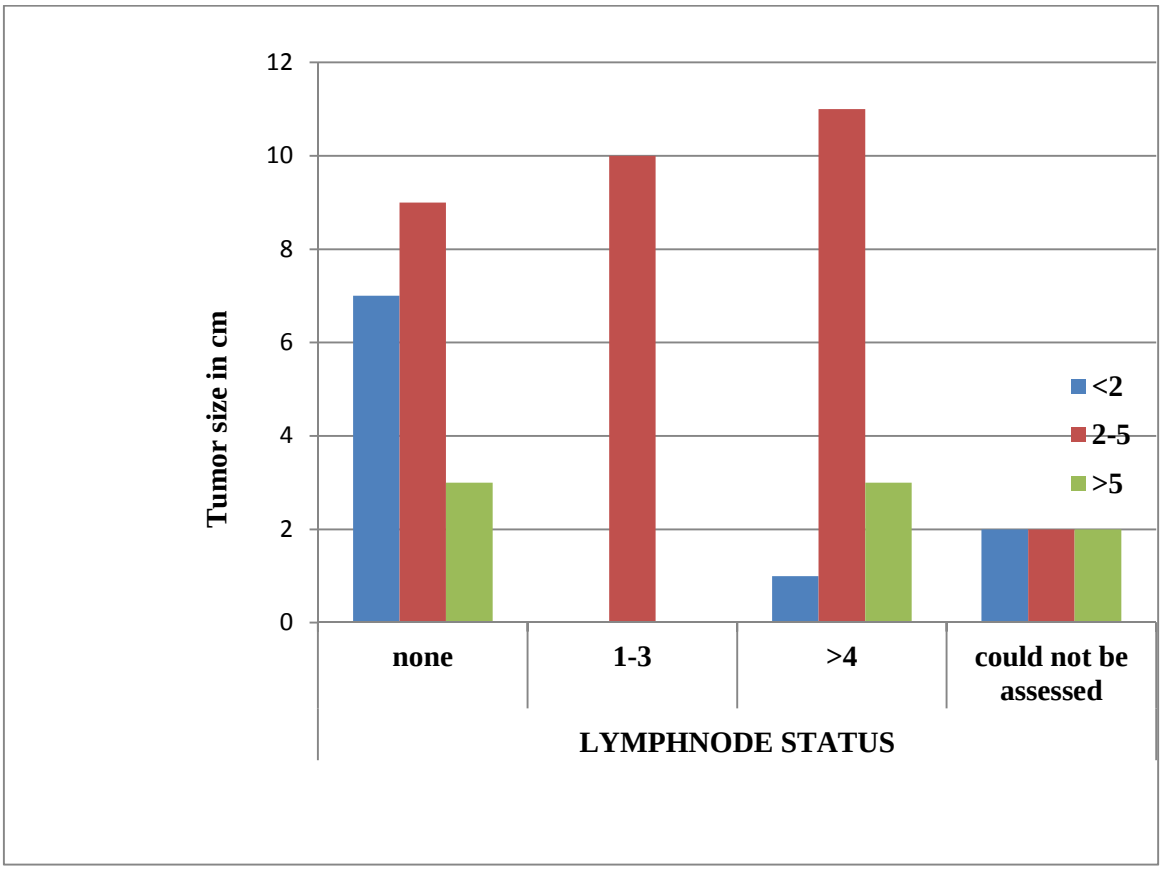
Among the 10 cases with tumour size < 2 cm, 7 cases did not exhibit any lymph node metastasis, 1 case showed > 4 lymph node involvement and lymph nodal status could not be assessed in 2 cases. Among the 32 cases with tumour largest diameter 2-5 cm, 9 cases showed no nodal metastasis, 10 cases showed 1-3 lymph node involvement and 11 cases showed > 4 nodes involvement. Out of the 8 cases with largest tumour diameter > 5 cm, 3 showed no nodal involvement, 3 showed > 4 nodes with metastasis and nodal status could not be assessed in 2 cases.

TABLE 16: CORRELATION OF TUMOR SIZE WITH LYMPH NODE STATUS

TUMOR SIZE	LYMPHNODE STATUS				P VALUE
	NONE	1-3	>4	COULD NOT BE ASSESSED	
<2 cm	7	0	1	2	0.038*
2-5 cm	9	10	11	2	
>5 cm	3	0	3	2	

The association between tumour size and lymph node status was assessed using Chi-Square test. The P value of 0.038* was obtained, which revealed a significant association between the both.

CHART 12: CORRELATION OF TUMOR SIZE WITH LYMPH NODE STATUS



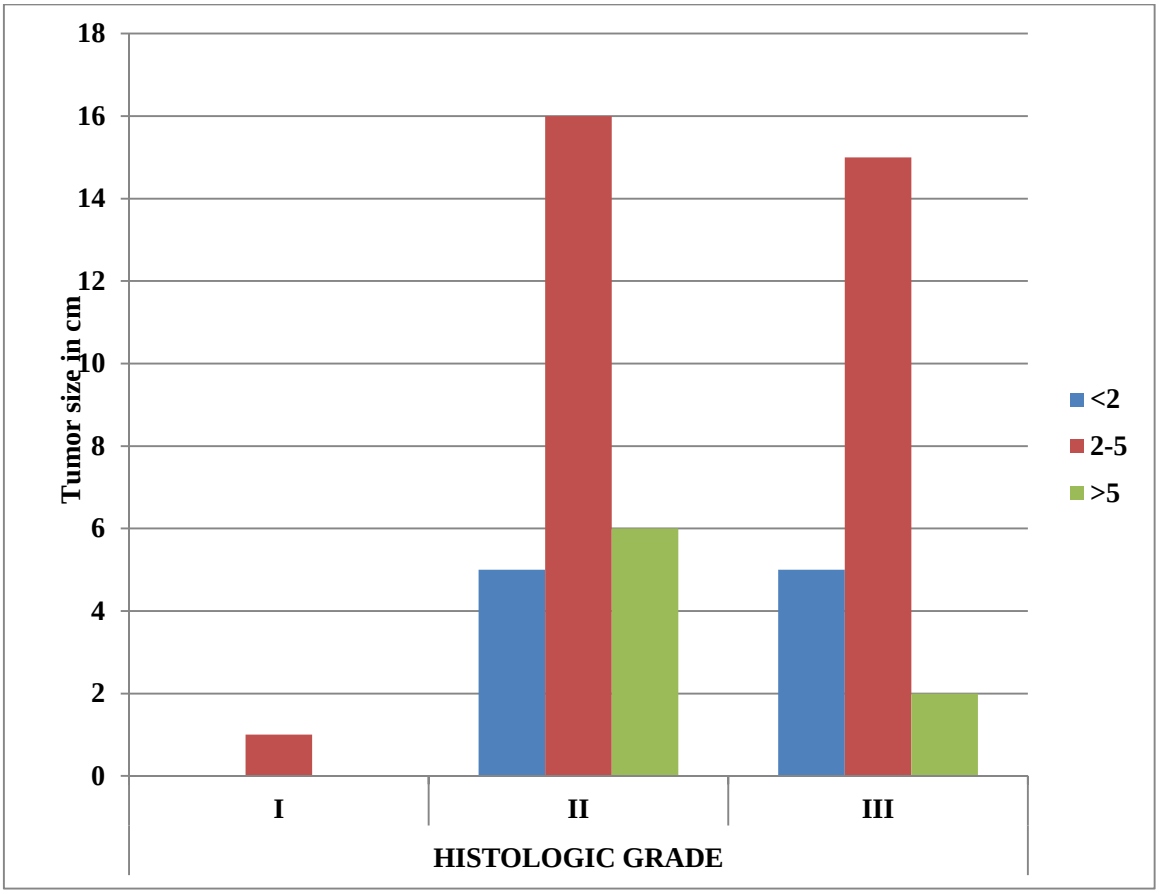
CORRELATION OF TUMOR SIZE WITH HISTOLOGIC GRADE

Among the 10 cases with largest tumour diameter < 2 cm, 4 cases were of histologic grade II, 5 were of histologic grade III and one was atypical medullary carcinoma. Among the 32 cases with tumour size 2-5 cm, 1 case was of grade I, 13 were of grade II, 15 were of grade III and 3 belonged to histologic types other than IDC NOS.

TABLE 17: CORRELATION OF TUMOR SIZE WITH HISTOLOGIC GRADE

TUMOR SIZE	HISTOLOGIC GRADE			P VALUE
	I	II	III	
<2	0	5	5	0.710
2-5	1	16	15	
>5	0	6	2	

CHART 13: CORRELATION OF TUMOR SIZE WITH HISTOLOGIC GRADE



The association between tumour size and histologic grade was determined using Chi-Square test. It revealed an insignificant P value of 0.694, proving that there is no significant association between them.

CORRELATION OF CK 5/6 EXPRESSION WITH CLINICOPATHOLOGIC PARAMETERS

We studied the association between CK 5/6 expression and clinicopathologic parameters using Chi-Square test and did not observe any positive association between them.

TABLE 18: CORRELATION OF CK 5/6 EXPRESSION WITH CLINICOPATHOLOGIC PARAMETERS- AGE AND TUMOR SIZE

PROGNOSTIC PARAMETERS		CK5/6		P VALUE
		POSITIVE	NEGATIVE	
AGE	<40 years	2	6	0.878
	40-50 years	3	15	
	>50 years	5	19	
TUMOR SIZE	<2 cm	3	7	0.568
	2-5 cm	5	27	
	>5 cm	2	6	

TABLE 19: CORRELATION OF CK 5/6 EXPRESSION WITH CLINICOPATHOLOGIC PARAMETERS- HISTOLOGIC TYPE, GRADE AND TUMOR NECROSIS

PROGNOSTIC PARAMETERS		CK5/6		P VALUE
		POSITIVE	NEGATIVE	
HISTOLOGIC TYPE	IDC NOS	8	38	0.134
	Atypical medullary	0	1	
	ILC	1	1	
	Medullary	1	0	
HISTOLOGIC GRADE	I	0	1	0.497
	II	7	20	
	III	3	19	
TUMOR NECROSIS	Present	7	28	1.000
	Absent	3	12	

TABLE 20: CORRELATION OF CK 5/6 EXPRESSION WITH CLINICOPATHOLOGIC PARAMETERS- STROMAL RESPONSE AND LYMPHNODE STATUS

PROGNOSTIC PARAMETERS		CK5/6		P VALUE
		POSITIVE	NEGATIVE	
STROMAL RESPONSE	Fibrosis	2	16	0.391
	Lymphocytic infiltration	6	13	
	Both	2	9	
	None		2	
LYMPHNODE STATUS	None	6	13	0.208
	1-3	1	9	
	≥ 4	1	14	
	Could not be assessed	2	4	

CORRELATION OF EGFR EXPRESSION WITH CLINICOPATHOLOGIC PARAMETERS

The association between EGFR expression and clinicopathologic parameters was studied using Chi-Square test and we observed no significant positive association between them.

TABLE 21: CORRELATION OF EGFR EXPRESSION WITH CLINICOPATHOLOGIC PARAMETERS- AGE AND TUMOR SIZE

PROGNOSTIC PARAMETERS		EGFR		P VALUE
		POSITIVE	NEGATIVE	
AGE	<40 years	3	5	0.068
	40-50 years	15	3	
	>50 years	16	8	
TUMOR SIZE	<2 cm	9	1	0.174
	2-5 cm	19	13	
	>5 cm	6	2	

TABLE 22: CORRELATION OF EGFR EXPRESSION WITH CLINICOPATHOLOGIC PARAMETERS- HISTOLOGIC TYPE, GRADE AND TUMOR NECROSIS

PROGNOSTIC PARAMETERS		EGFR		P VALUE
		POSITIVE	NEGATIVE	
HISTOLOGIC TYPE	IDC NOS	32	14	0.4
	Atypical medullary	1	0	
	ILC	1	1	
	Medullary	0	1	
HISTOLOGIC GRADE	I	0	1	0.305
	II	18	9	
	III	16	6	
TUMOR NECROSIS	Present	9	6	0.907
	Absent	13	6	

TABLE 23: CORRELATION OF EGFR EXPRESSION WITH CLINICOPATHOLOGIC PARAMETERS- STROMAL RESPONSE AND LYMPHNODE STATUS

PROGNOSTIC PARAMETERS		EGFR		P VALUE
		POSITIVE	NEGATIVE	
STROMAL RESPONSE	Fibrosis	12	6	0.735
	Lymphocytic infiltration	12	7	
	Both	8	3	
	None	2		
LYMPH NODE STATUS	None	12	7	0.343
	1-3	5	5	
	≥ 4	12	3	
	Could not be assessed	5	1	

DISCUSSION

Breast cancer is a heterogeneous disease encompassing a variety of distinct clinical, morphological and molecular features exhibiting a range of clinical characteristics and therapeutic implications[8,126].Triple-negative breast cancer is defined by the lack of expression of hormone receptors and Her2 overexpression. They are distinct because of their aggressive clinical behaviour and characteristic clinicopathologic prognostic factors[104].

Nielsen et al. studied the immunohistochemical profile for triple-negative tumours using an immunohistochemical panel consisting of basal cytokeratins and evaluated the prognostic significance of these markers on the tumors[41]. Cheang et al. studied the use of the immunohistochemical panel comprising of five biomarkers ER, PR, Her2, CK 5/6 and EGFR and found that it is a highly helpful tool as a immunohistochemical surrogate for the detection of basal-like breast cancer and hence their therapeutic strategy for treatment[98].

In the present study, the triple-negative breast cancer phenotype was correlated with the clinical and histopathologic parameters such as age, tumour size, histologic type, histologic grade, lymphovascular invasion, lymph nodal status, associated DCIS component, stromal response and the immunohistochemical expression of basal markers namely CK5/6 and EGFR.

Although the predominant age group affected in this current study was < 50 years (52%) followed by patients aged >50 years (48%) with a mean age of 50.4 years. In a study by Rao et al. the mean age was found to be 46.8 years[127].

Thike et al. observed a mean age of 53 years in their study[126]. Hashmi et al. Observed the mean age to be 48.4 years[128]. Tan et al. observed that Triple negative breast cancers are common in patients aged >40 years[129]. In the present study, we did not observe any significant correlation between the expression of CK 5/6 and EGFR and age of the patients.

In the present study, we observed an increased percentage of triple negative breast cancer to be of infiltrating ductal carcinoma IDC, NOS type (92%) followed by other histologic types such as invasive lobular carcinoma and medullary carcinoma. This finding suggests that TNBC can present in various histomorphologic types[126]. Rao et al. Thike et al. and Hashmi et al. also observed the similar findings in their study[126–128]. However, no significant correlation was found between the histologic type and expression of CK 5/6 and EGFR.

In our study, we observed the histologic grades of TNBC to be predominantly grades II and III, 46% and 44% respectively. In a study by Thike et al, they observed an incidence of 77% of grade III histology TNBC[126]. Hashmi et al also observed an increased frequency of grade III tumours of about 63.4%[128]. Rao et al study shows grade III tumours with 76% occurrence[127].

In the present study, the mean tumour size was 4.01 cm. A majority of tumours 64 % were in the range of 2-5 cm size along largest diameter. Kreike et al studied 97 cases of triple negative breast cancers and found 65% of tumours to be >2 cm in largest dimension[130]. Although Thike et al[126] found 70% cases to be > 2cm in size and observed a significant correlation between larger tumours and

CK 5/6 expression, our study did not show any significant correlation between CK 5/6 and EGFR expression and tumour size as observed by Rao et al and Tan et al[127,129].

In our study, we observed tumour necrosis in 70% of the cases studied, in the form of both focal and comedonecrosis. This finding is concordant with the observation by Thike et al and Hashmi et al who observed tumour necrosis in 98% and 96.4% of the cases respectively[126,128]. No significant correlation was observed between basal markers and tumour necrosis which is discordant with the observation by Rao et al who found a significant association between them[127].

In the current study, we observed DCIS component around the tumour in about 16% of the cases. Kreike et al also observed DCIS component in 18% of the TNBC cases[130]. Lymphovascular invasion was observed less frequently in various series with incidence ranging from 20-29%[126–128] only whereas in our current study, we observed lymphovascular invasion in 64% of cases, which is slightly on the higher side. Lymphocytic infiltration was seen in 46% of the cases and did not show statistically significant correlation with CK 5/6 and EGFR.

Lymph node metastasis in triple negative breast cancer has been studied and the results of various series show varied results. The results of some series showed high prevalence rates of node positive TNBC[48,95]. In our study, the lymph node metastases were observed in 50% of the cases.

We carried out a correlation test between tumour size and lymph node status and found a positive correlation with significant P value of 0.038. This finding was concordant with the observation by Thike et al who found that increasing incidence of nodal metastases with increasing tumour sizes[126] and discordant with the findings by Dent et al[104], who observed decreasing incidence of axillary lymph node involvement with increasing tumour sizes.

In the present study, we studied the expressions of CK5/6 and EGFR using immunohistochemistry. We observed that a large proportion of the triple negative breast cancers expressed at least one of the two markers- CK5/6 and/ or EGFR (72%). These results were in concordance with the results of Rao et al and Choccalingam et al who found 74% and 67.7% cases respectively with expression of CK 5/6 and/ or EGFR[125,127].

The basal cytokeratin CK 5/6 was expressed by less than a quarter of the total number of cases (20%), which is less when compared to other previous studies by Nielsen et al and Livasy et al[41,117]. The expression of EGFR was observed in 68% of the cases, which is concordant with the previous studies [117,125,127]. These observations entail an overlap between ‘triple negativity’ and ‘basalness’. Similar results were obtained in studies by Rakha et al and Choccalingam et al[48,125]. Foulkes et al suggest the term “core basal” phenotype to specify the tumours which have triple negativity for ER, PR and Her2 and express at least one of the basal markers CK 5/6 and/ or EGFR. These have a

worse clinical outcome than the tumours which lack expression of all the five markers[108].

The important histologic features of tumours with basal-like phenotype like high tumour grade, lymphocytic infiltration, scant tumour stroma, lymph node metastases, tumour necrosis in the form of geographic necrosis and comedo necrosis, high nuclear grade, increased mitotic rate, frequent apoptosis were also noted in the current study[10].

SUMMARY

This study was conducted in the Department of Pathology, Tirunelveli Medical College in 50 cases of triple-negative primary invasive breast carcinoma. The clinical and histopathologic parameters such as age, tumour size, histologic type, histologic grade, tumour necrosis, lymph node metastases, stromal reaction, lymphovascular invasion, DCIS component were assessed in haematoxylin and eosin stained sections of the tumour tissue, followed by immunohistochemical analysis using CK 5/6 and EGFR was performed.

Among the 50 cases, 46 cases (92%) were infiltrating ductal carcinoma NOS type, 2 cases (4%) were invasive lobular carcinoma, 1 case (2%) was medullary carcinoma and 1 case (2%) was atypical medullary carcinoma. Among the 50 cases, 1case (2%) was of grade I histology, 27 cases (54%) were under grade II, and 21 cases (42%) were grade III tumours.

Among the 50 cases, 32 cases (64%) had tumour size of 2-5 cm along the largest dimension, 32 cases (64%) showed lymphovascular invasion, 8 cases (16%) had associated in situ component around the tumour, 35 cases (70%) had areas of tumour necrosis, 23 cases (46%) had lymphocytic infiltrates and 23 cases (46%) had stromal fibrosis and 25 cases (50%) had lymph node metastases at the time of diagnosis.

Among the 50 cases, 36 cases (72%) expressed at least one of the basal markers CK 5/6 and/ or EGFR. 10 cases (20%) expressed CK 5/6 and 34 cases (68%) expressed EGFR. No statistically significant correlation was noted

between the expression of basal markers CK 5/6 and EGFR and the clinicopathologic prognostic parameters. A strong correlation was found between the tumour size and axillary lymph node metastases with a statistically significant P value of 0.038.

CONCLUSION

Triple-negative breast cancer subtype is a heterogeneous entity which comprises of tumours with distinct clinical and morphologic characteristics. Among these, the basal-like phenotype, as identified by the expression of basal markers CK 5/6 and EGFR, exhibits an aggressive clinical behaviour with poor therapeutic outcomes. The triple-negativity of tumours should not be used as a marker for basal-like tumours. With the use of the basal markers CK 5/6 and EGFR, a clinically aggressive phenotype with poor clinical outcome, the basal-like phenotype can be identified among the triple-negative breast cancer. A large majority of triple negative breast cancers express EGFR, which can be used as a potential target for treatment.

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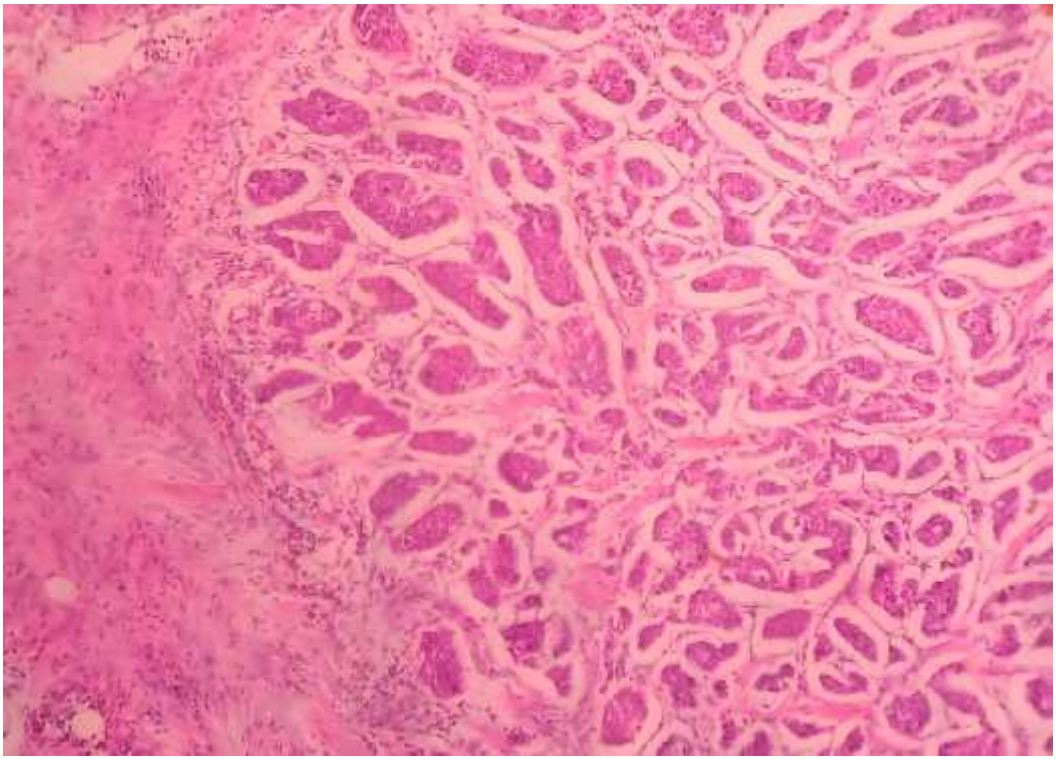


FIGURE 3(A) : INVASIVE DUCTAL CARCINOMA SHOWING TRABECULAR AND NESTED PATTERN OF GROWTH

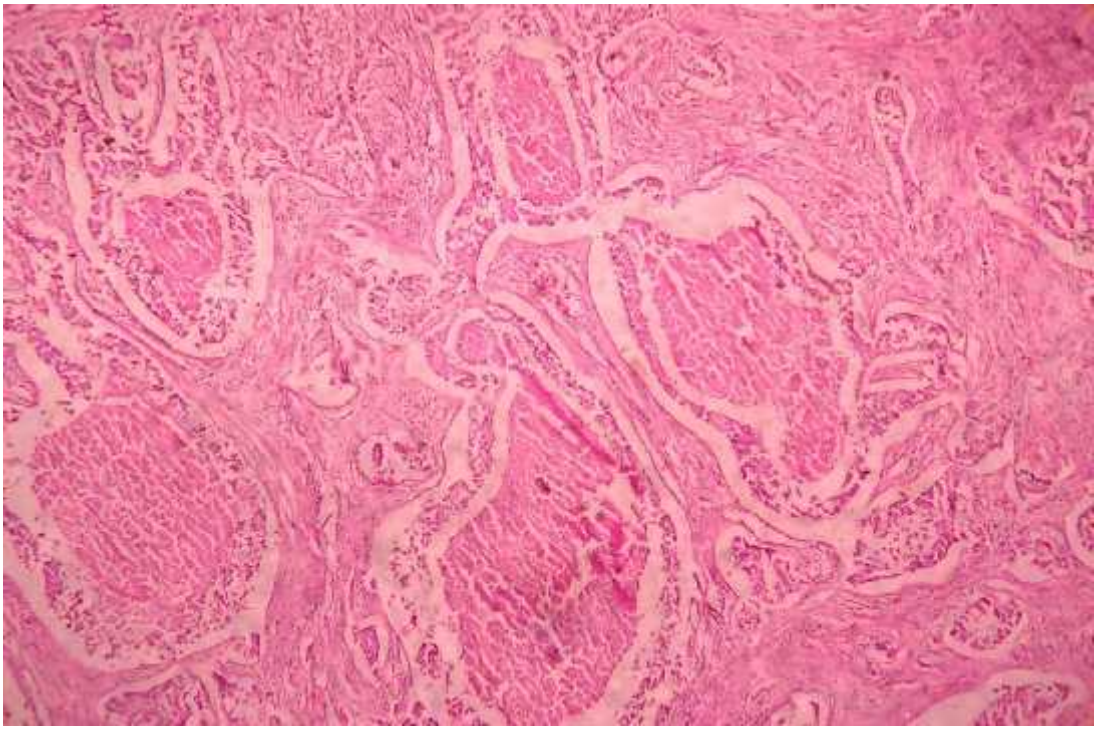


FIGURE 3 (B): COMEDO NECROSIS IN A CASE OF CARCINOMA OF BREAST

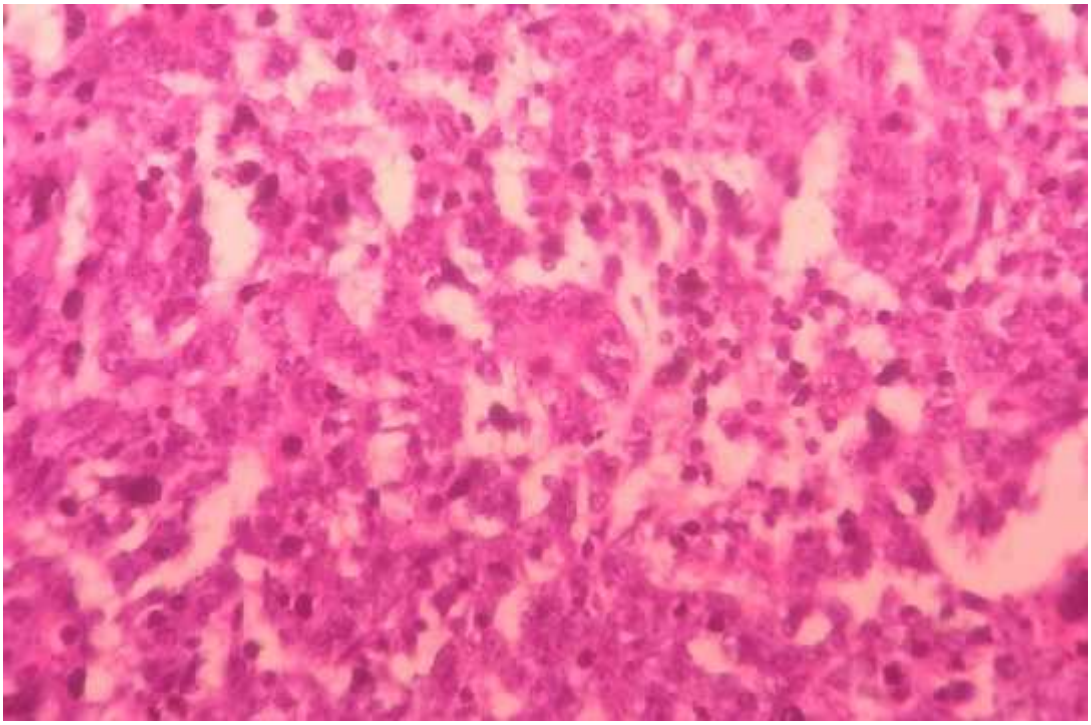


FIGURE 3 (C): HIGH NUCLEAR GRADE AND INCREASED MITOTIC RATE IN A CASE OF BREAST CARCINOMA (HE, x40)

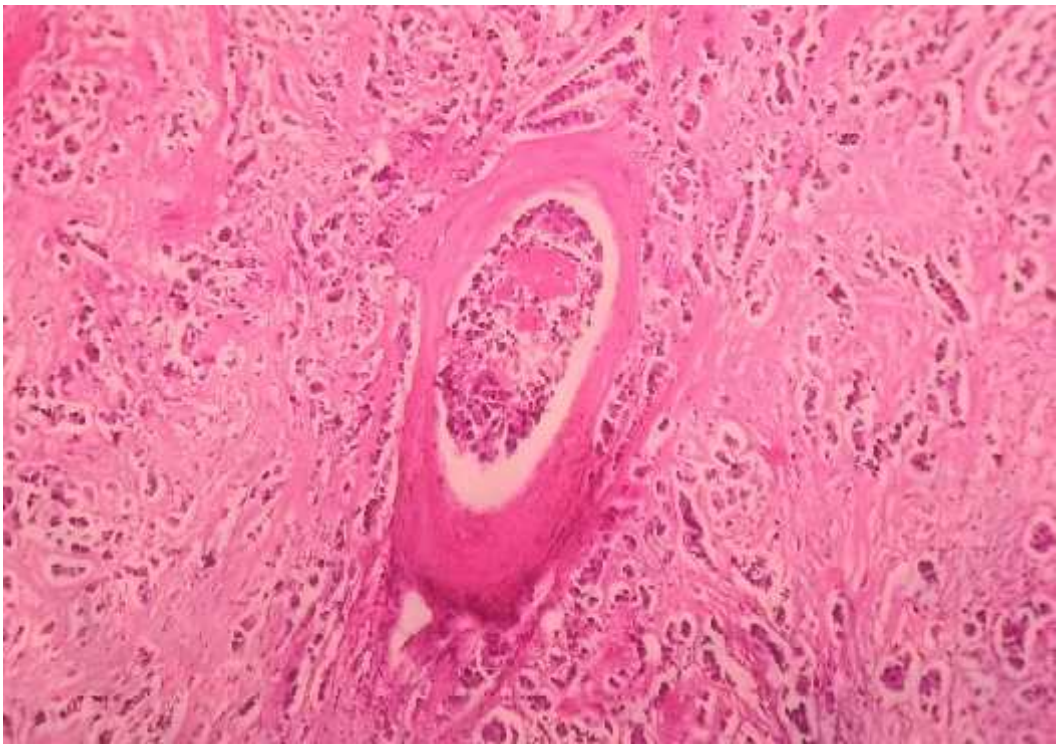


FIGURE 3 (D): INVASION OF MEDIUM SIZED BLOOD VESSEL IN A CASE OF IDC NOS TYPE

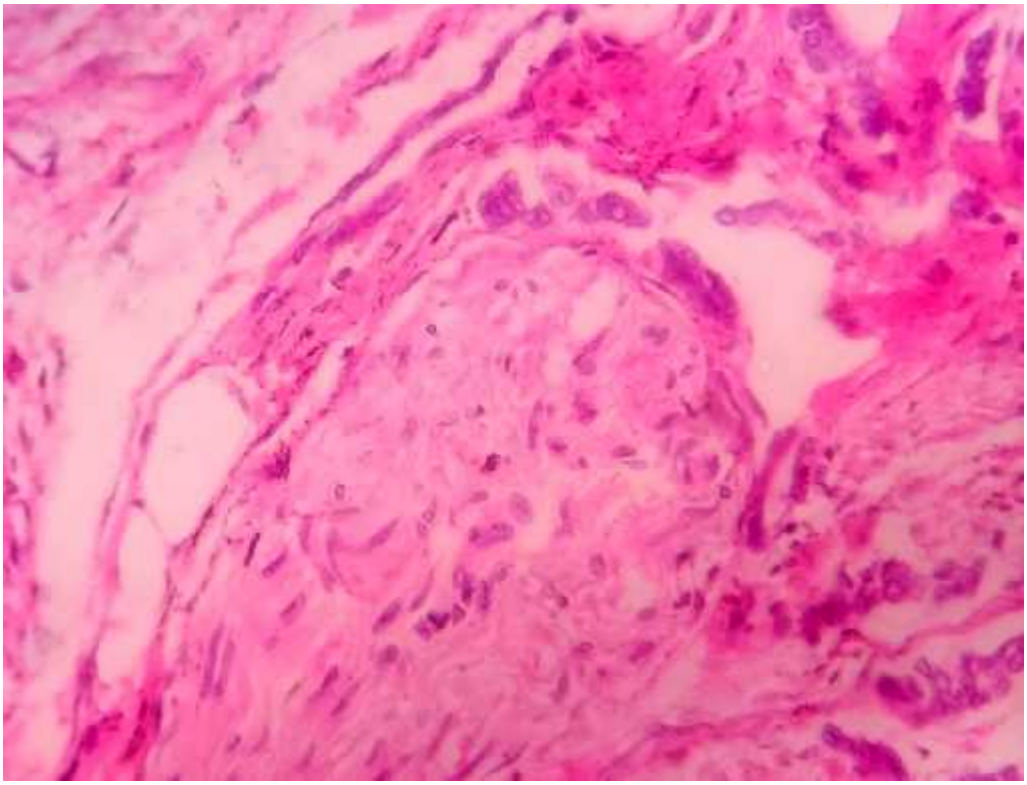


FIGURE 3 (E): PERINEURAL INVASION AT THE INVASIVE FRONT OF TUMOR IN A CASE OF DUCTAL CARCINOMA OF BREAST

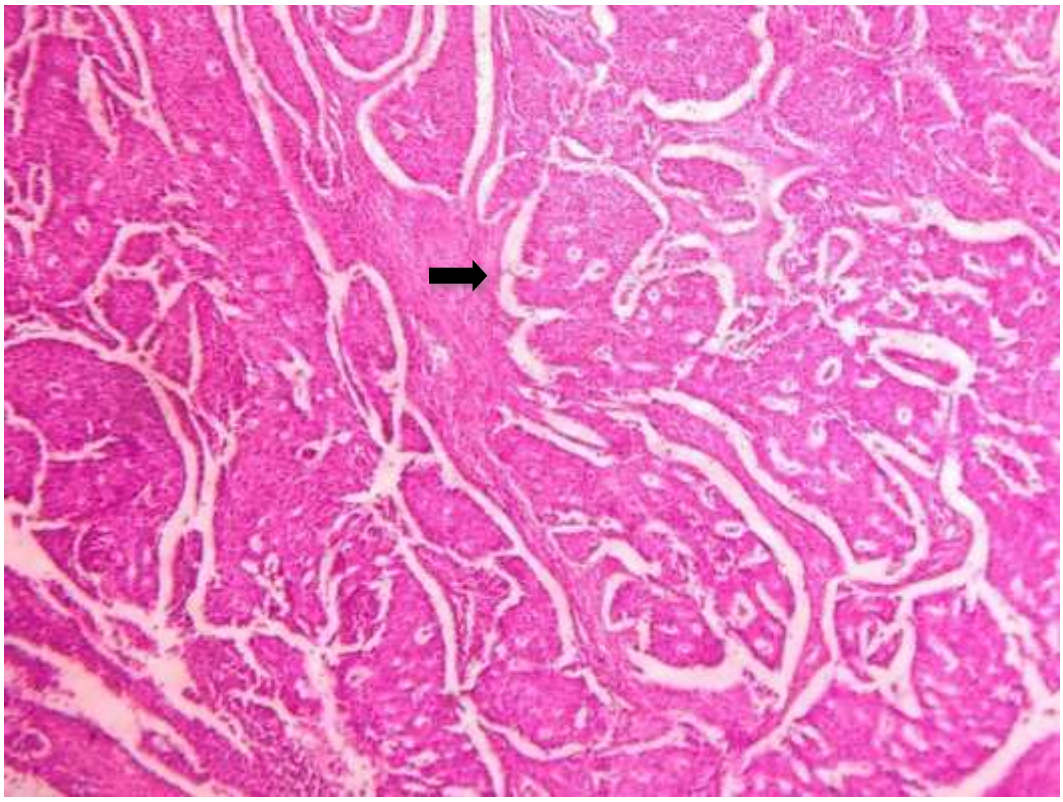


FIGURE 3 (F): INVASIVE DUCTAL CARCINOMA WITH DCIS COMPONENT

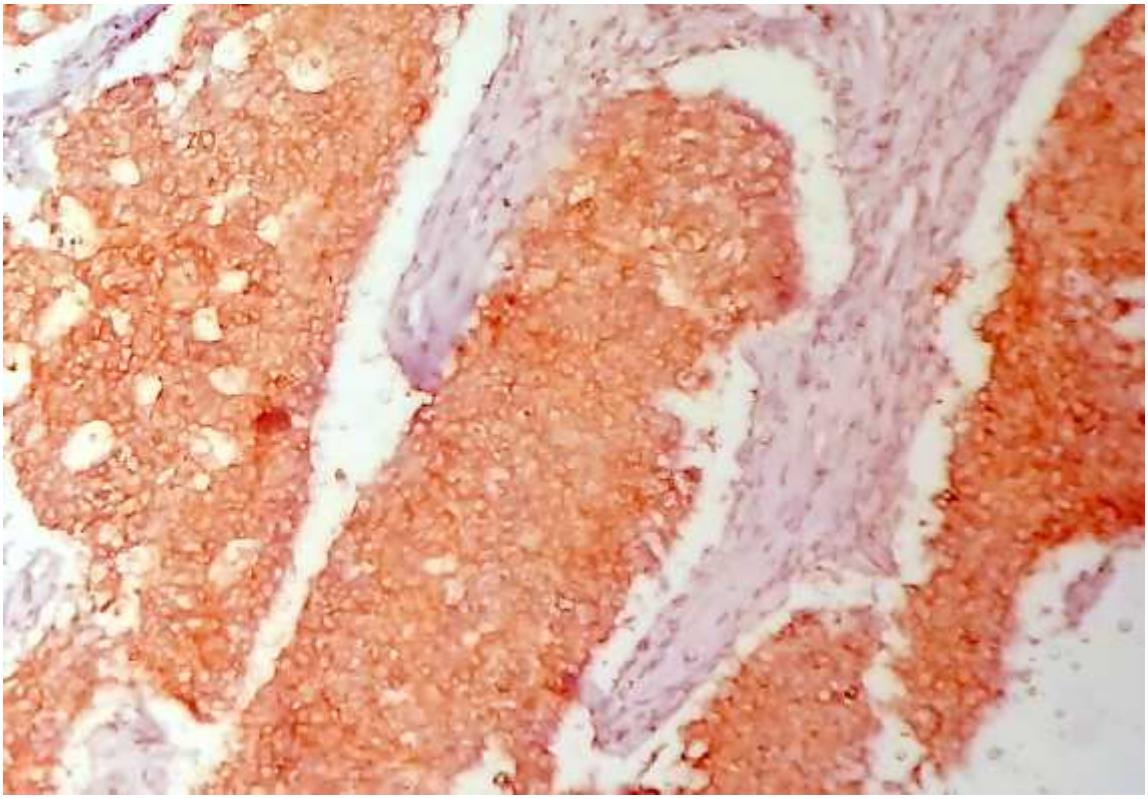


FIGURE 4 (A): IMMUNOHISTOCHEMICAL STAINING SHOWING INTENSE POSITIVITY FOR CYTOKERATIN 5/6

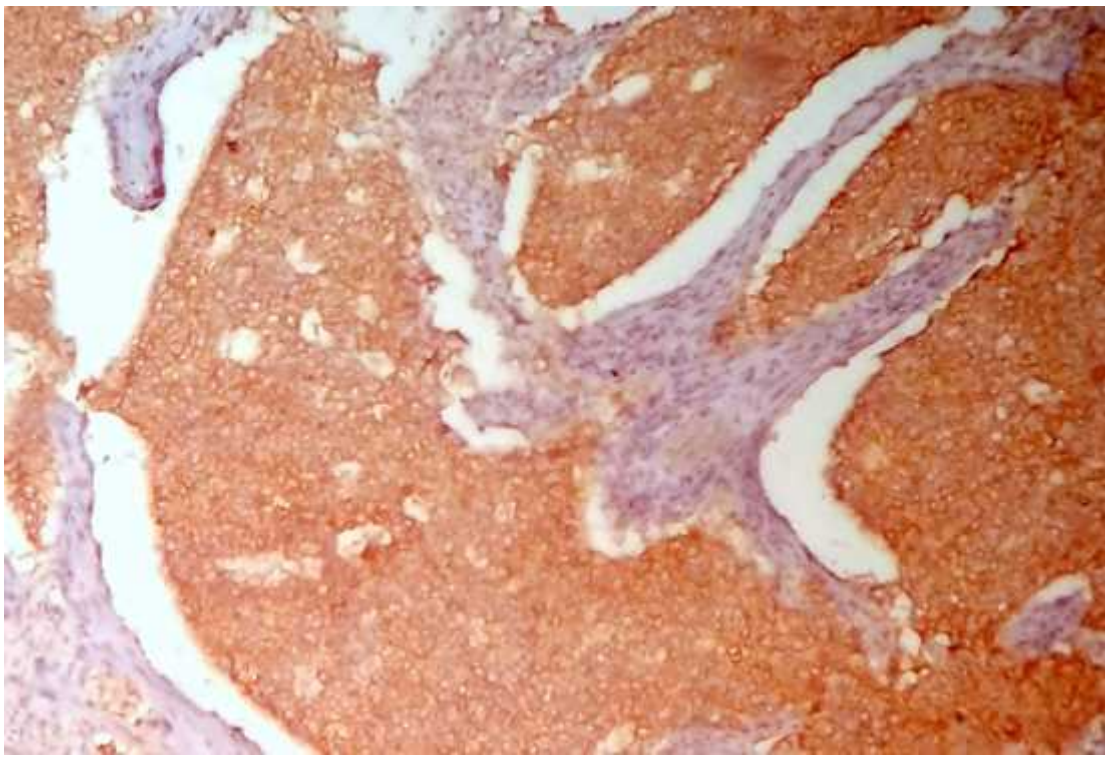


FIGURE 4 (B): IMMUNOHISTOCHEMICAL STAINING SHOWING INTENSE POSITIVITY FOR EGFR

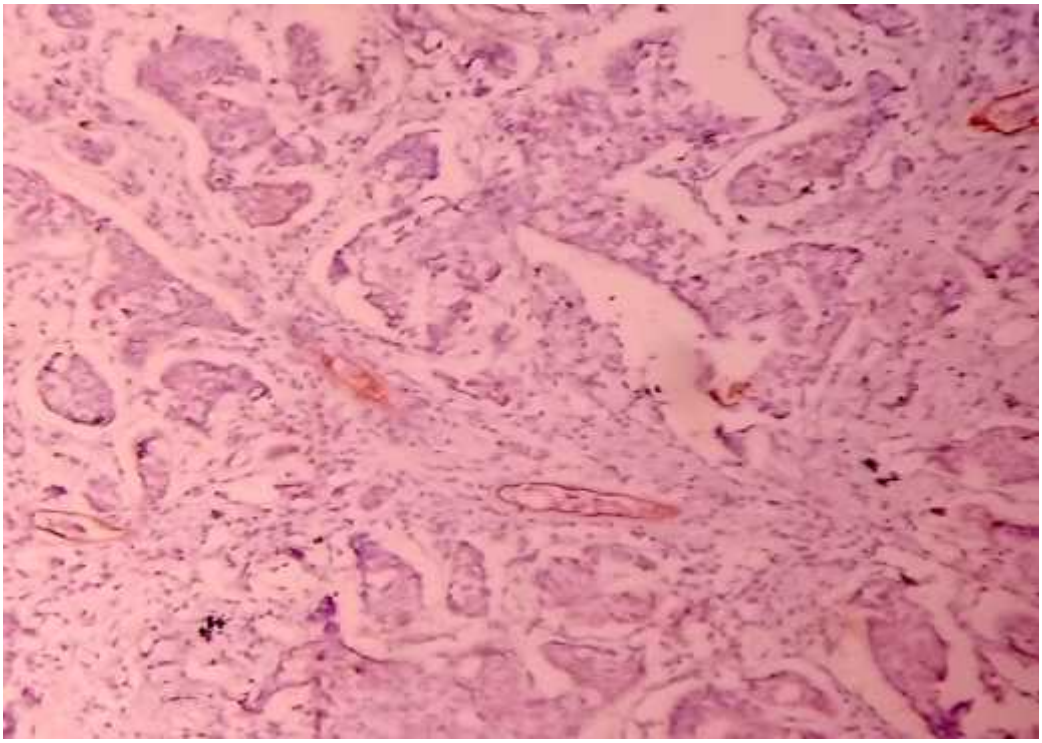


FIGURE 4 (C): IMMUNOHISTOCHEMISTRY SHOWING NEGATIVE STAINING FOR CYTOKERATIN 5/6 WITH POSITIVE STAINING OF INTERNAL CONTROL

ANNEXURE- I

WHO CLASSIFICATION OF TUMORS OF BREAST

Epithelial tumours

1. Invasive ductal carcinoma, not otherwise specified
    a. Pleomorphic carcinoma
    b. Carcinoma with melanotic features
    c. Carcinoma with choriocarcinomatous features
    d. Carcinoma with osteoclast like giant cells
2. Invasive lobular carcinoma
    a. Classic lobular carcinoma
    b. Solid lobular carcinoma
    c. Alveolar lobular carcinoma
    d. Pleomorphic lobular carcinoma
    e. Tubulolobular lobular carcinoma
    f. Mixed lobular carcinoma
3. Tubular carcinoma
4. Cribriform carcinoma
5. Carcinoma with medullary features
    a. Medullary carcinoma
    b. Atypical medullary carcinoma
    c. Invasive carcinoma NST with medullary features
6. Metaplastic carcinoma of no special type
    a. Low-grade adenosquamous carcinoma

b. Fibromatosis-like metaplastic carcinoma
c. Squamous cell carcinoma
d. Spindle cell carcinoma
e. Metaplastic carcinoma with mesenchymal differentiation
    i. Chondroid differentiation
    ii. Osseous differentiation
    iii. Other types of mesenchymal differentiation
f. Mixed metaplastic carcinoma
g. Myoepithelial carcinoma

7. Mucinous carcinoma
8. Carcinoma with signet-ring-cell differentiation
9. Carcinoma with neuroendocrine features
10. Carcinoma with apocrine differentiation
11. Invasive papillary carcinoma
12. Invasive micropapillary carcinoma
13. Adenoid cystic carcinoma
14. Mucoepidermoid carcinoma
15. Salivary gland/skin adnexal type tumours
16. Polymorphous carcinoma
17. Inflammatory carcinoma
18. Bilateral breast carcinoma and non-synchronous breast carcinoma
19. Exceptionally rare types and variants

    Secretory carcinoma

Oncocytic carcinoma

Sebaceous carcinoma

Lipid-rich carcinoma

Glycogen-rich clear cell carcinoma

Acinic cell carcinoma

Lobular neoplasia

Intraductal proliferative lesions

- Usual ductal hyperplasia
- Columnar cell lesions
- Atypical ductal hyperplasia
- Ductal carcinoma in situ

Intraductal papillary lesions

- Intraductal papilloma
- Intraductal papillary carcinoma
- Encapsulated papillary carcinoma
- Solid papillary carcinoma

Benign epithelial proliferations

1. Adenosis, sclerosing adenosis and apocrine adenosis
2. Microglandular adenosis, atypical microglandular adenosis and microglandular adenosis with carcinoma

3. Radial scar and complex sclerosing lesion
4. Tubular adenoma
5. Lactating adenoma
6. Apocrine adenoma
7. Ductal adenoma
8. Pleomorphic adenoma

Myoepithelial and epithelial–myoepithelial lesions

- Myoepithelial and epithelial–myoepithelial lesions
- Adenomyoepithelioma and adenomyoepithelioma with carcinoma

Mesenchymal tumours

1. Nodular fasciitis
2. Benign vascular lesions
3. Pseudoangiomatous stromal hyperplasia
4. Myofibroblastoma
5. Desmoid-type fibromatosis
6. Inflammatory myofibroblastic tumour
7. Lipoma
8. Granular cell tumour and benign peripheral nerve-sheath tumour
9. Angiosarcoma
10. Liposarcoma
11. Rhabdomyosarcoma

12. Osteosarcoma
13. Leiomyoma and leiomyosarcoma

Fibroepithelial tumours

- Fibroadenoma
- Phyllodes tumour
- Hamartoma

Tumours of the nipple

- Nipple adenoma
- Syringomatous tumour
- Paget disease

Lymphoid and haematopoietic tumours

- Diffuse large B-cell lymphoma
- Burkitt lymphoma
- T-cell lymphoma
- Extranodal marginal zone lymphomas of mucosa-associated lymphoid tissue
- Follicular lymphoma

Metastases to the breast

Tumours of the male breast

- Gynaecomastia
- Carcinoma of the male breast

ANNEXURE 2

PROCESSING FOR IMMUNOHISTOCHEMISTRY

1. 4-5µm thick sections are cut using microtome from the selected paraffin blocks.
2. The sections are taken on poly L-lysine coated adhesive slides. The slides were incubated at 60-70º C for one hour.
3. The slides are subjected to 2 changes of xylene 5 minutes each.
4. They are then transferred to absolute alcohol for 5 minutes followed by by 90% and 70% alcohol for 5 minutes to rehydrate the tissue sections.
5. The tissue sections are then placed in running tap water for 5 minutes and washed in distilled water
6. Antigen retrieval is performed using microwave oven in specific buffer solution - TRIS EDTA buffer
7. Then the sections are cooled to room temperature and the slides are washed with distilled water
8. Endogenous peroxidase activity is prevented by incubating the tissue sections with adequate use of peroxidase blocking solution in a humid chamber for 5 minutes. The sections are then washed in TRIS wash buffer.
9. The primary antibody (CK 5/6 and EGFR) is then added over the tissue sections and incubated for 30 minutes.
10. The tissue sections are then washed in TRIS wash buffer.
11. A primary amplifier is added for 15 minutes to enhance the application of primary antibody which is then followed by washing in TRIS wash buffer

12. The secondary antibody is added and incubated for 12 minutes and then washed with TRIS wash buffer
13. The working solution of DAB chromogen (1ml DAB buffer +1 drop DAB chromogen) is then added over the tissue and incubated for 2-5 minutes and then washed with 2 changes of distilled water.
14. Counterstaining of nuclei was done with Harris' haematoxylin for 30 seconds and washed in running tap water.
15. Dehydration is done by 2 changes each in increasing grades of alcohol.
16. Mounting is done by DPX mountant and observed under microscope.

BUFFER PREPARATIONS

1. **TRIS – EDTA BUFFER: pH- 9.0**

   Tris - 6.05 gm

   EDTA - 0.744 gm

   Distilled water - 1000 ml

2. **TRIS WASH BUFFER**

   Tris - 0.605 gm

   Sodium chloride - 8 gm

   1 N HCl - 4ml

   Distilled water - 1000 ml

PRECAUTIONS

1. The glassware used should be dry and clean.
2. All the buffer solutions used should be prepared fresh and the pH should be adjusted accordingly
3. The staining procedures are never allowed to dry so they are performed under a humidified chamber.
4. The DAB chromogen should be handled and disposed carefully as it is a carcinogen.
5. The primary and secondary antibody, DAB chromogen, peroxidase block, and amplifier, everything should be stored at 4-6ºC.

ANNEXURE- III

ALLRED SCORING GUIDELINES FOR ER AND PR

Proportion score

Proportion score is done by calculating the proportion of tumour cells with stained nuclei.

0 = no nuclear staining

1 = <1% nuclear staining

2 = 1%-10% nuclear staining,

3 = 11%-33% nuclear staining

4 = 34%-66% nuclear staining

5 = 67%-100% nuclear staining

Intensity of staining

0 = no staining

1 = weak staining

2 = moderate staining

3 = strong staining

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

Interpretation:

0,2 – Negative

≥ 3 - Positive

GRADING OF THE IMMUNOHISTOCHEMICAL STAINING FOR HER 2 OVEREXPRESSION

SCORE	STAINING PATTERN	HER 2 OVEREXPRESSION ASSESSMENT
0	No staining at all or very slight partial membrane staining in less than 10% of tumour cells.	Negative
1+	Faint barely perceptible membrane staining in more than 10% of tumour cells. Cells stained in only part of the membrane.	Negative
2+	Weak to moderate complete membrane staining observed in more than 10% of tumour cells.	Weakly Positive
3+	Strong complete membrane staining in more than 30% of tumour cells	Strongly Positive

SCORING FOR CYTOKERATIN (CK5/6)

Cytokeratin 5/6 was scored as positive if any faint or strong cytoplasmic and/or membranous immunostaining was observed in the neoplastic tumour cells.

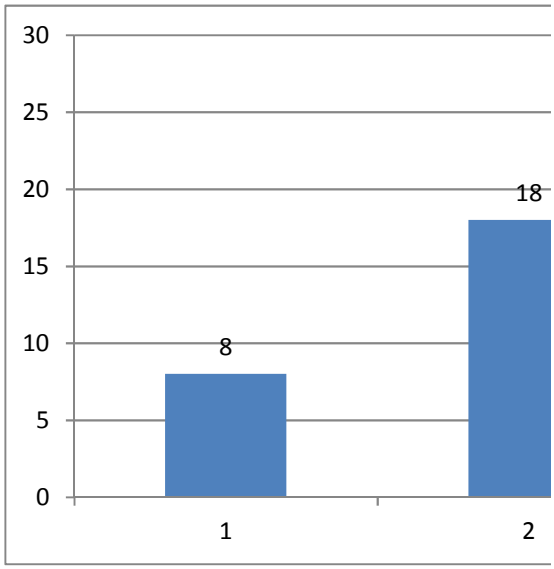
SCORING FOR EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR)

EGFR was scored as positive if more than 1% of the tumour cells showed membrane immunoreactivity of any intensity.

S. NO	PATH NO	AGE (Yrs)	MENOPAUSE	TYPE OF SURGERY	TUMOR SIZE (CM)	NECROSIS	NO. OF NODES	LARGEST NODE (CM)	SMALLEST NODE (CM)	HISTOLOGICAL TYPE	GRADE	LV INVASION	PERINEURAL INVASION	INSITU COMPONENT	NECROSIS	STROMAL REACTION	MARGINS	SKIN	NIPPLE & AREOLA	INVOLVED LYMPHNODES	CK5/6	EGFR	CK5/6/EGFR
1	2207/15	55	attained	MRM	3.5	absent	12	2	0.3	IDC NOS	III	present	absent	absent	present	fibrosis	free	involved	involved	7	negative	negative	-/-
2	472/15	55	attained	completion mastectomy	7	present	1	2	-	IDC NOS	II	present	absent	absent	present	lymphocytic infiltration	free	free	free	free	negative	negative	-/-
3	1358/15	40	-	simple mastectomy with axillary clearance	3	absent	1	0.3	-	medullary carcinoma	-	absent	absent	absent	present	fibrosis	free	free	free	free	positive	negative	+/-
4	2381/15	56	attained	MRM	2	absent	2	0.5	0.4	IDC NOS	II	absent	absent	absent	present	lymphocytic infiltration	free	free	free	free	negative	negative	-/-
5	1547/15	40	attained	MRM	5	present	22	2	1	IDC NOS	II	present	absent	absent	present	lymphocytic infiltration	free	free	free	free	negative	negative	-/-
6	1218/15	50	attained	MRM	5	absent	8	1	0.2	IDC NOS	III	present	absent	absent	present	fibrosis	free	free	free	3	negative	negative	-/-
7	1717/15	52	attained	MRM	4	absent	5	3	0.5	IDC NOS	III	present	absent	absent	absent	fibrosis & lymphocytic	free	free	free	free	negative	positive	-/+
8	2683/15	36	-	MRM	11	absent	12	1.5	0.3	IDC NOS	II	absent	absent	absent	present	lymphocytic infiltration	free	free	free	free	negative	negative	-/-
9	3320/15	47	-	MRM	2	absent	-	-	-	IDC NOS	II	absent	absent	absent	absent	fibrosis	free	free	free	-	positive	positive	+/+
10	3520/15	60	attained	MRM	9	absent	5	1	0.3	IDC NOS	II	present	absent	absent	absent	fibrosis	free	free	free	5	positive	positive	+/+
11	2840/15	56	attained	MRM	2.5	absent	11	2	0.5	IDC NOS	III	present	absent	absent	absent	fibrosis & lymphocytic	free	free	free	9	negative	positive	-/+
12	2598/15	47	-	mastectomy with axillary clearance	8	absent	-	-	-	IDC NOS	II	absent	absent	present	present	fibrosis	involved	free	free	-	negative	positive	-/+
13	1850/15	44	-	MRM	3.5	present	19	4	0.3	IDC NOS	III	present	absent	absent	present	fibrosis & lymphocytic	free	free	free	12	negative	positive	-/+
14	3846/15	48	-	MRM	2	absent	-	-	-	IDC NOS	II	present	absent	present	absent	lymphocytic infiltration	free	free	free	-	negative	positive	-/+
15	3628/15	51	attained	MRM	4	absent	10	1.5	0.5	IDC NOS	II	absent	absent	absent	present	fibrosis & lymphocytic	free	free	free	free	positive	positive	+/+
16	939/15	55	-	simple mastectomy with axillary clearance	5	present	3	2	0.5	IDC NOS	II	present	absent	absent	present	fibrosis & lymphocytic	free	free	free	3	negative	negative	-/-
17	3209/16	56	attained	MRM	7	absent	9	2	0.8	IDC NOS	II	absent	absent	present	present	fibrosis	free	free	free	free	positive	positive	+/+
18	2027/16	44	-	MRM	2	present	7	2.5	1	IDC NOS	III	absent	absent	absent	present	lymphocytic infiltration	free	free	free	free	positive	positive	+/+
19	2006/15	33	-	MRM	3	absent	8	1	0.3	ILC	-	present	absent	absent	present	fibrosis & lymphocytic	free	free	free	-	positive	negative	+/-
20	2980/15	57	attained	bilateral MRM	9	present	-	-	-	IDC NOS	II	present	absent	absent	present	lymphocytic infiltration	free	involved	free	-	negative	positive	-/+
20	2980/15	57	attained	bilateral MRM	4	present	8	2	0.3	IDC NOS	II	present	absent	present	present	lymphocytic infiltration	free	involved	free	8	negative	positive	-/+
21	3607/15	42	-	simple mastectomy with axillary clearance	6	absent	14	2	0.5	IDC NOS	III	present	absent	absent	present	-	free	free	free	14	negative	positive	-/+
22	3358/15	65	attained	simple mastectomy with axillary clearance	4	absent	4	2	0.5	IDC NOS	II	present	present	absent	absent	fibrosis	free	free	free	2	negative	positive	-/+
23	1261/16	22	-	MRM	1.5	absent	5	1	0.3	IDC NOS	III	absent	absent	absent	present	lymphocytic infiltration	free	free	free	free	negative	positive	-/+
24	763/16	42	-	MRM	4.5	absent	4	1	0.2	IDC NOS	III	present	absent	absent	present	fibrosis & lymphocytic	free	free	involved	4	negative	positive	-/+
25	1260/16	50	attained	MRM	3.5	absent	8	1	0.5	IDC NOS	I	absent	absent	absent	absent	fibrosis	free	free	free	free	negative	negative	-/-
26	1362/16	50	-	MRM	1	absent	8	1	0.3	IDC NOS	III	present	absent	present	absent	fibrosis	free	free	free	free	positive	positive	+/+
27	775/16	56	attained	MRM	4	absent	4	1.5	0.2	IDC NOS	II	present	absent	present	present	lymphocytic infiltration	free	free	free	2	negative	positive	-/+
29	208/16	38	-	MRM	4	absent	7	1.5	0.5	IDC NOS	III	present	absent	present	present	fibrosis	free	free	free	free	negative	positive	-/+
30	671/16	60	attained	MRM	4	absent	4	1.5	0.2	IDC NOS	III	absent	absent	absent	present	fibrosis	free	free	free	1	positive	positive	+/+
31	762/16	63	attained	MRM	1.5	present	10	1	0.2	IDC NOS	III	absent	absent	absent	present	fibrosis	free	involved	involved	free	negative	positive	-/+
32	210/16	46	-	MRM	3	absent				IDC NOS	III	present	absent	absent	present	fibrosis	free	free	free	-	negative	positive	-/+
33	3375/16	70	attained	simple mastectomy with axillary clearance	3	absent	11	1.5	0.2	IDC NOS	III	present	absent	absent	absent	fibrosis & lymphocytic	free	free	free	4	negative	negative	-/-
34	3615/16	45	-	MRM	4	absent	12	2.5	0.5	IDC NOS	II	present	absent	absent	absent	lymphocytic infiltration	free	free	free	4	negative	negative	-/-
35	1526/16	65	attained	MRM	1	present	19	3.5	0.5	IDC NOS	II	absent	absent	absent	present	lymphocytic infiltration	free	free	free	free	negative	positive	-/+
36	3605/15	53	-	MRM	3.5	present	7	2.5	0.5	IDC NOS	III	present	absent	absent	present	fibrosis	free	free	free	2	negative	negative	-/-
37	1599/16	35	-	MRM	2.5	absent	14	1.5	0.5	IDC NOS	III	present	absent	present	present	-	free	free	free	1	negative	positive	-/+
38	1620/16	47	-	MRM	4	absent	21	3	0.8	IDC NOS	III	present	absent	absent	present	fibrosis	free	free	free	8	negative	positive	-/+
39	4104/16	47	-	MRM	2	present	9	2	0.5	IDC NOS	III	absent	absent	absent	absent	fibrosis & lymphocytic	involved	free	free	5	negative	positive	-/+
40	1949/16	49	-	MRM	4	present	16	1.5	0.5	IDC NOS	II	present	present	absent	absent	lymphocytic infiltration	free	free	free	5	negative	positive	-/+
41	1598/16	50	-	MRM	3	absent	5	1.5	0.5	ILC	-	absent	absent	absent	present	lymphocytic infiltration	free	free	free	free	negative	positive	-/+
42	4007/16	48	-	MRM	2	absent	2	1	0.5	atypical medullary carcinoma	-	present	absent	absent	present	fibrosis & lymphocytic	free	free	free	free	negative	positive	-/+
43	3653/16	52	attained	MRM	3.5	present	12	2	0.5	IDC NOS	III	absent	absent	absent	absent	fibrosis	involved	free	free	2	negative	negative	-/-
44	4115/16	51	attained	MRM	4	present	12	2.5	0.5	IDC NOS	II	absent	absent	absent	absent	fibrosis	free	free	free	free	negative	negative	-/-
45	1999/16	38	-	MRM	4	absent	13	4.5	0.5	IDC NOS	III	present	absent	absent	absent	lymphocytic infiltration	free	free	free	1	negative	negative	-/-
46	1146/16	52	attained	MRM	2.5	present	5	0.5	0.3	IDC NOS	II	present	absent	absent	present	lymphocytic infiltration	free	free	free	free	positive	positive	+/+
47	41/16	48	-	MRM	3	present	10	2	0.5	IDC NOS	II	present	absent	absent	present	lymphocytic infiltration	free	free	free	7	negative	positive	-/+
48	4747/16	62	attained	MRM	5	present	14	2.5	0.8	IDC NOS	II	present	absent	absent	present	fibrosis	free	free	free	8	negative	positive	-/+
49	8002/16	70	attained	MRM	7	present	16	3	0.5	IDC NOS	III	present	absent	absent	present	fibrosis & lymphocytic	free	free	involved	12	negative	positive	-/+
50	3833/14	65	attained	MRM	4	present	6	2	0.5	IDC NOS	II	absent	absent	absent	present	lymphocytic infiltration	free	free	free	1	negative	positive	-/+

AGE_GRP

		Frequency	Percent
Valid	1	8	16%
	2	18	36%
	3	24	48%
	Total	50	100



MENO

		Frequency	Percent
Valid	1	25	50%
	2	25	50%
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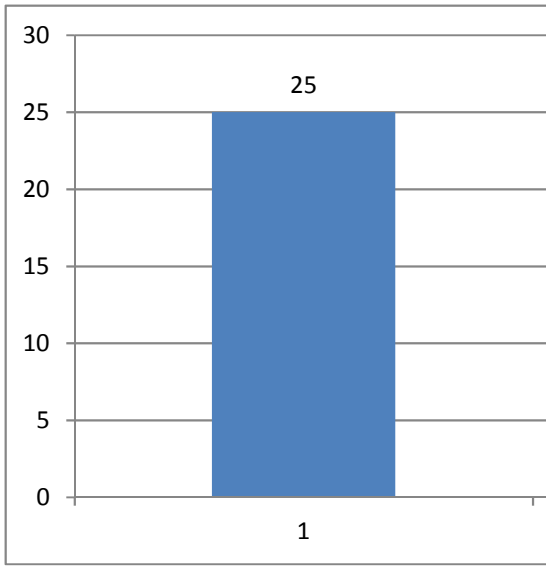
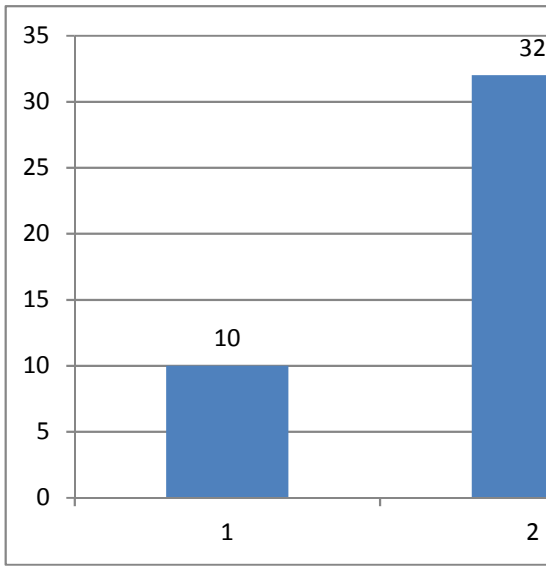


SIZE

		Frequency	Percent
Valid	1	10	20%
	2	32	64%
	3	8	16%
	Total	50	100

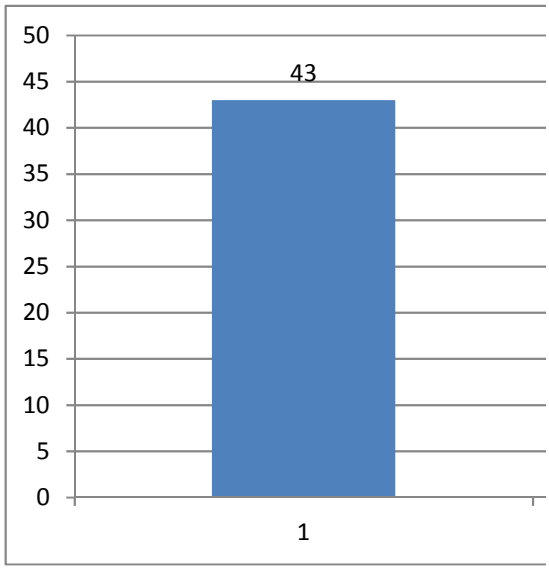
SURG

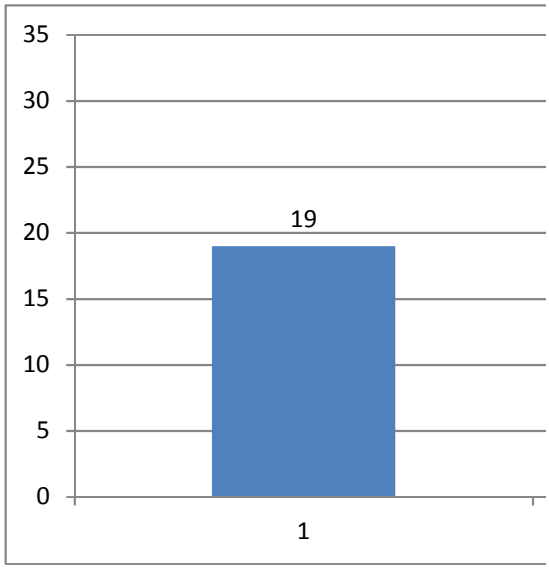
		Frequency	Percent
Valid	1	43	86%
	2	7	14%
	Total	50	100

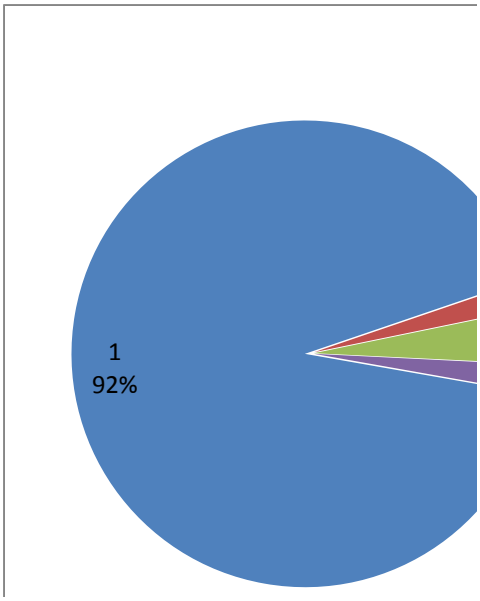


Gross Necrosis

		Frequency	Percent
Valid	1	19	38%
	2	31	62%
	Total	50	100







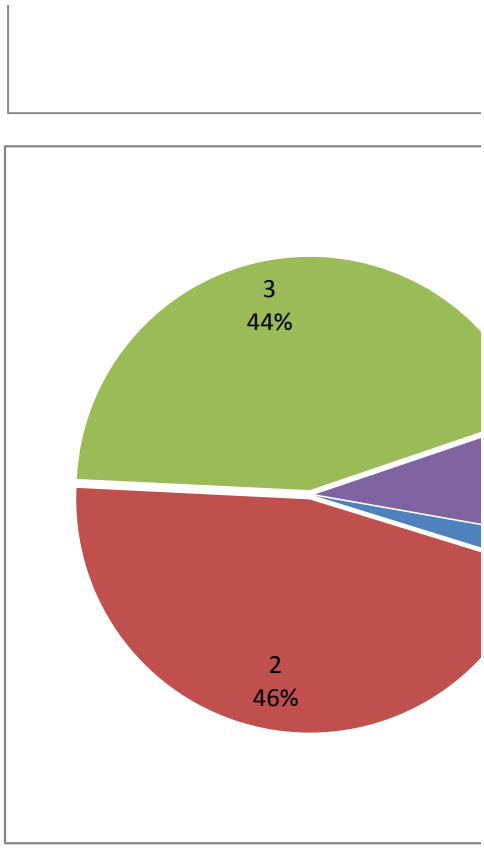
HISTO

		Frequency	Percent
Valid	1	46	92%
	2	1	2%
	3	2	4%
	4	1	2%
	Total	50	100

GRADE

		Frequency	Percent

Valid	1	1	2%
	2	23	46%
	3	22	44%
Missing	4	4	8%
Total		50	100

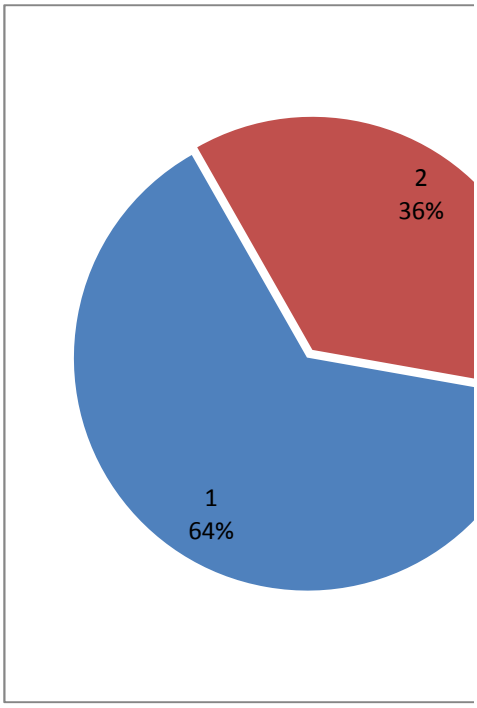


LVINV

		Frequency	Percent
Valid	1	32	64%
	2	18	36%
	Total	50	100

PERINE

		Frequency	Percent
Valid	1	2	4%
	2	48	96%
	Total	50	100

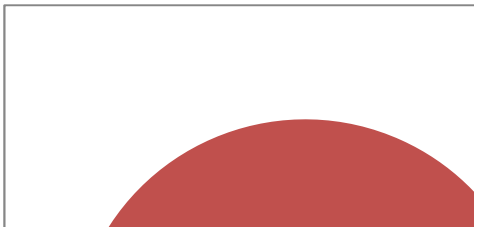


INSITU

		Frequency	Percent
Valid	1	8	16%
	2	42	84%
	Total	50	100

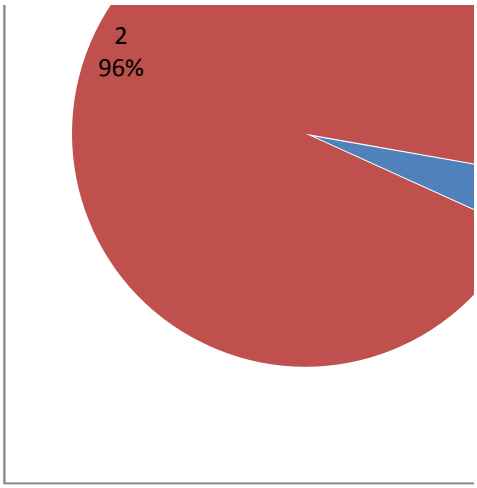
Micro Necrosis

		Frequency	Percent
Valid	1	35	70%
	2	15	30%
	Total	50	100



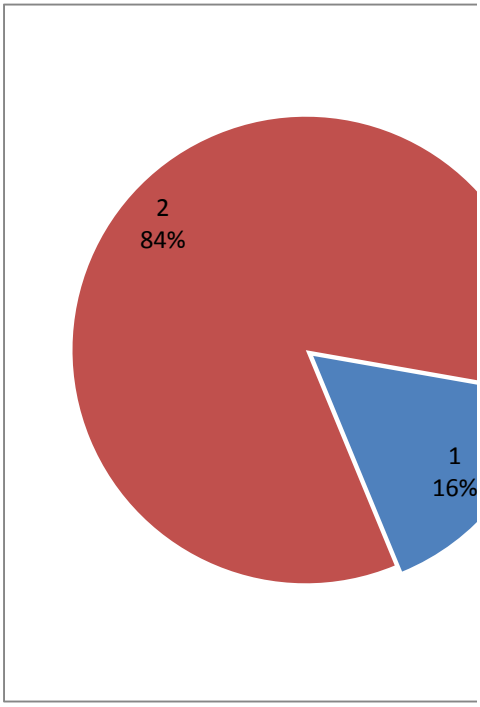
STROM

		Frequency	Percent
Valid	1	1	2%
	2	23	23%
	3	22	22%
	4	4	8%
Total		50	100



MARGI2

		Frequency	Percent
Valid	1	47	94%
	2	3	6%
	Total	50	100

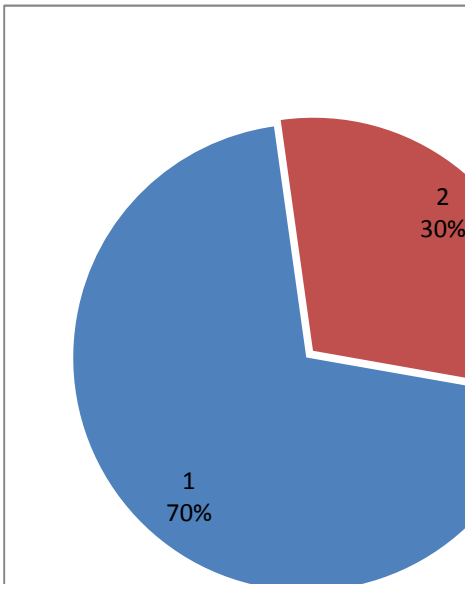


SKIN2

		Frequency	Percent
Valid	1	46	92%
	2	4	8%
	Total	50	100

Nipple

		Frequency	Percent
Valid	1	46	92%
	2	4	8%
	Total	50	100

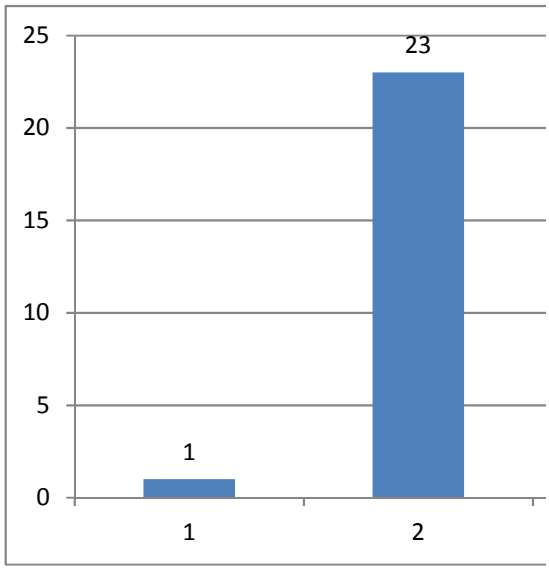


LYMPHNO

		Frequency	Percent
Valid	1	19	38%
	2	10	20%
	3	15	30%
	4	6	12%
	Total	50	100

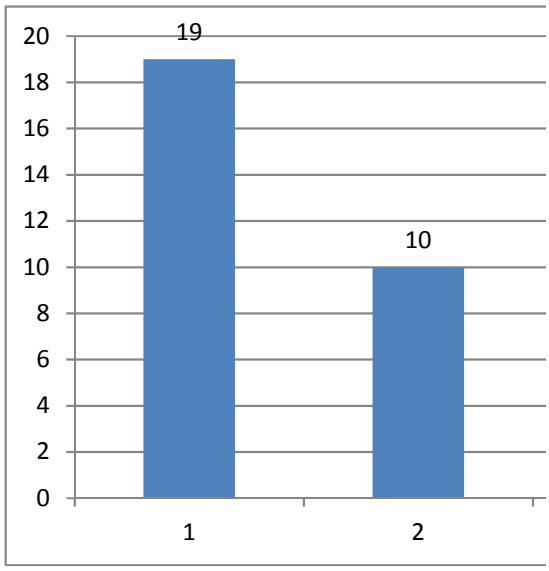
CK56

		Frequency	Percent
Valid	1	10	20%
	2	40	80%
	Total	50	100



EGFR

		Frequency	Percent
Valid	1	34	68%
	2	16	32%
	Total	50	100



CK56EGFR

		Frequency	Percent
Valid	1	8	16%
	2	2	4%
	3	26	52%
	4	14	28%
	Total	50	100

ECADH

		Frequency	Percent
Valid	1	10	20%
	2	7	14%
	3	13	26%
	4	20	40%
	Total	50	100

Crosstab

Count

		LYMPHNO				P value
		1	2	3	4	
Tumor Size	1	7		1	2	0.038
	2	9	10	11	2	
	3	3		3	2	

Crosstab
Count

		GRADE				P value
		1	2	3	4	
Tumor Size	1		4	5	1	0.694
	2	1	13	15	3	
	3		6	2		

ECADH * LYMPHNO Crosstabulation
Count

		LYMPHNO				P value
		1	2	3	4	
ECADH	1	4	2	3	1	0.253
	2	5		2		
	3	4	3	6		
	4	6	5	4	5	

Crosstab
Count

		CK56		P value
		1	2	
AGE_GRP	1	2	6	0.878
	2	3	15	
	3	5	19	

Crosstab
Count

		CK56		P value
		1	2	
SIZ_CAT	1	3	7	0.568
	2	5	27	

	3	2	6	

Crosstab
Count

		CK56		P value
		1	2	
HISTO	1	8	38	0.134
	2		1	
	3	1	1	
	4	1		

Crosstab
Count

		CK56		P value
		1	2	
GRADE	1		1	0.685
	2	5	18	
	3	3	19	

Crosstab
Count

		CK56		P value
		1	2	
NECROS2	1	7	28	1.000
	2	3	12	

STROM * CK56 Crosstabulation
Count

		CK56		P value
		1	2	
STROM	fib	2	16	0.391
	lym	6	13	
	both	2	9	
	none		2	

Crosstab
Count

		CK56		P value
		1	2	
LYMPHNO	1	6	13	0.208
	2	1	9	
	3	1	14	
	4	2	4	

Crosstab
Count

		EGFR		P value
		1	2	
AGE_GRP	1	3	5	0.068
	2	15	3	
	3	16	8	

Crosstab
Count

		EGFR		P value
		1	2	
SIZ_CAT	1	9	1	0.174
	2	19	13	
	3	6	2	

Count

		EGFR		P value
		1	2	
HISTO	1	32	14	0.4
	2	1		
	3	1	1	
	4		1	

Crosstab
Count

		EGFR	P value

		1	2	
GRADE	1		1	0.303
	2	16	7	
	3	16	6	

Crosstab
Count

		EGFR		P value
		1	2	
NECROS2	1	25	10	0.427
	2	9	6	

STROM * EGFR Crosstabulation
Count

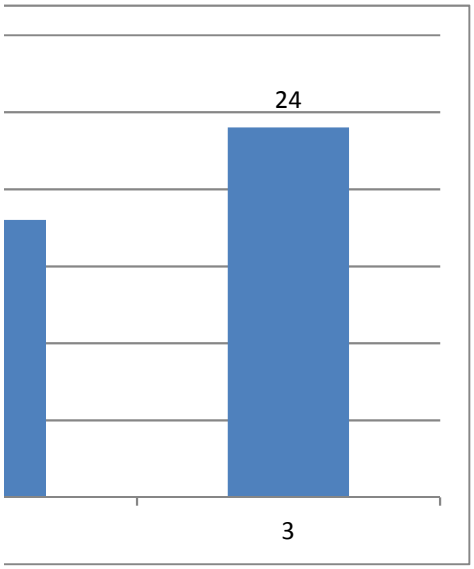
		EGFR		
		1	2	P value
STROM	fib	12	6	0.735
	lym	12	7	
	both	8	3	
	none	2		

Crosstab
Count

		EGFR		P value
		1	2	
LYMPHNO	1	12	7	0.343
	2	5	5	
	3	12	3	
	4	5	1	

CK56EGFR

		Frequency	Percent
Valid	1	8	16%
	2	2	4%
	3	26	52%
	4	14	28%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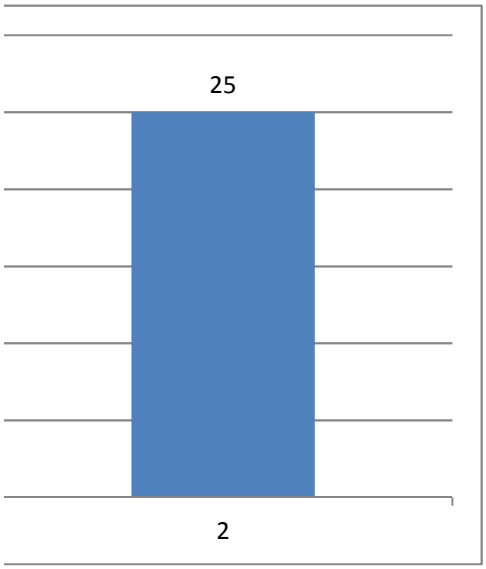
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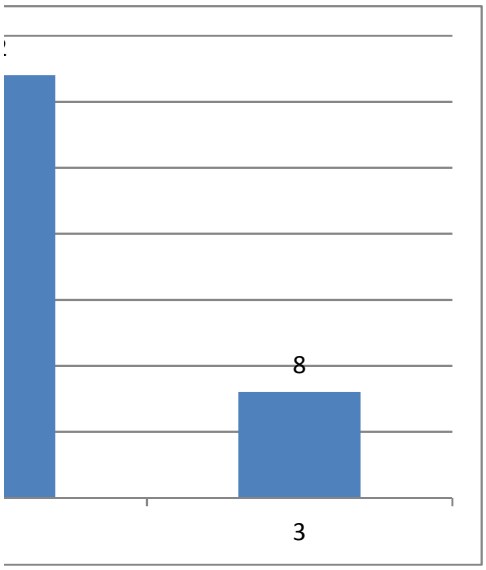
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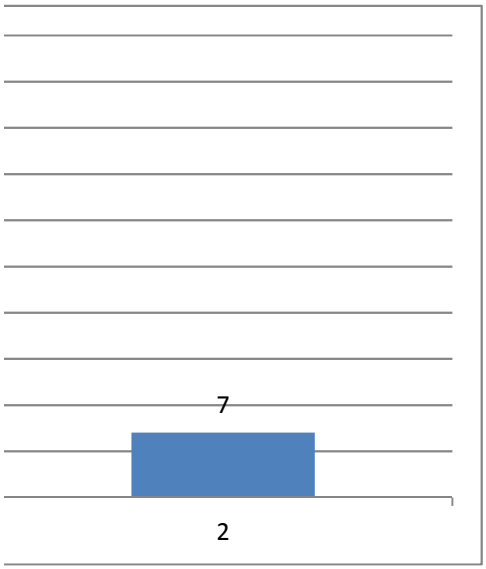
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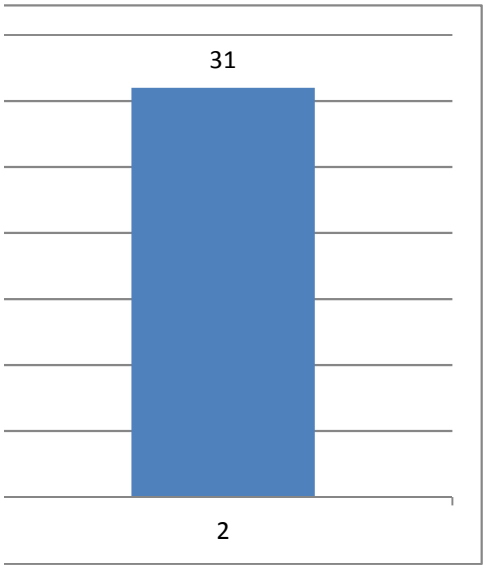
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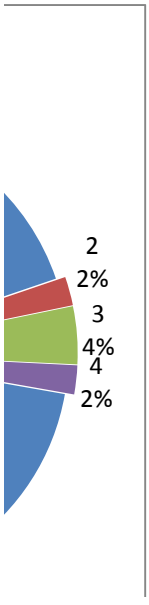
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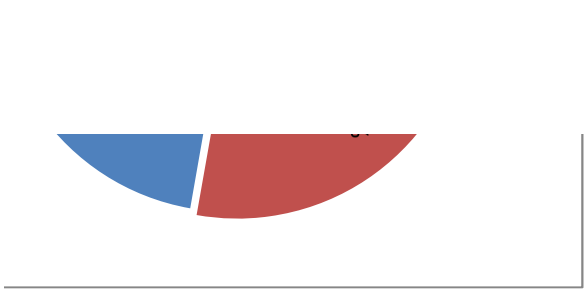

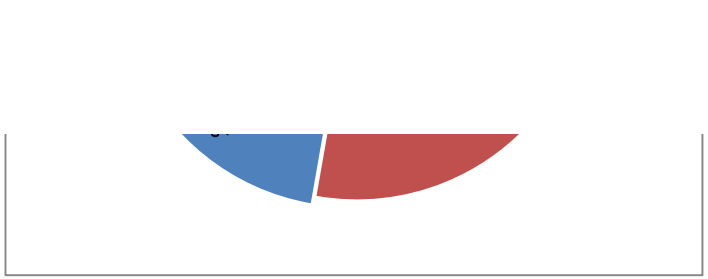

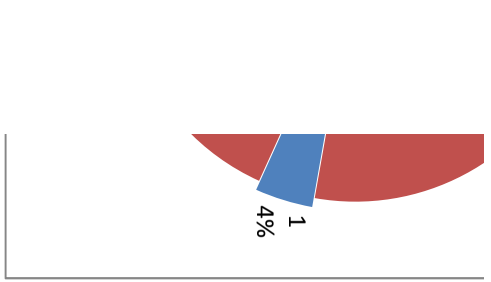
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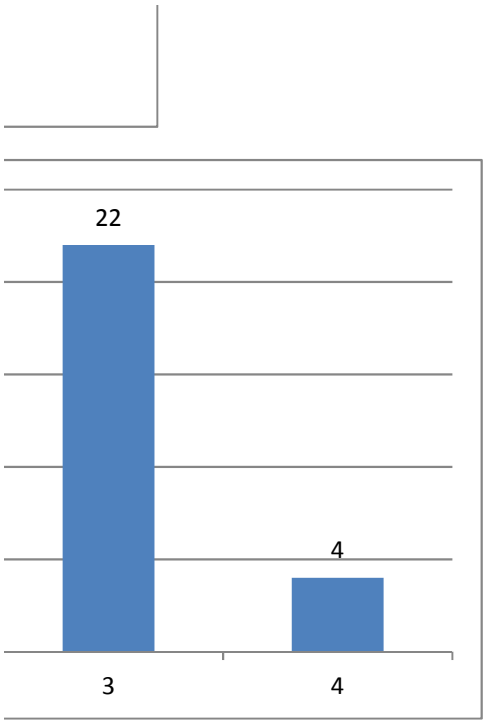
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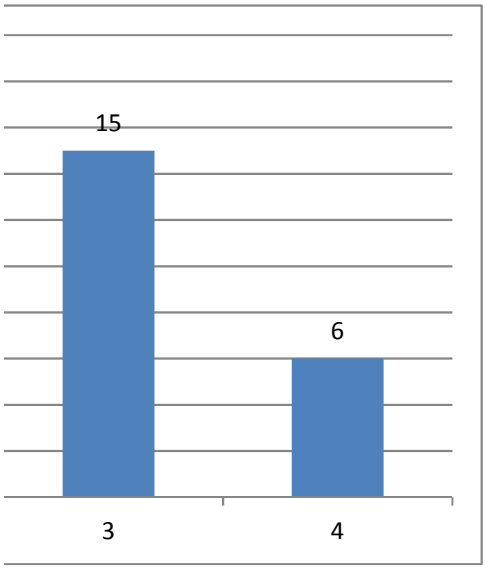
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S. NO	PATH NO	AGE (Yrs)	MENOPAUSE	TYPE OF SURGERY	TUMOR SIZE (CM)	NECROSIS	NO. OF NODES	LARGEST NODE (CM)	SMALLEST NODE (CM)	HISTOLOGICAL TYPE	GRADE	LV INVASION	PERINEURAL INVASION	INSITU COMPONENT	NECROSIS	STROMAL REACTION	MARGINS	SKIN	NIPPLE & AREOLA	INVOLVED LYMPHNODES	CK5/6	EGFR
1	2207/15	55	attained	MRM	3.5	absent	12	2	0.3	IDC NOS	III	present	absent	absent	present	fibrosis	free	involved	involved	7	negative	negative
2	472/15	55	attained	completion mastectomy	7	present	1	2	-	IDC NOS	II	present	absent	absent	present	lymphocytic infiltration	free	free	free	free	negative	negative
3	1358/15	40	-	simple mastectomy with axillary clearance	3	absent	1	0.3	-	medullary carcinoma	-	absent	absent	absent	present	fibrosis	free	free	free	free	positive	negative
4	2381/15	56	attained	MRM	2	absent	2	0.5	0.4	IDC NOS	II	absent	absent	absent	present	lymphocytic infiltration	free	free	free	free	negative	negative
5	1547/15	40	attained	MRM	5	present	22	2	1	IDC NOS	II	present	absent	absent	present	lymphocytic infiltration	free	free	free	free	negative	negative
6	1218/15	50	attained	MRM	5	absent	8	1	0.2	IDC NOS	III	present	absent	absent	present	fibrosis	free	free	free	3	negative	negative

S. NO	7	8	9	10	11	12
PATH NO	1717/15	2683/15	3320/15	3520/15	2840/15	2598/15
AGE (Yrs)	52	36	47	60	56	47
MENOPAUSE	attained	-	-	attained	attained	-
TYPE OF SURGERY	MRM	MRM	MRM	MRM	MRM	mastectomy with axillary clearance
TUMOR SIZE (CM)	4	11	2	9	2.5	8
NECROSIS	absent	absent	absent	absent	absent	absent
NO. OF NODES	5	12	-	5	11	-
LARGEST NODE (CM)	3	1.5	-	1	2	-
SMALLEST NODE (CM)	0.5	0.3	-	0.3	0.5	-
HISTOLOGICAL TYPE	IDC NOS	IDC NOS	IDC NOS	IDC NOS	IDC NOS	IDC NOS
GRADE	III	II	II	II	III	II
LV INVASION	present	absent	absent	present	present	absent
PERINEURAL INVASION	absent	absent	absent	absent	absent	absent
INSITU COMPONENT	absent	absent	absent	absent	absent	present
NECROSIS	absent	present	absent	absent	absent	present
STROMAL REACTION	fibrosis & lymphocytic	lymphocytic infiltration	fibrosis	fibrosis	fibrosis & lymphocytic	fibrosis
MARGINS	free	free	free	free	free	involved
SKIN	free	free	free	free	free	free
NIPPLE & AREOLA	free	free	free	free	free	free
INVOLVED LYMPHNODES	free	free	-	5	9	-
CK5/6	negative	negative	positive	positive	negative	negative
EGFR	positive	negative	positive	positive	positive	positive

S. NO	PATH NO	AGE (Yrs)	MENOPAUSE	TYPE OF SURGERY	TUMOR SIZE (CM)	NECROSIS	NO. OF NODES	LARGEST NODE (CM)	SMALLEST NODE (CM)	HISTOLOGICAL TYPE	GRADE	LV INVASION	PERINEURAL INVASION	INSITU COMPONENT	NECROSIS	STROMAL REACTION	MARGINS	SKIN	NIPPLE & AREOLA	INVOLVED LYMPHNODES	CK5/6	EGFR
13	1850/15	44	-	MRM	3.5	present	19	4	0.3	IDC NOS	III	present	absent	present	present	fibrosis & lymphocytic	free	free	free	12	negative	positive
14	3846/15	48	-	MRM	2	absent	-	-	-	IDC NOS	II	present	absent	present	absent	lymphocytic infiltration	free	free	free	-	negative	positive
15	3628/15	51	attained	MRM	4	absent	10	1.5	0.5	IDC NOS	II	absent	absent	absent	present	fibrosis & lymphocytic	free	free	free	free	positive	positive
16	939/15	55	-	simple mastectomy with axillary clearance	5	present	3	2	0.5	IDC NOS	II	present	absent	absent	present	fibrosis & lymphocytic	free	free	free	3	negative	negative
17	3209/16	56	attained	MRM	7	absent	9	2	0.8	IDC NOS	II	absent	absent	present	present	fibrosis	free	free	free	free	positive	positive

S. NO	18	19	20	20
PATH NO	2027/16	2006/15	2980/15	2980/15
AGE (Yrs)	44	33	57	57
MENOPAUSE	-	-	attained	attained
TYPE OF SURGERY	MRM	MRM	bilateral MRM	bilateral MRM
TUMOR SIZE (CM)	2	3	4	9
NECROSIS	present	absent	present	present
NO. OF NODES	7	8	8	-
LARGEST NODE (CM)	2.5	1	2	-
SMALLEST NODE (CM)	1	0.3	0.3	-
HISTOLOGICAL TYPE	IDC NOS	ILC	IDC NOS	IDC NOS
GRADE	III	-	II	II
LV INVASION	absent	present	present	present
PERINEURAL INVASION	absent	absent	absent	absent
INSITU COMPONENT	absent	absent	absent	present
NECROSIS	present	present	present	present
STROMAL REACTION	lymphocytic infiltration	fibrosis & lymphocytic	lymphocytic infiltration	lymphocytic infiltration
MARGINS	free	free	free	free
SKIN	free	free	involved	involved
NIPPLE & AREOLA	free	free	free	free
INVOLVED LYMPHNODES	free	-	-	8
CK5/6	positive	positive	negative	negative
EGFR	positive	negative	positive	positive

S. NO	PATH NO	AGE (Yrs)	MENOPAUSE	TYPE OF SURGERY	TUMOR SIZE (CM)	NECROSIS	NO. OF NODES	LARGEST NODE (CM)	SMALLEST NODE (CM)	HISTOLOGICAL TYPE	GRADE	LV INVASION	PERINEURAL INVASION	INSITU COMPONENT	NECROSIS	STROMAL REACTION	MARGINS	SKIN	NIPPLE & AREOLA	INVOLVED LYMPHNODES	CK5/6	EGFR
21	3607/15	42	-	simple mastectomy with axillary clearance	6	absent	14	2	0.5	IDC NOS	III	present	absent	absent	present	-	free	free	free	14	negative	positive
22	3358/15	65	attained	simple mastectomy with axillary clearance	4	absent	4	2	0.5	IDC NOS	II	present	present	absent	absent	fibrosis	free	free	free	2	negative	positive
23	1261/16	22	-	MRM	1.5	absent	5	1	0.3	IDC NOS	III	absent	absent	absent	present	lymphocytic infiltration	free	free	free	free	negative	positive
24	763/16	42	-	MRM	4.5	absent	4	1	0.2	IDC NOS	III	present	absent	absent	present	fibrosis & lymphocytic	free	free	involved	free	negative	positive
25	1260/16	50	attained	MRM	3.5	absent	8	1	0.5	IDC NOS	I	absent	absent	absent	absent	fibrosis	free	free	free	free	negative	negative
26	1362/16	50	-	MRM	1	absent	8	1	0.3	IDC NOS	III	present	absent	present	absent	fibrosis	free	free	free	free	positive	positive

S. NO	27	29	30	31	32	33
PATH NO	775/16	208/16	671/16	762/16	210/16	3375/16
AGE (Yrs)	56	38	60	63	46	70
MENOPAUSE	attained	-	attained	attained	-	attained
TYPE OF SURGERY	MRM	MRM	MRM	MRM	MRM	simple mastectomy with axillary clearance
TUMOR SIZE (CM)	4	4	4	1.5	3	3
NECROSIS	absent	absent	absent	present	absent	absent
NO. OF NODES	4	7	4	10		11
LARGEST NODE (CM)	1.5	1.5	1.5	1		1.5
SMALLEST NODE (CM)	0.2	0.5	0.2	0.2		0.2
HISTOLOGICAL TYPE	IDC NOS	IDC NOS	IDC NOS	IDC NOS	IDC NOS	IDC NOS
GRADE	II	III	III	III	III	III
LV INVASION	present	present	absent	absent	present	present
PERINEURAL INVASION	absent	absent	absent	absent	absent	absent
INSITU COMPONENT	present	present	absent	absent	absent	absent
NECROSIS	present	present	present	present	present	absent
STROMAL REACTION	lymphocytic infiltration	fibrosis	fibrosis	fibrosis	fibrosis	fibrosis & lymphocytic
MARGINS	free	free	free	free	free	free
SKIN	free	free	free	involved	free	free
NIPPLE & AREOLA	free	free	free	involved	free	free
INVOLVED LYMPHNODES	2	free	1	free	-	4
CK5/6	negative	negative	positive	negative	negative	negative
EGFR	positive	positive	positive	positive	positive	negative

S. NO	34	35	36	37	38	39
PATH NO	3615/16	1526/16	3605/15	1599/16	1620/16	4104/16
AGE (Yrs)	45	65	53	35	47	47
MENOPAUSE	-	attained	-	-	-	-
TYPE OF SURGERY	MRM	MRM	MRM	MRM	MRM	MRM
TUMOR SIZE (CM)	4	1	3.5	2.5	4	2
NECROSIS	absent	present	present	absent	absent	present
NO. OF NODES	12	19	7	14	21	9
LARGEST NODE (CM)	2.5	3.5	2.5	1.5	3	2
SMALLEST NODE (CM)	0.5	0.5	0.5	0.5	0.8	0.5
HISTOLOGICAL TYPE	IDC NOS	IDC NOS	IDC NOS	IDC NOS	IDC NOS	IDC NOS
GRADE	II	II	III	III	III	III
LV INVASION	present	absent	present	present	present	absent
PERINEURAL INVASION	absent	absent	absent	absent	absent	absent
INSITU COMPONENT	absent	absent	absent	present	absent	absent
NECROSIS	absent	present	present	present	present	absent
STROMAL REACTION	lymphocytic infiltration	lymphocytic infiltration	fibrosis	-	fibrosis	fibrosis & lymphocytic
MARGINS	free	free	free	free	free	involved
SKIN	free	free	free	free	free	free
NIPPLE & AREOLA	free	free	free	free	free	free
INVOLVED LYMPHNODES	4	free	2	1	8	5
CK5/6	negative	negative	negative	negative	negative	negative
EGFR	negative	positive	negative	positive	positive	positive

S. NO	PATH NO	AGE (Yrs)	MENOPAUSE	TYPE OF SURGERY	TUMOR SIZE (CM)	NECROSIS	NO. OF NODES	LARGEST NODE (CM)	SMALLEST NODE (CM)	HISTOLOGICAL TYPE	GRADE	LV INVASION	PERINEURAL INVASION	INSITU COMPONENT	NECROSIS	STROMAL REACTION	MARGINS	SKIN	NIPPLE & AREOLA	INVOLVED LYMPHNODES	CK5/6	EGFR
40	1949/16	49	-	MRM	4	present	16	1.5	0.5	IDC NOS	II	present	present	absent	absent	lymphocytic infiltration	free	free	free	5	negative	positive
41	1598/16	50	-	MRM	3	absent	5	1.5	0.5	ILC	-	absent	absent	absent	present	lymphocytic infiltration	free	free	free	free	negative	positive
42	4007/16	48	-	MRM	2	absent	2	1	0.5	atypical medullary carcinoma	-	present	absent	absent	present	fibrosis & lymphocytic	free	free	free	free	negative	positive
43	3653/16	52	attained	MRM	3.5	present	12	2	0.5	IDC NOS	III	absent	absent	absent	absent	fibrosis	involved	free	free	2	negative	negative
44	4115/16	51	attained	MRM	4	present	12	2.5	0.5	IDC NOS	II	absent	absent	absent	absent	fibrosis	free	free	free	free	negative	negative

S. NO	PATH NO	AGE (Yrs)	MENOPAUSE	TYPE OF SURGERY	TUMOR SIZE (CM)	NECROSIS	NO. OF NODES	LARGEST NODE (CM)	SMALLEST NODE (CM)	HISTOLOGICAL TYPE	GRADE	LV INVASION	PERINEURAL INVASION	INSITU COMPONENT	NECROSIS	STROMAL REACTION	MARGINS	SKIN	NIPPLE & AREOLA	INVOLVED LYMPHNODES	CK5/6	EGFR
45	1999/16	38	-	MRM	4	absent	13	4.5	0.5	IDC NOS	III	present	absent	absent	absent	lymphocytic infiltration	free	free	free	1	negative	negative
46	1146/16	52	attained	MRM	2.5	present	5	0.5	0.3	IDC NOS	II	present	absent	absent	present	lymphocytic infiltration	free	free	free	free	positive	positive
47	41/16	48	-	MRM	3	present	10	2	0.5	IDC NOS	II	present	absent	absent	present	lymphocytic infiltration	free	free	free	7	negative	positive
48	4747/16	62	attained	MRM	5	present	14	2.5	0.8	IDC NOS	II	present	absent	absent	present	fibrosis	free	free	free	8	negative	positive
49	8002/16	70	attained	MRM	7	present	16	3	0.5	IDC NOS	III	present	absent	absent	present	fibrosis & lymphocytic	free	free	involved	12	negative	positive
50	3833/14	65	attained	MRM	4	present	6	2	0.5	IDC NOS	II	absent	absent	absent	present	lymphocytic infiltration	free	free	free	1	negative	positive

-/-	-/-	-/-	-/+	-/-	-/-	**CK5/6/EGFR**

+/-	+/-	+/+	+/+	-/-	+/-	**CK5/6/EGFR**

+/+	-/-	+/+	+/-	+/-	**CK5/6/EGFR**

CK5/6/EGFR
+/+
+/-
-/+
-/+

+/+	-/-	+/-	+/-	+/-	+/-	**CK5/6/EGFR**

-/-	+/-	+/-	+/+	+/-	+/-	**CK5/6/EGFR**

+/-	+/-	+/-	-/-	+/-	-/-	**CK5/6/EGFR**

-/-	-/-	+/-	+/-	+/-	**CK5/6/EGFR**

+/-	+/-	+/-	+/-	+/+	-/-	**CK5/6/EGFR**