EXPRESSION AND CLINICOPATHOLOGIC CORRELATION OF BASAL CYTOKERATINS IN BREAST CANCER

DISSERTATION

Submitted for

M.D IN PATHOLOGY

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

DEPARTMENT OF PATHOLOGY

PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH

PEELAMEDU, COIMBATORE-641004

TAMILNADU, INDIA

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CERTIFICATE

This is to certify that the dissertation work entitled **"EXPRESSION AND CLINICOPATHOLOGIC CORRELATION OF BASAL CYTOKERATINS IN BREAST CANCER"** submitted by **Dr. Seyed Rabia** is a work done by her during the period of study in the department from 31/05/2012 to 30/05/2015. This work was done under the guidance of **Dr. S.Vidhya Lakshmi**, Associate Professor, Department of Pathology, PSGIMS&R**.**

Dr. Prasanna N Kumar
Professor & HOD
Department of Pathology
PSGIMS&R
Coimbatore-04

Dr. S. Ramalingam
Principal
PSGIMS&R
Coimbatore-04

CERTIFICATE

This is to certify that the thesis entitled "**EXPRESSION AND CLINICOPATHOLOGIC CORRELATION OF BASAL CYTOKERATINS IN BREAST CANCER**" submitted by **Dr. Seyed Rabia** to The Tamilnadu Dr MGR Medical University for the award of the degree of Doctor of Medicine in Pathology, is a bonafide record of research work carried out by her under the supervision of **Dr. S. Vidhya Lakshmi,** Associate Professor of Pathology. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma.

Dr Seyed Rabia
Post-Graduate,
Department of Pathology,
PSGIMSR,
Coimbatore-04.

Dr. S. Vidhya Lakshmi
Associate Professor,
Department of Pathology,
PSGIMSR,
Coimbatore-04.

PSG Institute of Medical Sciences & Research
Institutional Human Ethics Committee

POST BOX NO. 1674, PEELAMEDU, COIMBATORE 641 004, TAMIL NADU, INDIA
Phone : 91 422 - 2598822, 2570170, Fax : 91 422 - 2594400, Email : psgethics2005@yahoo.co.in

October 19, 2012

To
Dr Seyed Rabia
I year Post Graduate
Department of Pathology
PSG IMS & R
Coimbatore

Ref.: Your study entitled 'Expression and clinicopathologic correlation of basal cytokeratins in breast cancer'

Ref.2: Our letter dated 20.09.2012
Documents submitted by you on 04.10.2012

Sub.: Ethics Committee Approval for the study

The Institutional Human Ethics Committee, PSG IMS & R, Coimbatore -4, has reviewed your proposal on 18th September, 2012 in its expedited review meeting held at College Council Room, PSG IMS&R, between 3.00 pm and 4.30 pm, and discussed your application to conduct the study entitled:

"Expression and clinicopathologic correlation of basal cytokeratins in breast cancer"

The following documents were received for review:

1. Duly filled application form
2. Confidentiality Statement
3. Data Collection Tool
4. CV

After due consideration, the Committee has decided to approve the above study.

The members who attended the meeting held on 18.09.2012, at which your proposal was discussed, are listed below:

Name	Qualification	Responsibility in IHEC	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
Dr P Sathyan	DO, DNB	Clinician, Chairperson	Male	No	Yes
Dr S Bhuvaneshwari	M.D	Clinical Pharmacologist Member - Secretary	Female	Yes	Yes
Dr Sudha Ramalingam	M.D	Epidemiologist	Female	Yes	Yes

PSG Institute of Medical Sciences & Research
Institutional Human Ethics Committee
POST BOX NO. 1674, PEELAMEDU, COIMBATORE 641 004, TAMIL NADU, INDIA
Phone : 91 422 - 2598822, 2570170, Fax : 91 422 - 2594400, Email : psgethics2005@yahoo.co.in

		Alt. Member - Secretary			
Dr Y S Sivan	Ph D	Member - Social Scientist	Male	Yes	Yes
Dr D Vijaya	Ph D	Member – Basic Scientist	Female	Yes	Yes

The approval is valid for one year.

We request you to intimate the date of initiation of the study to IHEC, PSG IMS&R.

This Ethics Committee is organized and operates according to Good Clinical Practice and Schedule Y requirements.

Non-adherence to the Standard Operating Procedures (SOP) of the Institutional Human Ethics Committee (IHEC) and national and international ethical guidelines shall result in withdrawal of approval (suspension or termination of the study). SOP will be revised from time to time and revisions are applicable prospectively to ongoing studies approved prior to such revisions.

Kindly note this approval is subject to ratification in the full board review meeting scheduled on 26.10.2012.

Yours truly,

19.10.12

SECRETARY
PSG IMS & R
COIMBATORE-641 004
INSTITUTIONAL HUMAN ETHICS COMMITTEE

Dr S Bhuvaneshwari
Member - Secretary
Institutional Human Ethics Committee

INTRODUCTION

Breast cancers are a diverse group of diseases that vary remarkably in terms of clinical presentation, histology, behaviour and genetic characteristics.[1]

Since the 1930s, there has been a steady increase in the incidence of breast cancers worldwide. This increase continued steadily into the early nineties. It contributes to 22% of all female malignancies worldwide (SEER committee) and 26% in the developed world.[2] Globalization is now titling this balance; adaptation of a western lifestyle and improved access to diagnostic modalities has been implicated in the increased rates in Asia, including India.

Two established reasons have been attributed to high rates of incidence.

i) Increase in awareness and mammographic screening.

ii) Use of hormone replacement therapy in post menopausal women.

As per the WHO's International Agency for Research on Cancer, the number of new cases of female breast cancers in India in the year 2012 was 144,937. This figure is lesser than the number of women diagnosed in the USA in the same year (232,714). However, the mortality rate is nearly 50% in the Indian cohort whereas

ACKNOWLEDGEMENT

I start in the name of the Almighty, who has given me more than I have asked for and more than I deserve.

This dissertation was brought to life through the constant support, passion and help rendered by my Guide, Dr S Vidhya Lakshmi, Associate Professor of Pathology. I am immensely grateful to her for her enthusiasm and encouragement through the last three years.

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It is with gratitude that I thank Dr V. Nirmala, Emeritus Professor and Dr. S. Shanthakumari, Professor of Pathology, for their support and tutelage through the years.

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None of this would be a reality if not for my husband. He pushed me into taking the entrance exams and post-graduation. I thank him for sacrificing constantly so I can achieve my dreams in comfort.

I am grateful and thankful to God for my family and in particular, my parents for their trust and moral support. I couldn’t thank them enough.

Last, but definitely not the least, I’d like to thank my mini-pathologist and my baby girl, Zahra. I thank her for grounding me and making me see the bigger picture in life. Her bedtime stories have consisted of excerpts from Robbins and she also wakes me up to study (unintentionally, of course) and I thank her for that.

ABSTRACT OF THE THESIS TITLED "EXPRESSION AND CLINICOPATHOLOGIC CORRELATION OF BASAL CYTOKERATINS IN BREAST CANCER"

Introduction: Treatment for breast cancer is based on the expression of the immunomarkers such as ER, PR and HER2/neu. Cases which are negative to all the three immunomarkers, are called Triple Negative Breast Cancers (TNBC) and they have a poor prognosis. Recent studies have shown that some of the TNBCs express cytokeratins CK 5/6 (subcategorizing them as basal-like breast cancers) and these respond well to anthracycline-based chemotherapy.

Aim and Objectives: To study the expression of basal cytokeratins CK 5/6 in breast carcinomas reported in our centre and to correlate with histological type, grade, size, clinical features and ER, PR and HER2/neu status.

Methods: Tissues of 44 cases of breast carcinoma diagnosed between June 2009 and May 2014 were retrieved. Immunohistochemical staining for CK 5/6 was done and it was correlated with parameters such as histopathological type, grade, size, invasion and ER, PR and HER2/neu status.

Results: Eight of the breast carcinomas (18%) were categorized as Triple Negative Breast Cancers (TNBC) as they were negative for ER, PR and HER2/neu. Four of the TNBCs (50%), were positive for CK 5/6. Significant statistical correlation was observed between the size of the tumour and positive CK 5/6 expression. All CK 5/6 positive cases were of high grade.

Conclusion: The routine use of CK 5/6 is recommended in all cases of TNBCs, as 50% of them are positive for these markers. Patients in this subcategory could benefit from anthracycline-based chemotherapy.

Key Words: Triple Negative Breast Cancers, Cytokeratin 5/6, basal-like breast carcinoma.

INTRODUCTION

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As per the WHO's International Agency for Research on Cancer, the number of new cases of female breast cancers in India in the year 2012 was 144,937. This figure is lesser than the number of women diagnosed in the USA in the same year (232,714). However, the mortality rate is nearly 50% in the Indian cohort whereas only one woman out 5-6 patients dies of breast cancer in the US.[3]

The WHO further stresses that India has the maximum mortality rate when compared to the other countries under study. Persistent efforts to improve diagnostic and therapeutic modalities have contributed to the relatively better cure rates in the USA. Thus, breast cancer research has become the need of the hour with introduction of more studies tailored to the Indian population.

GLOBOCAN cancer fact sheet released in 2012 also shows that though the incidence is lesser, the burden is somewhat similar owing to India's large population. Similarly, the mortality rate was devastatingly high. Even in 2008, GLOBOCAN statistics showed that carcinoma cervix was the most common female cancer. It was estimated by the same agency that breast carcinomas would surpass and claim this dubious honour soon. Currently, breast carcinomas are the commonest cancers occuring in Indian females and contribute to 21.5% of all cancer deaths.[3]

Pap screening and improved surgical techniques and chemotherapy has contributed to lowering the fatalities of cervical cancer. Similar results can be achieved in breast cancer by implementing early detection by compulsory mammography. However, with a 5-year prevalence rate of 22.2%, India has a sizeable population of women living with breast carcinomas. The treatment has been revolutionized by the introduction of targeted therapy.

Breast cancers that express Estrogen and Progesterone receptors can be treated by hormonal manipulation.[4] Targeted therapy towards HER2/neu has great success and Trastuzumab has been introduced as an adjuvant drug in those showing overexpression of HER2neu.[5]

A subset of breast cancers have been found to show no expression of any of the above mentioned markers. These have been labeled as Triple Negative Breast Cancers (TNBCs). Though hormonal manipulation is of no use in this subset, they have been found to show expression of other markers such as basal cytokeratins and EGFR.[1]

They also have greater sensitivity to anthracycline-based chemotherapy[6] despite poor pathologic complete response.

Our study focuses on identifying the cases of breast cancer at our centre and performing immunohistochemical studies of the basal cytokeratin CK 5/6 in them. We further propose to study their expression and correlate with various clinicopathological parameters.

AIMS AND OBJECTIVES

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1. To study the clinicopathological profile of invasive breast carcinomas diagnosed in the department of pathology during the study period.
2. To categorize the tumours based on the histological type and grade.
3. To observe the immunohistochemical expression of hormone receptors and HER2/neu in various categories of breast cancers.
4. To analyze the expression of Cytokeratin CK 5/6 in breast cancers and correlate with clinical and pathological parameters.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

GROSS ANATOMY OF THE ADULT BREAST[10]:

The mature adult breast rests on the pectoralis major muscle from which it is separated by the pectoralis fascia. It is situated with the long axis diagonal to the chest wall and extension into the axilla as the tail of Spence is seen. The boundaries of the breast are as follows:

i. Laterally, it extends over Serratus anterior.
ii. Inferiorly, over External oblique muscle and Superior rectus sheath
iii. Medially, it is limited by the sternum.

The superficial fascia is seen continuous with the cervical fascia superiorly and with the superficial abdominal fascia of Cooper inferiorly. Suspensory ligaments of Cooper, which anchor the skin and nipple to the breast, are fibrous strands extending from the dermis into the parenchyma. Retromammary space containing loose areolar tissue is formed by the space between the deep membranous layer superficial and the fascia of both pectoralis major and serratus anterior. The pectoralis major muscle extends into the dome-shaped pyramidal axillary space to form the axillary fascia. These anatomical landmarks are important during dissection for identifying neoplastic or inflammatory infiltrative processes.

The arterial supply of the breast is by the internal thoracic, axillary and intercostal arteries. The venous draining tends to be more varied, the superficial and deep venous complexes formed mainly by branches of the axillary and internal thoracic veins.

Lymphatics of the breast mainly drain into the axillary nodes. This constitutes 75% of the overall lymphatic flow. Rotter's nodes (located in the interpectoral fascia), internal thoracic nodes and posterior intercostal nodes constitute other areas of drainage. Drainage into supraclavicular, infraclavicular and intramammary nodes is also seen.

FUNCTIONAL GROSS ANATOMY

The breast parenchyma is composed of 15-25 lobes based on the major lactiferous ducts draining into the nipple. Each lobe consists of a complex morphofunctional unit composed of Terminal duct/lobule unit (TDLU) and the large duct system. The large ducts branch into TDLUs; the terminal duct further branches into a grape-like cluster of acini to form lobule. The TDLU is embedded in a myxoid-appearing, hormone responsive stroma with absence of elastic fibres while the larger duct is enveloped by less hormone-responsive, elastic connective tissue. The breast parenchyma has variable

proportions of fat and stroma depending on age and individual predisposition. The plasticity is suggestive of hormonal regulation.[11]

The TDLU carries out the secretory activity of the mammary gland. It is formed by the lobule and terminal ductule, further connecting to the subsegmental duct, segmental duct and collecting (lacteriferous) duct in sequence. The unit then empties into the nipple. Beneath the nipple, the lacteriferous duct is dilated to form the lactiferous sinus.

MICROSCOPIC ANATOMY

The duct orifice, which opens out into the nipple, is lined by stratified squamous epithelium. The luminal aspect of the ductal-lobular system is lined by columnar or cuboidal cells. These cells are predominantly involved in secretory activity. Myoepithelial cells lie between the epithelial layer and the basal membrane, invading the luminal epithelium through slender processes. Spindle-shaped myoepithelial cells form a continuous layer, parallel to the long axis. These cells contract to assist milk flow during lactation. Toker cells are basally located clear cells which are related to Paget's disease.[12]

The stroma shows sparse distribution of lymphocytes, plasma cells, mast cells and histiocytes. Periductal histiocytes, termed ochrocytes, are seen associated with inflammatory and proliferative conditions.

The histology of the lobules is inconstant due to changes associated with menstruation, pregnancy, lactation, aging, menopause, and hormone intake.

During pregnancy, increased number of secretory acini is seen as the terminal duct proliferates under the influence of the hormones oestrogen and progesterone. Prolactin, human chorionic somatomammotropin, thyroxine and corticosteroids also play an important role in proliferation of the breast. The lobules enlarge and the acini dilate, while the interlobular septa remain the same. The lining epithelium is cuboidal to low columnar vacuolated cells. The intralobular stroma is not as prominent and shows an influx of lymphocytes, plasma cells and eosinophils. The acini produce a protein-rich fluid called colostrum which dilates them further.

In the puerperal period and during lactation, the prolactin activity which was suppressed during pregnancy by oestrogens and progesterone becomes predominant. Milk-distended acini form almost the entire breast. On histological examination, the acini are filled with vacuolated eosinophilic material and lined by flattened epithelium. At the end of lactation, the

epithelial cells undergo apoptosis and the lobules regress and atrophy. Even though a decrease in the size of the breast is noted, the number and size of lobules will be permanently increased.

Menopause is associated with a sharp drop in levels of estrogen and progesterone. This hormonal alteration manifests in the breast with decreased in the cellularity and number of lobules along with epithelial atrophy. Shrinkage, as well as cystic dilation of the lobules is noted. Perivascular and periductal elastosis is seen. The breast of the elderly appears radiolucent.

The nipple consists of large collecting ducts opening out through the lactiferous orifices. Sebaceous glands, independent of pilar unit can be seen. The stroma is dense and fibrous and ereticle smooth muscular tissue is seen embedded within it.

Montgomery Tubercles are protuberances in the areola, numbering around ten to twenty. They are formed by lacteriferous ducts associated with a sebaceous apparatus. These become prominent during pregnancy and their epidermis shows increased melanin in the basal layer.

A few unusual conditions are seen in normal breast:

1. Pregnancy-like changes in the absence of hormonal intake or pregnancy: The cells resemble those seen in Arias-Stella reaction (have abundant vacuolated cytoplasm and apically located large, hyperchromatic nuclei) while the lumen are dilated. An association has been found with in situ and invasive carcinoma.
2. Clear cell change of ductal/lobular epithelium: also referred to as lamprocytosis[10], this condition is associated with lobular enlargement. The lobules are lined by large clear cells almost obliterating the lumen. The cytoplasm is granular, vacuolated or clear and does not feature the “decapitation” secretion that is seen in pregnancy-induced changes.

EMBROYOLOGY

Mammary ridges (also called milk lines) are thickened endometrium on the ventral surface of a 5-week fetus that extends from axilla to upper medial portion of thigh. Most of the ridge involute usually but persistence has been noted in the form of ectopic mammary glandular tissue.

At around 15 weeks of gestation, mesenchymal condensation occurs around the epithelial breast bud. Cords of epithelium grow down into the

mesenchyme and these develop into lobes. Fibrovascular tissue ensconcing the lobes evolves from the papillary layer of the fetal dermis. Myoepithelial cells are seen arising from basal cells. More collagenized stroma originates from the reticular dermis and encompasses the lobular architecture forming the suspensory ligaments of Cooper which anchor the breast parenchyma to the overlying skin. Adipocytic differentiation occurs between 20 and 32 weeks. Towards the end of gestation, canalization of epithelial cords and branching of the glandular structures is seen. Mammary pit is the primitive homologue of the nipple. If the evagination of the mammary pit does not process normally, congenitally inverted nipple is seen.

Testosterone influences breast development after 15^{th} week. Towards the end, maternal and placental steroid hormones and prolactin induce secretory activity. Palpable breast enlargement and secretion of “witch’s milk” in the newborn is due to the persistence of these hormones in their circulation. After a month, this secretory activity subsides and the breast regresses into an inactive state until puberty.

During puberty, thelarche occurs under the cyclical influence of estrogen and progesterone. Ducts proliferate and stromae differentiate on estrogenic stimulation. Lobule formation, which remained quiescent until the onset of ovulation is now initiated. Differentiation and growth is maximum during

adolescence and further accentuated during pregnancy. The adolescent male breast is composed of ducts lined by flattened epithelium in a fibroadipose stroma.[2]

CARCINOMA OF THE BREAST

DEFINITION

Invasive breast carcinomas are a group of malignant neoplasms that arise from the epithelium and tend to invade the adjacent tissue, lymph nodes and metastasize to distant sites. Adenocarcinomas, originating from the mammary parenchymal epithelium, are the highest in incidence. These carcinomas originate from the cells of the terminal duct lobular unit (TDLU). Breast carcinomas encompass a variety of morphological phenotypes and histological types, each of these with their own prognostic values.

Breast carcinoma is the commonest non-skin malignancy seen in women worldwide. Most cancer deaths are attributed to breast carcinoma, second only to bronchogenic carcinoma. Statistics issued by the SEER committee in 2012 shows that in the USA, nearly 226,000 women were diagnosed with invasive breast cancer, 63,000 with carcinoma in situ, and almost 40,000 women succumbed to the disease[2]. Overall, it constitutes 22% of all malignancies in women worldwide. In developed and affluent countries, it accounts for 26% of all female cancers, the next common subtype having only half the incidence. The risk rates are much lesser in Eastern Asia and

Sub-Saharan African countries but a steady increase in incidence has been noted. This has been attributed to environmental factors, adoption of a western lifestyle (delayed pregnancy, fewer pregnancies and decreased breastfeeding) and improved access to diagnostic modalities.[13]

Studies done in 1990 showed that the age-specific incidence rates varied 10-fold worldwide. DCIS and smaller, stage I lesions have been detected more frequently since 1980, owing to the widespread use of mammography.

A study done in reduction mammoplasty specimens by Dotto et al, taking 516 consecutive cases showed 92 (18%) usual ductal hyperplasia (UDH), 28 (5%) DIN1, 17 (3%) LIN and 1 (0.2%) tubular carcinoma. No cases of high-grade DIN or invasive carcinoma were identified.[14]

Even though these carcinomas can be seen in any age group, they are rare before the age of 25. The incidence increases after the age of 30 and the perimenopausal age group is most commonly affected. Hormonally responsive tumours are seen increasing with age while the incidence of ER-negative cancers remains constant. Detection rates have increased in the post-menopausal age group due to

i. Mammographic screening which preferentially detects ER-positive tumours.

ii. usage of post-menopausal hormone replacement therapy. Less than 20% of cancers in this age group are ER-negative, Her2neu positive.

The survival rates started improving during the 1970s, but implementation of population screening programs and neoadjuvant targeted therapy in the turn of the century has led to early diagnosis, successful treatment and remission. The mortality rate which was around 30% in 1994 has steeply declined and is now 20%.

In a registry maintained by Cancer Institute (W.I.A.), Chennai between the years 2006 and 2008, breast cancers formed 26.3% of all malignancies in females, emphasizing the burden in the local population also.[15]

Aetiology And Risk Factors

These are as listed below:

1. **Germline mutations**.
2. **First degree relatives** with breast cancer:

   Even in the absence of a germline mutation, first degree relatives of those affected with breast carcinoma are found to be at an increased risk of the same.

3. **Menstrual history**:

Early menarche and late menopause are two conditions associated with increased estrogenic exposure and hence, a higher risk of breast cancer.

4. **Reproductive history:**

The following factors have been implicated in breast carcinoma.

i. nulliparity and infertility.

ii. late age at first childbirth.

iii. lack of breastfeeding → Lactation causes differentiation of the Terminal Duct Lobule Unit (TDLU) and suppresses ovulation. The protective impact of lactation is seen best in those who breastfed over long periods of time, preferably over two years. This is common practice in developing countries where frequent and longer periods of nursing can be seen.

5. Exogenous hormones:

The National Toxicology Program, in 2002 labeled estrogen a carcinogen.[16] Post menopausal Hormone Replacement Treatment is associated with an increased risk of breast cancer, especially if estrogen is given in conjunction with progesterone for a long period. The tumours that have estrogen as the aetiology tend to be small ER-

positive tumours. These findings are further accentuated by the decreased risk of developing breast cancer (up to 75%) in those who undergo oopherectomy. Tamoxifen (Selective Estrogen Receptor Modulator) and Aromatase inhibitors, both of which are associated with decreased risk of breast cancer. Oral contraceptives, which in the past have been implicated in the aetiogenesis of breast cancer, have been evaluated recently. The relative risk has been attributed to detection bias and found to be statistically insignificant.

6. **Nutrition:**

Earlier studies showed that a high-fat, meat-based diet increased the relative risk of breast cancer; this has led to many studies being conducted on a large scale. The conclusion has been that diet plays a minimal role in the aetiopathogenesis of breast carcinoma. However, post-menopausal obese women are at an increased risk, probably due to the excess fat deposits synthesizing estrogen. Younger obese women (<40 years) tend to have anovulatory cycles, with associated diseases such as PCOD. These women have lower progesterone levels and are at a decreased risk.

7. **Environmental Toxins:**

Smoking and organochlorine pesticides are being investigated and their effects are inconclusive, as of now. Alcohol intake was found to cause a mild increase in breast carcinoma rates.

8. **Physical activity:**

Cohorts of individuals who have been active throughout their lifetime have been found to have decreased predisposition to malignancies. Additionally, physical activity has been found to confer some protection to post menopausal women. Post-menopausal women with a sedentary lifestyle and obesity develop insulin resistance. Increased insulin levels lead to elevation of sex steroids, particularly androgen and these are converted to estrogen in the adipose tissue.

9. **Benign Breast Disease:**

The absolute lifetime risk of developing breast cancer in patients with benign breast diseases are as follows [17]:-

i) Fibrocystic changes: 3%

ii) Proliferative disease without atypia: 5 to 7%

iii) Proliferative disease with atypia: 13 to 17%

iv) Carcinoma in situ: 25 to 30%

10. Radiation exposure:

People who have undergone radiation exposure in the form of cancer treatment regimens or nuclear accidents are at increased risk. This is especially relevant in young women who have undergone chest irradiation as part of Hodgkin's lymphoma treatment protocol (20 to 30% risk) [18]

11. Race/Ethinicity/Country of Birth:

The variation in the frequency of germline mutated genes leads to the ethnicity divide. As stated earlier, the rates in developing nations, although on a rise, are still lesser than the rates in the western world.

12. Contralateral breast/Endometrial carcinoma:

There is a 1% risk of developing breast cancer in the contralateral breast in survivors. Endometrial carcinoma, which shares many risk factors with breast carcinoma, is mostly due to prolonged estrogen exposure.[19]

13. Breast density:

Increased density is associated with a 4 to 6-fold higher risk of breast cancer. This tends to be familial and is also seen in people with less parity, late childbirth and hormone replacement therapy. Failure of involution causes increased breast density in the elderly.

PATHOGENESIS

Breast cancers arise from cells with numerous genetic aberrations that are acquired by hormonal exposure or are inherited as germline mutations. The penetration of these genes further depends on other environmental factors. Sporadic cases, on the other hand, are not free from genetic factors. The aetiopathogenesis is complex and multifactorial, as elaborated earlier.

Familial Breast cancers:

13% of all breast cancers can be attributed to an identifiable inherited susceptibility gene or genes. This group is responsible for cancers that affect multiple first-degree relatives, the young and are multicentric.[20] These tumour suppressor genes (BRCA1, BRCA2, TP53, and CHEK2) either undergo sporadic mutation or defective copies of these genes are inherited.[21]

A landmark event in the molecular study of breast cancer was the identification of BRCA genes. 16% of familial breast cancers are due to mutation of these genes, which are seen in certain ethnic populations such as

Ashkenazi Jews (up to 2% affected). Carriers of the mutated genes have a 70 to 80% risk of developing breast cancer by 70 years of age.

80 to 90% of single gene familial breast cancers and roughly 3% of all breast cancers are due to BRCA1 and BRCA2 mutations.

BRCA1 mutations also predispose to the development of ovarian cancer (nearly 20 to 40% of cancers). BRCA 2 is seen more commonly in male breast cancer. BRCA1 encoded protein works in the following ways to suppress tumorigenesis:-

i) homologous recombination DNA repair

ii) Checkpoint control of cell cycle

iii) Ubiquitylation

iv) Chromatin remodeling

v) DNA decatenation

BRCA 2 protein is responsible for DNA repair, cytokinesis and meiosis. Loss of repair function of DNA double-stranded breaks result in tumours with medullary features; i.e, high grade, high mitotic rate, necrosis, 'triple negative' with a basal-like gene expression and TP53 mutation. BRCA2

mutation associated cancers tend to be more heterogenous and are hormone-receptors positive.

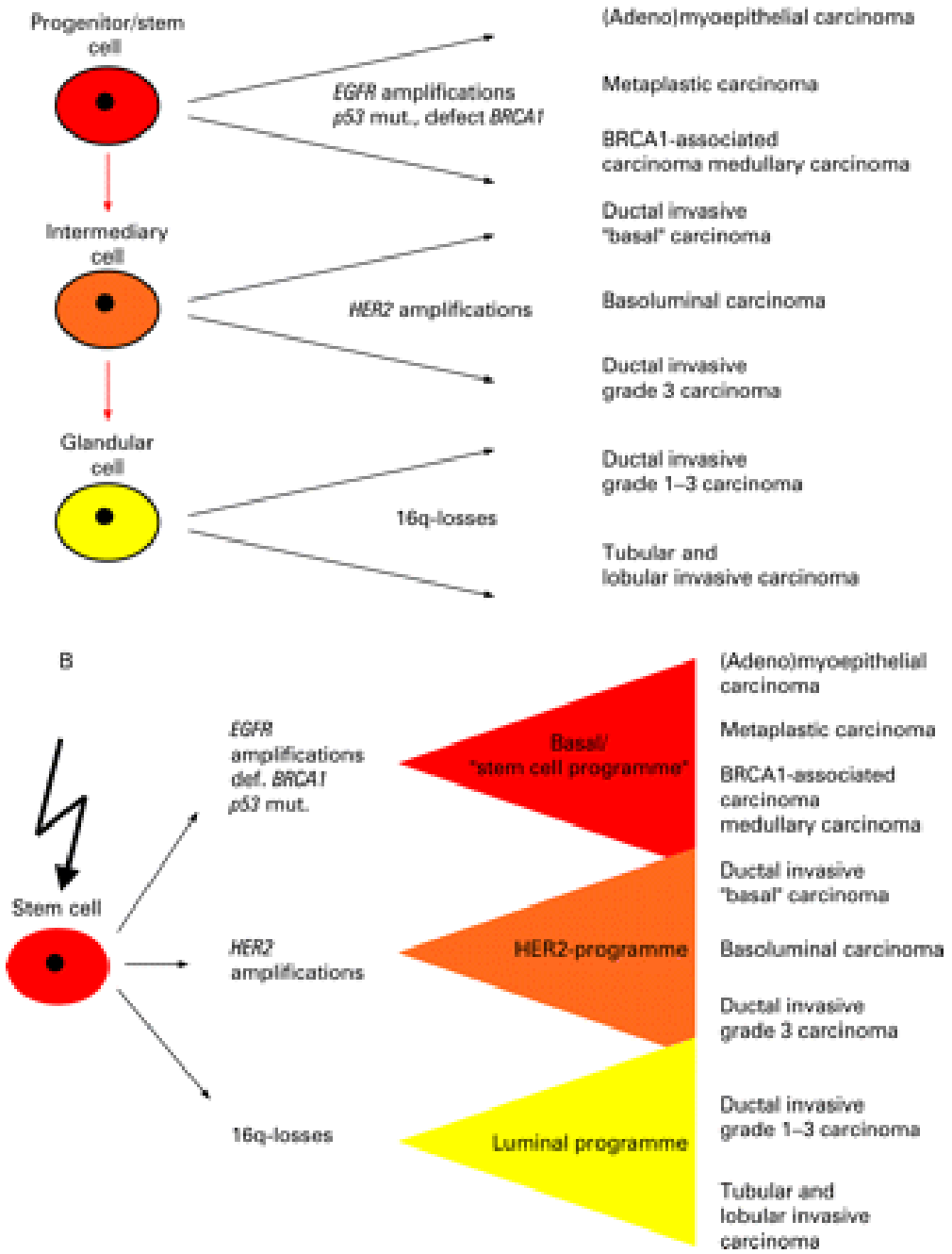
Fewer than 1% of familial breast cancers are caused by mutations in the following tumour suppressor genes i)PTEN (Cowden) ii)STK11 (Peutz-Jeghers) iii)ATM (Ataxia Telengectasia)

8% of familial breast cancers are a consequence of germline mutations in TP53 and CHEK2. Genomic integrity is maintained by aforementioned tumour suppressor genes. Once mutated, the cell either undergoes apoptosis or is repaired. ATM detects damage and along with p53 and CHEK 2, causes arrest of the cell cycle. CHEK 2 and both BRCA genes repair double stranded DNA breaks. Impairment of any of the above genes result in propagating cells with permanent DNA damage.[22]

MOLECULAR SUBTYPES OF INVASIVE BREAST CANCER[23]

	Luminal A	Luminal B	HER2/neu	Basal-like
Pattern of gene expression	Luminal cytokeratins and hormone receptors-high expression	Expression of luminal cytokeratins seen. Expression of hormone receptors and related genes-moderate to weak.	Low expression of ER. High expression of HER2/neu and 17q12	High expression of basal cytokeratins and epithelial genes. Low expression of hormone receptors
Clinical and biological features	ER/PR-positive. HER2/neu- negative.	ER/PR-positive. HER2/neu expression-variably positive. Higher grade and proliferation than Luminal A	ER/PR negative. HER2/neu positive. High grade, TP53 mutations present and higher likelihood of nodal metastasis	Triple-negative (ER, PR, HER2/neu) TP53, BRCA1 mutations seen.
Histological correlation	Tubular carcinoma, Cribriform carcinoma, IDC, NOS Classic lobular carcinoma	Micropapillary carcinoma	High grade IDC, NOS	High grade IDC Metaplastic and medullary carcinoma

Treatment	Response to endocrine therapy-good.	Response to endocrine therapy-> less satisfactory than Luminal A	Responds to Trastuzumab	No response to endocrine therapy or Trastuzumab
Chemotherapy	Response is variable	Response better than that of Luminal A	Responds to anthracycline-based chemotherapy	Responds to Cisplatin and PARP inhibitors
Prognosis	Good	Not as good as luminal A	Generally poor	Generally poor
Immunomarkers	ER and/or PR+	ER and/or PR+	ER, PR- HER2+	ER, PR, HER2 – CK5/6 or EGFR +


Progenitor/stem cell
Intermediary cell
Glandular cell
EGFR amplifications
p53 mut., defect BRCA1
HER2 amplifications
16q-losses
(Adeno)myoepithelial carcinoma
Metaplastic carcinoma
BRCA1-associated carcinoma medullary carcinoma
Ductal invasive "basal" carcinoma
Basoluminal carcinoma
Ductal invasive grade 3 carcinoma
Ductal invasive grade 1–3 carcinoma
Tubular and lobular invasive carcinoma
B
Stem cell
EGFR amplifications def. BRCA1 p53 mut.
HER2 amplifications
16q-losses
Basal/ "stem cell programme"
HER2-programme
Luminal programme
(Adeno)myoepithelial carcinoma
Metaplastic carcinoma
BRCA1-associated carcinoma medullary carcinoma
Ductal invasive "basal" carcinoma
Basoluminal carcinoma
Ductal invasive grade 3 carcinoma
Ductal invasive grade 1–3 carcinoma
Tubular and lobular invasive carcinoma

HISTOLOGICAL SUBTYPES OF BREAST CARCINOMA:

WHO histological classification of tumours of the breast[13]

Epithelial tumours:

Invasive ductal carcinoma, not otherwise specified

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

Invasive lobular carcinoma

Tubular carcinoma

Invasive cribriform carcinoma

Medullary carcinoma

Mucinous carcinoma and other tumours with abundant mucin

- Mucinous carcinoma
- Cystadenocarcinoma and columnar cell mucinous carcinoma
- Signet ring cell carcinoma

Neuroendocrine tumours

- Solid neuroendocrine carcinoma
- Atypical carcinoid tumour

- Small cell / oat cell carcinoma
- Large cell neuroendocrine carcinoma

Invasive papillary carcinoma

Invasive micropapillary carcinoma

Apocrine carcinoma

Metaplastic carcinomas

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

Lipid-rich carcinoma

Secretory carcinoma

Oncocytic carcinoma

Adenoid cystic carcinoma

Acinic cell carcinoma

Glycogen-rich clear cell carcinoma

Sebaceous carcinoma

Inflammatory carcinoma

Lobular neoplasia

- Lobular carcinoma in situ

Intraductal proliferative lesions

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

Microinvasive carcinoma

Intraductal papillary neoplasms

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

Benign epithelial proliferations

- Adenosis including variants
  - Sclerosing adenosis
  - Apocrine adenosis
  - Blunt duct adenosis
  - Microglandular adenosis

Adenomyoepithelial adenosis

Radial scar / complex sclerosing lesion

Adenomas

Tubular adenoma

Lactating adenoma

Apocrine adenoma

Pleomorphic adenoma

Ductal adenoma

Myoepithelial lesions

Myoepitheliosis

Adenomyoepithelial adenosis

Adenomyoepithelioma

Malignant myoepithelioma

Mesenchymal tumours

Haemangioma

Angiomatosis

Haemangiopericytoma

Pseudoangiomatous stromal hyperplasia

Myofibroblastoma

Fibromatosis (aggressive)

Inflammatory myofibroblastic tumour

Lipoma

    Angiolipoma

Granular cell tumour

Neurofibroma

Schwannoma

Angiosarcoma

Liposarcoma

Rhabdomyosarcoma

Osteosarcoma

Leiomyoma

Leiomyosarcoma

Fibroepithelial tumours

Fibroadenoma

Phyllodes tumour

    Benign

    Borderline

    Malignant

Periductal stromal sarcoma, low grade

Mammary hamartoma

Tumours of the nipple

Nipple adenoma

Syringomatous adenoma

Paget disease of the nipple

Malignant lymphoma

Diffuse large B-cell lymphoma

Burkitt lymphoma

Extranodal marginal-zone B-cell lymphoma of MALT type

Follicular lymphoma

Metastatic tumours

Tumours of the male breast

Gynaecomastia

Carcinoma

- Invasive
- In situ

Invasive ductal carcinoma, Not Otherwise Specified

IDC, NOS are tumours that express components of specialized subtypes but are not in entirety, the tumour as a whole. Microscopic foci of tubular/medullary/metaplastic/colloid and apocrine carcinoma may be seen within a single tumour.

The two main determinants are the histological origins of the tumour.

1. Cell of origin: they are classified as ductal or lobular.
2. a. epithelial (in situ)

   b. stroma (invasive)

Ductal Carcinoma In Situ (DCIS)

DCIS is a malignancy of ductal and lobular epithelial cells that are limited by the basement membrance. This spreads through the ductal system, extensively involving breast sectors.

This is more easily picked up by mammography, which serves as a screening tool for early lesions of breast carcinoma. The only symptoms associated with DCIS are nipple discharge and a vague palpable mass (owing to periductal fibrosis).

The architecture is not as predictive of the outcome as the degree of cytological atypia. Younger women tend to be more symptomatic with extensive involvement and *lobular cancerization.*

Types of DCIS:

The morphological subtypes are grouped under high-grade comedocarcinomas and low grade DCIS.

Comedocarcinoma:

These lesions are associated with larger size, central location and multicentricity. On gross, the lesion is composed of thick-walled ducts with intervening normal breast parenchyma. On compression, necrotic plugs extrude from the centre.

Microscopically, the lesion is composed of ducts with pleomorphic cells arranged in solid sheets with abundant mitoses. The ducts are widened with a central necrotic focus. Concentric fibrosis is seen surrounding the ducts with a preserved myoepithelial compartment. Comedocarcinomas and other subtypes which are ER, PR negative and have aneuploidy fall under Grade III.

All cases of comedocarcinoma have to be assessed for:

i) degree of intraductal spread.

ii) Stromal invasion.

These should be extensively searched for and if present, the relative proportions of the in situ and invasive components have to be calculated.

If the intraductal component exceeds 25% or more of the infiltrating tumour, the term Extensive Intraductal carcinoma has been recommended. A study showed that 21% of DCIS have an occult focus of invasion. This explains the presence of lymph node metastasis without a palpable primary.

Low Grade DCIS

These lack central necrosis and severe nuclear atypia. The following subtypes can be appreciated on histological examination.

i) (In situ) Papillary carcinoma:

On gross, they present as a well-circumscribed mass which may undergo cystic change on occasion. These have to be clearly distinguished from the more common papillomas and other benign lesions. Microscopically, these tumours have cells that are uniform in size and shape with large hyperchromatic nuclei exhibiting frequent mitoses. There is absence of

apocrine metaplasia, myoepithelial cells, other architectural patterns and benign breast disease. Lack of stroma favours papillary carcinoma. Solid, micropapillary and papillary carcinoma with transitional type epithelium are the variants described in literature. The micropapillary variant consists of papillae lacking a central fibrovascular core.

ii) Solid DCIS:

The lumina are filled with proliferating neoplastic cells that are larger than LCIS cells. These cells have prominent cytoplasmic borders and a more uniform nuclear morphology.

iii) Cribriform DCIS:

These are associated with the formation of uniform round spaces with the tumour clusters, imparting a sieve-like or cookie-cutter appearance. Roman bridges and trabecular bars are noted occasionally.

iv) Clinging carcinoma

is a controversial variant that shows malignant cells lining glands in one or two layers. The higher graded tumours are associated with individual cell necrosis.

Lobular cancerization is the phenomenon where an identifiable lobule shows features of DCIS.

Any case of DCIS must be thoroughly examined, especially if worrisome features are present e.g. nuclear debris, cell ghosts, inspissated secretions and necrosis. Any of the following outcomes may be seen:

1. May not transform into invasive cancer within the lifespan of the individual.
2. Grades and cytological atypia dictate the disease progression.
3. Invasive breast cancer may result without undergoing the sequence from DCIS.

Lobular Carcinoma in situ:

This condition is incidentally discovered and has high rates of multicentricity (70% of the cases) and bilaterality (30 to 40%). Also called lobular neoplasia, the histological examination of these tumours show distension of the lobules which are filled with uniform, small round cells with monomorphic nuclei. These cells are dyscohesive, a feature attributed to the loss of E-cadherin, a tumour suppressing adhesion protein. Pagetoid spread can be seen, but there is no skin involvement, necrosis or

calcification in these tumours. They tend to be ER/PR positive and HER2/ neu negative.

Invasive carcinoma may eventually develop ib 25-30% of women, with the risk being high in both ipsilateral as well as the contralateral breast.

Microinvasive carcinoma[13]

These tumours are predominantly non-invasive, but one or more small infiltrative foci are seen into the stroma. They occur in conjunction with DCIS and account for less than one percent of all breast cancers. Histologically, they are described as tumours that are less than or equal to 1 mm size. The stroma shows periductal lymphocytic infiltrate or desmosplasia and there is preserved basement membrane.

Invasive Ductal Carcinomas, Not Otherwise Specified

These are a heteregenous group of tumours that do not exhibit specific characteristics to denote a histological subtype. These neoplasms account to between 40 to 75% of all breast cancers.[24]

Macroscopy:

They vary in size from 0.1 to 10 cm. On gross, they are vaguely circumscribed with stellate configuration. The cut surface is grey white, firm and gritty.

Histopathology:

Microscopic features of these tumours are highly variable. The atypical cells are arranged in groups, trabeculae or in solid/synctial pattern. Single file appearance may be appreciated but the cytological features differ from lobular carcinoma. The nuclear features here tend to be pleomorphic with variability in the mitotic figures. Foci of DCIS are often seen (up to 80% of the cases).

Mixed type carcinoma:

After relevant sampling and thorough histological examination, a tumour is classified as ductal, NOS if the pattern is seen in over 50% of its mass. If the ductal component forms 10 to 49% of the tumour, it is classified as either mixed ductal and lobular or mixed ductal and specific tumour subtype.

Pleomorphic carcinoma:

This variant of ductal carcinoma occurs and is composed of pleomorphic tumour cells, with bizarre and giant forms comprising more than half of the tumour, superimposed on a background of pure adenocarcinoma or spindle/squamous differentiation. These tumours have a high rate of metastasis, lymphovascular invasion, cavitation and necrosis. Giant cells comprise more than 75% of tumour cells. They are classified as Grade 3 malignancies. Mitoses are increased and these tumours are negative for ER, PR and positive for TP53 and S100.

Carcinoma with osteoclastic giant cells:

These tumours have stromal osteoblastic giant cells and are associated with inflammation and increased vascularity. The carcinomatous component is IDC usually with the giant cells interspersed with the gland formation or are seen forming rosettes around the neoplastic cells.

The prognosis is similar to, or even better than Infiltrating Ductal Carcinoma, NOS. The osteoclasts are believed to originate from Tumour Associated Macrophages (TAMs).

Carcinoma with choriocarcinomatous features:

In some instances, patients with IDC, NOS had high serum levels of beta-HCG with nearly 60% of the tumour cells taking up the beta-HCG stain, on immunohistochemical studies. Histological differentiation is however rare and this subtype is seen in women in the sixth and seventh decade.

Carcinoma with melanotic features:[25]

Cases of breast parenchymal tumour with components of both ductal carcinoma and malignant melanoma have been reported. The presence of melanin by itself could be due to epidermal invasion and not a sign of tissue differentiation. Most breast melanotic tumours are secondary deposits.

Prognosis of IDC:

It depends on variables such as grade, size of the tumour, lymphovascular invasion and hormone and growth factor receptor positivity[25]. Various studies have shown that the 10-year survival rate is between 35 and 50%. 70 to 80% of the tumours are ER positive and only 15-30% is HER2neu positive.

INVASIVE LOBULAR CARCINOMA

These tumours are composed of dispersed cells arranged single or in linear cords ("single file" pattern) in a fibrous stroma. They form 5-15% of all breast carcinomas. These tumours have been peaking over the last twenty years in older women and this has been attributed to post-menopausal hormone replacement therapy. Mammographically, these tumours have distorted architecture and lack microcalcification. Bilaterality and multicentricity is frequently observed.

Macroscopy:

These tumours are poorly circumscribed due to the diffuse pattern of the neoplastic cells. Invasive Lobular Carcinomas are larger than their ductal counterparts.

Microscopy:

These neoplastic cells are small, uniform and are dispersed singly or in "Indian-file" pattern. Periductal concentric whorls may be seen. These cells have round nuclei, scant cytoplasm, occasionally with mucoid inclusions. LCIS is seen 90% of the cases. Mitoses are rare.

Variants:

i. Solid pattern.

ii. Alveolar variant: clusters of at least twenty cells with the cytomorphology that of lobular carcinoma

iii. Pleomorphic lobular carcinoma:

These variants have high degree of atypia. Signet ring cells, apocrine or histiocytoid differentiation may be seen.

iv. Tubulolobular carcinoma (TLC): while the cytomorphology is essentially that of lobular carcinoma, these cells are found in a tubular pattern. In contrast to pure tubular carcinoma, TLC if found associated with an increased rate of axillary metastasis. E-cadherin status helps in making the differentiation.

Immunohistochemistry:

70 to 95% ER positivity rate is observed, higher than that of Infiltrating Ductal Carcinoma, NOS. The rate is 100% in the Alveolar variant and low (10%) in Pleomorphic type. PR and HER2/neu expression is lower in Infiltrating lobular carcinoma than their ductal counterparts.

Genetics:

E (epithelial)-cadherin gene has the following functions:

i) maintains cohesion between the epithelial cells

ii) cell differentiation and tumour suppression factor.

Deletion of 16q results in loss of E-cadherin expression and this is the striking feature of lobular carcinoma.[26] Loss of E-cadherin expression and heterozygosity of 16q can also be seen in LCIS and Mixed ductal-lobular carcinoma.

Prognosis of Lobular carcinoma:

ILC tend to metastasize to bone, GIT, the female genital tract and meninges. In contrast, Infiltrating Ductal Carcinoma involves the lungs and has a higher rate of axillary nodal metastasis.

The prognosis is better in the classical type than in the variants.

Tubular Carcinoma

These account to less than 2% of all invasive breast cancers. On mammography, their spicular nature leads to early detection. These small-sized tumours tend to occur in older women with lesser chances of nodal metastases.

Macroscopy:

Most tumours measure less than 1 cm across. The pure type has a stellate configuration with central yellowish areas (indicative of stromal elastosis) while the sclerosing type is more poorly defined.

Microscopy:

The neoplasm is made up of tubules lined by a single layer of cells with clear lumina. Majority are rounded with occasional angulation. Minimal pleomorphism is noted. Tubular carcinoma is effectively ruled out if marked pleomorphism or multilayering is noted despite the characteristic architecture. Myoepithelial cells are not seen. The stroma is desmoplastic with occasional calcification. Controversy prevails over the percentage cut off of tubular architecture needed to establish diagnosis. Current WHO advises that a 90% purity should warrant the diagnosis of tubular carcinoma while those having 50 to 90% should be considered mixed-type.

Immunohistochemistry:

ER and PR are generally positive while HER2 and EGFR are usually negative.

Prognostically, this subtype is associated with excellent prognosis on long-term follow-up. Recurrence and metastasis is rare.

INVASIVE CRIBRIFORM CARCINOMA

The growth pattern in the pure form of these tumours consists almost entirely of invasive cribriform pattern. Less than 50% is of tubular pattern. The cells are arranged in islands which have a sieve-like or cookie-cutter appearance. Apical snouting and infrequent mitoses is seen. Desmoplasia is often observed. Minor tubular component (forming less than 50% of the tumour) is allowed. However, if carcinomas other than tubular form a component, they are referred to as mixed carcinomas.[27] 100% and 69% of cribriform carcinomas are ER and PR positive, respectively.

Adenoid cystic and carcinoid are considered in the differentials. Adenoid cystic tumours have admixed myoepithelial cell population and luminal secretions while carcinoid are identified by the presence of argyrophilic granules. Cribriform DCIS have an intact myoepithelial layer and are more contained.

Prognosis: the outcome in patients with these tumours has been very favourable, especially when compared with IDCs.

MEDULLARY CARCINOMA:

These tumours are well-circumscribed and are composed of diffuse sheets of cells with no glandular arrangement, lacking stroma and with a dense lymphoplasmacytic infiltration. Women in the fifth decade of life are commonly affected, with these tumours accounting to 17% of all breast carcinomas. Radiographically, they are strikingly well-delineated, leading to a benign differential diagnosis. The histology must fall into any of these five patterns:

1) At least 75% of the tumour mass should be composed of cells arranged in synctial sheets. Focal necrosis and squamoid differentiation can be seen.
2) No gland or tubule formation is allowed.
3) Diffuse infiltration of lymphocytes that is dense enough to obscure the tumour or in sparse amounts.
4) The cells have abundant cytoplasm and moderately to markedly pleomorphic vesicular nuclei with numerous mitoses.
5) These tumours are well-circumscribed, a feature that is obvious on low-power histological examination.

Immunohistochemistry:

These tumours have a high proliferative index and are both ER and HER2 negative. These tumour cells have been found to be correspond to T-lymphocytes. BRCA1 mutations have shown an association with these tumours. The prognosis has been better in these cases than IDCs due to limited spread.

MUCIN-PRODUCING CARCINOMAS:

These tumours are often seen in women over the age of 60 years. On mammography, these tumours are well-delineated with lobulation. On gross, the external surface is bosselated while the cut surface is gelatinous and glistening. Histologically, proliferating tumour cells are seen in clusters, floating in pools of mucin. Thin fibrovascular septae divide the tumours. The mucin stains positively with mucicarmine. Pure mucinous carcinomas are of cellular and hypocellular type. Neuroendocrine differentiation and intracytoplasmic mucin is seen in the cellular type. The proportion of non-mucinous type must be recorded in mixed tumours.

These tumours are ER and PR positive as a general rule. The pure form has a better prognosis than the mixed subtype. Death due to mucin emboli leading to cerebral infarction has been reported in literature.

Mucinous adenocarcinoma and columnar cell mucinous carcinoma:

These tumours are composed of tall columnar cells with bland basal nuclei and abundant intracytoplasmic mucin. Depending on their consistency, they are classified as Mucinous Cystadenocarcinoma (Cystic) or columnar cell mucinous carcinoma (solid)

Two types of signet ring carcinoma are seen:

i) Lobular type, with intracytoplasmic mucin
ii) Related to diffuse gastric carcinoma with intracytoplasmic acidic mucin.

NEUROENDOCRINE TUMOURS:

They comprise 2 to 5% of all breast carcinomas. These tumours occur in the sixth decade or later and are occasionally also seen in males.

Patients with small cell carcinomas are detected in advanced stage. These tumours infiltrate or are expansile with a gelatinous cut surface.

Histopathologically, the cells are arranged in sheets or lobules with peripheral palisading.[28] 26% of the tumours are mucin producing.

Types:

Solid:

Here, the cells are spindled, plasmacytoid or large with intervening delicate fibrovascular septae. Carcinoid-like rosette formation may be seen. Mitoses varies from 4 to 12/hpf with focal necrosis and neuroendocrine granules.

Small cell/oat cell carcinoma:

Similar to their counterparts in the lung, these tumours are composed of cells with scanty cytoplasm and hyperchromatic nuclei with nuclear streaming and overlapping.

Large cell Neuroendocrine tumours:

This variant shows increased mitoses (18 to 65/hpf). Foci of necrosis are often seen.

Differential diagnosis:

Breast primary neuroendocrine tumours should be differentiated from metastatic carcinoids and small cell carcinomas. Breast Neuroendocrine tumours are Cytokeratin 7 positive and Cytokeratin 20 negative whereas both markers are negative in pulmonary carcinoids. ER, PR, GCDFP 15 are expressed in breast cancers. Breast neuroendocrine tumours show positivity

for E-cadherin while lobular carcinomas, a close differential of small cell carcinomas are negative. Neuroendocrine markers are positive in at least 50% of the cells. Neuron specific enolase (NSE) is more sensitive than chromogranin A and synatophysin. TTF-1 and somatostatin receptors have also been demonstrated.

Electron microscopy demonstrated dense core granules (chromogranin) and clear vesicles (synaptohysin). Mucinous differentiation bodes good prognosis. Histological grading is an important parameter for assessing prognosis with small cell neuroendocrine tumours considered undifferentiated carcinomas.

INVASIVE PAPILLARY CARCINOMA:

They comprise less than 1% of all breast carcinomas and have relatively good prognosis. These affect post-menopausal women. They are well-circumscribed in two-thirds of the cases.

Histologically, these tumours often exhibit Grade 2 histology. The cells are arranged in papillary pattern with focal solid areas. Calcification is noted, especially in cases with DCIS. More than 75% of the cases have DCIS, usually of the papillary type. Lymphatic invasion and nipple involvement are occasionally seen.

INVASIVE MICROPAPILLARY CARCINOMA

These tumours have high rates of axillary node involvement. The neoplastic cells are arranged in clusters within large lacunae. Inside-out morphology or reverse polarity is seen often within the clusters. Vascular invasion is another common feature.

APOCRINE CARCINOMA

More than 90% of the neoplastic cells must show morphological and immunohistochemical evidence of apocrine features to warrant this diagnosis. Any morphological type (tubular/medullary/ papillary/ neuroendocrine) can express apocrine differentiation. Two types of cells have been recognized: Type A cells have abundant intensely eosinophilic granular cytoplasm. This type, also referred to as myoblastomatoid type, has granules which are PAS positive with diastase resistance and they resemble granular cell tumours.

Type B cells have foamy cytoplasm, reminiscent of sebaceous cells and histiocytes. These tumours are ER, PR negative and GCDFP-15 positive.

METAPLASTIC CARCINOMA

These tumours have an admixed population of squamous, spindled or mesenchymal cells. They are classified as purely epithelial or mixed epithelial and mesenchymal tumours.

Pure epithelial cells have the following subtypes 1)Squamous cells (Large cell keratinizing, spindle cell, acantholytic) 2)Adenosquamous

Mixed epithelial-mesenchymal tumours are

1) Carcinoma with chondroid metaplasia
2) Carcinoma with osseous metaplasia
3) Carcinosarcoma

SQUAMOUS CELL CARCINOMA

Keratinizing, non-keratinizing, spindle and acantholytic cells may be seen in varying combinations. Desmoplastic stromal reaction is pronounced. Metastatic foci show squamous differentiation as well. Immunohistochemistry is required to confirm the epithelial nature of spindled and acantholytic variants. Broad and High Molecular Weight Cytokeratins serve this purpose.

Adenosquamous carcinoma:

These tumours have a discernible glandular population admixed with solid nests of squamous cells. The squamous cells are hormone receptor negative while the glandular component's positivity depends on the degree of differentiation.

Mixed epithelial/mesenchymal metaplastic carcinomas:

These tumours are also called matrix-producing carcinomas and consist of both carcinomatous and mesenchymal elements. Grading depends on the nuclear features. The spindle cells may be positive for cytokeratins focally. ER, PR may show positivity in the ductal elements depending on the differentiation.

The differential diagnosis depends on the sarcomatous portion of the tumours.

LIPID-RICH CARCINOMA

In these tumours, more than 90% of the cells have abundant intracytoplasmic neutral lipid. These tumours must be differentiated from other tumours with clear cytoplasm. They tend to be high grade (Grade III) and invasive. The cytoplasm stains negatively for mucins

SECRETORY CARCINOMA

These tumours are well-circumscribed and small. Pushing margins are identified.

Histologically, the central portions of the tumour show prominent hyalinization. Tubuloalveolar and focal papillae lined by cells with vacuolated cytoplasm are seen. Prominent nucleoli and scanty mitotic figures are other features.

ONCOCYTIC CARCINOMA

These tumours have an oncocytic cell population comprising more than 70%. These swollen cells have more than 60% of the cell comprising of mitochondria, which impart the granular eosinophilic character to the cytoplasm.

Differential diagnosis: Immunophenotyping is necessary to distinguish between oncocytic myoepithelial, apocrine and neuroendocrine tumours.

ADENOID CYSTIC CARCINOMA

These are of low aggressive potential unlike their salivary gland counterparts. They present as discrete nodules which may be tender or cystic. Microscopically, three patterns have been described: trabecular-

tubular, solid and cribriform. Two types of cells are noted; the basaloid cells and luminal cells. Adenoid cystic carcinomas tend to be negative for hormone receptors. These tumours have to be differentiated from benign collagenous spherulosis and cribriform carcinomas.

ACINIC CELL CARCINOMA

These rare tumours show serous (acinic cell) differentiation. Histologically, cells are arranged in microcystic and microglandular patterns. The cells have abundant granular eosinophilic cytoplasm with an irregular ovoid nucleus. The mitotic count can be high (up to 15/hpf).

GLYCOGEN-RICH CLEAR CELL CARCINOMA (GRCC)

In this tumour, abundant intracytoplasmic glycogen is seen in over 90% of the cells. The tumours that come in the differential are lipid-rich carcinoma, adenomyoepithelioma, metastatic clear cell carcinoma and clear cell hidradenoma. Very few of these tumours are of Grade 1, with most falling into 2 or 3.

SEBACEOUS CARCINOMA

These are a rare subset of tumours similar to adnexal tumours with sebaceous differentiation. Cutaneous derivation must be ruled out. The cells

are arranged in nests or lobules with abundant vacuolated cytoplasm. A second population of small spindle cells with eosinophilic cytoplasm is seen at the periphery. Squamoid morules are occasionally seen.

INFLAMMATORY CARCINOMA

These cancers have a characteristic clinical presentation. Lymphatic obstruction by the underlying IDC or infiltration into the dermal lymphatics results in this. These tumours are automatically staged as T4d. It is important to recognize that these are not true inflammatory conditions but malignancies which often have Grade 3 morphology. The survival rates are very poor for this subtype.

NOTTINGHAM MODIFICATION OF SCARFF-BLOOM-RICHARDSON GRADING:[8]

This is a semi-quantitative grading system proposed by Ellis and Elston.

Tubule formation

1 point	>75%
2 points	10-75%
3 points	<10%

Nuclear pleomorphism

1 point	minimal pleomorphism
2 points	moderate pleomorphism
3 points	marked pleomorphism

Mitotic count:

Assignment of mitotic counts depends on the field area under examination:

Field diameter (mm)	0.44	0.59	0.63
Field area (mm2)	0.152	0.274	0.312
Mitotic count			
1 point	0-5	0-9	0-11
2 points	6-10	10-19	12-22
3 points	>11	>20	>23

GRADES:

Grade 1: well-differentiated breast carcinoma: 3-5 points

Grade 2: moderately differentiated breast carcinoma: 6-7 points

Grade 3: poorly differentiated breast carcinomas: 8-9 points

TNM STAGING OF BREAST CARCINOMAS:[29]

Primary Tumor (T)

TX Primary tumor cannot be assessed

T0 No evidence of primary tumor

Tis: Carcinoma in situ

Tis (DCIS): Ductal carcinoma in situ

Tis (LCIS) Lobular carcinoma in situ

Tis (Paget's): Paget's disease of the nipple NOT without association with

IDC, DCIS or LCIS

Carcinomas in the breast parenchyma along with Paget's diease are staged based on the size and characteristics of the underlying parenchymal disease. However, the presence of Paget's disease should be noted.

T1 Tumor ≤ 2 cm in greatest dimension

T1mi Tumor ≤ 1 mm in greatest dimension

T1a Tumor > 1 mm but ≤ 0.5 cm in greatest dimension

T1b Tumor > 0.5 cm but ≤ 1 cm in greatest dimension

T1c Tumor > 1 cm but ≤ 2 cm in greatest dimension

T2 Tumor > 2 cm but ≤ 5 cm in greatest dimension

T3 Tumor > 5 cm in greatest dimension

T4 Tumor of any size; extension into the chest wall and/or into the skin with ulceration is noted.

Note: Dermal invasion alone does not qualify as T4

T4a Extension into the chest wall, more than just

pectoralis muscle adherence/invasion

T4b Ulceration or ipsilateral satellite nodules and/or

peau d'orange of the skin, not meeting criteria for inflammatory carcinoma

T4c Both T4a and T4b

T4d Inflammatory carcinoma

Staging of N:

Nx: Regional lymph nodes cannot be assessed

N0: No regional lymph node metastases

N1: Metastases in movable ipsilateral level I, II axillary nodes

N2a: Metastases in fixed/matted ipsilateral level I, II axillary lymph nodes

N2b: Metastases only in ipsilateral internal mammary nodes but with absence of clinically evident level I, II axillary lymph node metastases

Staging of M

N3a: Metastases to ipsilateral infraclavicular node(s)

N3b: Metastases to ipsilateral internal mammary node(s) and axillary lymph node(s)

N3c: Metastases to ipsilateral supraclavicular lymph node(s)

PATHOLOGIC (PN)

pNX: Regional nodes cannot be assessed due to previous removal or for similar reasons

pN0: No regional nodal metastasis on histopathological examination

pN0(i−): with negative immunohistochemistry.

pN0(i+) Tumour cells in regional lymph node(s) less than 0.2 mm (detected by H&E or IHC)

pN0(mol−): negative molecular findings by using RT-PCR

pN0(mol+): Positive molecular findings on using RT-PCR, but without histological or immunohistochemical findings.

pN1a: Metastases in 1–3 axillary lymph nodes, with at least one being greater than 0.2 cm

pN1b: Metastases in internal mammary nodes detected by sentinel lymph node biopsy and not clinically detected

pN1c: Metastases in 1–3 axillary lymph nodes, internal mammary lymph nodes detected by sentinel lymph node biopsy and not clinically detected.

pN2a: Metastases in 4–9 axillary nodes, with at least 1 tumor deposit > 0.2 cm.

pN2b: Metastases in clinically detected internal mammary lymph nodes in without axillary nodal metastases.

pN3a: i) Metastases present in ≥10 axillary lymph nodes (with at least 1 tumor deposit >2.0 mm). ii) Metastases to infraclavicular lymph node.

pN3b: i) Metastases in ipsilateral internal mammary nodes (clinically detected) with involvement of one or more axillary lymph nodes.ii) Metastases in >3 axillary lymph nodes and in internal mammary lymph nodes with sentinel lymph node biopsy but not clinically seen.

pN3c: Metastasis in the ipsilateral supraclavicular node.

Distant metastasis (M):

M0: No evidence of distant metastases (clinically and radiographically).

cM0 (i+): No radiographic/clinical evidence of distant metastases, but molecularly or microscopic detection tumor deposits in circulating blood, bone marrow, non-regional nodal tissue that are ≤0.2 mm

M1: Distant detectable metastases determined clinically or radiographically and/or histological evidence of deposits >0.2 mm.

TNM STAGING OF BREAST CARCINOMAS:[29]

Stage	T	N	M
0	Tis	N0	M0
IA	T1[b]	N0	M0
IB	T0	N1mi	M0
	T1[b]	N1mi	M0
IIA	T0	N1[c]	M0
	T1[b]	N1[c]	M0
	T2	N0	M0
IIB	T2	N1	M0
	T3	N0	M0
IIIA	T0	N2	M0

Stage	T	N	M
	T1[b]	N2	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
IIIB	T4	N0	M0
	T4	N1	M0
	T4	N2	M0
IIIC	Any T	N3	M0
IV	Any T	Any N	M1

PROGNOSTIC AND PREDICTIVE FACTORS

The major prognostic factors are the ones that determine the staging of breast cancer.

1) **Invasive versus in situ**: In situ carcinomas have a chance of being completely cured in a majority of women in comparison with their invasive counterparts that have a greater incidence of invasion and death.
2) **Distant metastases**: Complete cure is usually not possible in these tumours as a rule. The histological and molecular subtype determines the timing of distant metastases[30,31].
3) **Lymph node status:**

   This is the most important prognostic factor if distant metastases is absent[32]. The prognosis worsens as more lymph nodes show tumour deposits. Even micrometastasis (<0.2 cm) demonstrated by immunohistochemistry show shortened disease-free periods. Sentinel node biopsies, with use of radiotracer or dyes, are indicative of nodal spread in most cases. However, if the sentinel node is the internal mammary node, the procedure cannot be done.[33,34]

4) **Tumour size:**

Assessing microscopic size (indicative of both invasive and in situ components) is more predictive of nodal metastasis. Tumours become palpable once they are over 2 cm in size. However, at this stage, the survival rate is less than 77%

5) **Locally advanced disease:**

Skin and skeletal muscle involvement interferes with complete surgical resection and increases the stage of the disease.

6) **Inflammatory carcinoma:**

Dermal lymphatic involvement can lead to "peau d'Orange" appearance due to skin thickening in Inflammatory breast cancers. These cancers, seen commonly in young and black women, have an extremely poor prognosis.[35]

MINOR PROGNOSTIC FACTORS:

1) **Histological type:**

Similarly-sized tumours of tubular, mucinous, cribriform, papillary, adenoid cystic subtypes are found to have better prognosis than IDC of the non-specific type.[36]

2) **Histological grade:**

The long term survival drops from 70% for Grade I carcinomas to

45% for Grade II carcinomas. Higher grade tumours show better response to certain types of chemotherapy.

3) **ER/PR status:**

If both ER and PR are positive, the response to hormonal therapy is 80%

4) **HER2neu:**

Overexpression is associated with lesser rate of survival but targeted therapy such as trastuzumab and Lapatinib have been developed.

5) **Lymphovascular invasion:**

Presence of tumour cells within small vascular channels have been found in over half the invasive tumours and are predictive of lymph node invasion and recurrence. Extensive involvement of dermal lymphatics is seen in inflammatory carcinoma.

6) **Proliferative rate:**

This can be assessed by:-

i) Light microscopy-assessing mitotic count.

ii) Flow cytometry- S-phase fraction can be gated and assessed.

iii) Ki67/Mib index- detects cells in G1 through M phases of the cell cycle (actively proliferating cells).

iv) Thymidine labelling index

v) RT-PCR: Highly proliferative tumours have poorer prognosis but good response to chemotherapy.

7) **Perineural invasion:**

This feature has been observed in invasive tumours.

8) **Tumour necrosis:**

If observed within the first two years of diagnosis, this parameter has been associated with poor prognosis.

9) **Tumour stroma:**

Foci of fibrosis within the centre of a tumours has been associated with poor prognosis.

10) **Response to neoadjuvant chemotherapy:**

Prior to surgery, the patient is treated with chemotherapy to downstage the carcinoma. Good response has good prognosis. High grade tumours with necrosis and negative HER2 status tend to respond well to chemotherapy.

11) **Gene expression:**

The expression of BRCA 1 is associated with worse prognosis. Allison et al summarized that Luminal A molecular subtype is associated with good prognosis, Luminal B with intermediate prognosis and both HER2 and Basal subtypes with worse prognosis.

Immunohistochemistry:

Markers used in breast cancers are of two types: prognostic and predictive markers.

The routine markers used in surgical pathology for cases of breast cancer are ER, PR and HER2/neu.

As per the ASCO guidelines, a panel of markers has been recommended by Harris et al.[37] CA 15-3 and CA 27.29 are used for screening, diagnosis and also for surveillance. Estrogen and Progesterone receptors are recommended for all primary breast carcinomas and for metastatic carcinomas. Similarly, HER2 estimation is recommended in every case of primary breast cancer. It helps in selecting patients for Trastuzumab therapy and predicts response taxane therapy. A special mention is made about Oncotype DX which helps in identifying patients manageable with Tamoxifen alone or if adjuvant chemotherapy is necessary.

Estrogen Receptors:

These weakly predict the prognosis but strongly predict response to endocrine therapy.[38] Nadji et al, in a study of nearly 6000 cases have shown that nearly all cases of Grade I tumours are ER positive, as are tubular, lobular and mucinous carcinomas. Progesterone receptors are independent

markers whose positivity signals better disease free survival. As these are therapeutic targets, quantitative assessments are critical. However, studies have demonstrated the need for meticulous standardization of technique and analysis, so that results are uniform.[39] Factors that lead to variations are

i)Type of specimen and fixative:

Mann et al have shown that 9% of resection specimens show false negative ER due to inadequate fixation.[40] A minimum of 6 to 8 hours of fixation has been recommended prior to immunohistochemical studies. This problem can be circumvented by comparing with the ER-status of the adjacent non-neoplastic breast parenchyma.

ii)Antibody used:

SP1 rabbit monoclonal antibody has been shown by studies to have the most robust response.[41] Other clones of anti-ER antibodies that are recommended are 1D5 and 6F11. Studies by Harvey et al have shown that immunohistochemistry is better than Ligand-Binding Assay for predicting response to Tamoxifen therapy.[42]

Traditionally, the charcoal and sucrose gradient assays have been used. Quicgel method has been utilized in standardization. Two parameters are evaluated in immunohistochemistry.

i. Number of tumour cell nuclei stained

ii. intensity of the reaction.

The Allred scoring system is a nine-point system established through a landmark study conducted by Harvey et al. A score of 3 or more has been considered as ER positivity.[38,43]

Score for proportion staining	Score for staining intensity
0=No nuclear staining	0=No staining
1 = <1% nuclei staining	1=Weak staining
2 = 1-10% nuclei staining	2 = Moderate staining
3 = 11-33% nuclei staining	3 = Strong staining
4 = 34-66% nuclei staining	
5 = 67 – 100% nuclei staining	

The discovery of these markers has been a cornerstone in breast cancer therapeutics. Radical estrogen-ablative therapies such as oopherectomy, pituaitary gland and adrenal removal have been abandoned in favour of Tamoxifen.[44] Not only do these markers predict response, but also provide clues during diagnosis of metastatic tumour with unknown primary. Cases of

DCIS that are hormone positive have been controlled with the use of Tamoxifen.[45]

However, the expression of ER alone does not predict the response to treatment. To increase the usefulness, a PR expression study must also be done in conjunction.

Laake at al[46] in 2000 established prognostic indices using ER scoring:

Score 0: Endocrine treatment will definitely not work.

Score 2-3: 20% chance of response to endocrine treatment

Score 4-6: 50% chance of response

Score 7-8: 75% response rates.

Elderly women have shown some tumour shrinkage after endocrine treatment, even though ER scoring is low.

Human Epidermal Receptor Protein-2:

This is both a predictive and prognostic marker. Synonymous with c-erbB-2, this oncogene protein is transmembrane glycoprotein. These epidermal growth factor receptor proteins are expressed in various normal epithelia (including mammary ducts). Their overexpression due to amplification of

the HER2 gene is seen in a subset of breast malignancies. Since 10 to 20% of primary breast malignancies show overexpression[39], it is now widely utilized as a predictive marker. Both immunohistochemistry and FISH studies are used to identify. Trastuzumab is a custom-made chemotherapeutic molecule that targets the oncogene product. Their use in early breast cancers has shown reduction of recurrence risk by 50% and mortality by one-third[39]. This anti-c-erbB2 antibody was introduced 13 years after the discovery of c-erb-B2.

Discordance rates between immunohistochemistry and FISH results up to 20% have been noted. In order to address this issue, the ASCO/CAP guidelines for reporting HER2 are as follows:

FDA Scoring System for HER2[47]

Negative = 0	No immunostaining
1+	Weak immunostaining, <30% of the tumour cells
Equivocal = 2+	Complete membranous staining, either uniform or weak in at least 10% of cells
Positive = 3+	Uniform intense membranous staining at least 30% of cells.

Strong membranous staining is taken as positive and diffuse cytoplasmic or background staining is disregarded. In equivocal cases, FISH studies have been recommended.

As per ASCO/CAP guidelines, the relative exclusion criteria are i) use of fixatives other than Neutral Buffered Formalin ii) Core needle biopsies fixed for less than one hour and large specimens less than 6 hours. ii) Any specimen fixed longer than 48 hours. The absolute rejection criterion is the presence of crush or edge artifact.

Basal-like Breast Carcinoma:

The definition of basal cells in the breast has been changing and argued over in the past years. In 2009, Rakha et al did a review of Basal-Like Breast Cancers[1]. They cite that in addition to the myoepithelial cells and luminal secretory cells, a third population of 'basal' cells are located in the luminal aspect of the TDLUs and large ducts. [48]

These cells express High Molecular Weight Cytokeratins (or 'basal' cytokeratins) but lack evidence of myoid differentiation. In normal breast, the term basal refers to both the myoepithelial cells and the aforementioned subset. In breast cancer, tumour cells expressing high-molecular weight

cytokeratins are of ‘basal’ origin. The basal differentiation of tumours was described as early as 1982 by Moll et al.[49]

These tumours are associated with Grade 3 histology; the tumour cells are highly pleomorphic and seen arranged in sheets with necrosis and inflammation, with lack of in situ components.[50] They form around 15% of all breast carcinomas and have poor prognosis. Lack of response to Tamoxifen, Aromatase inhibitors and Trastuzumab is distinctive.

These aggressive tumours tend to occur in younger women, with only cytotoxic chemotherapy being the treatment modality, apart from surgery.[51]

Histologically, a great degree of heterogeneity is noted in Basal-like breast cancers. Even though high-grade IDC is seen most frequently, prognostically favourable variants such as adenoid cystic have been associated with this genetic subtype.

These were initially diagnosed by gene expression profiling through microarray-based techniques. However, this is not feasible in every set up. With low histological concordance rates and only expensive methods of recognition, it has important to develop practical biomarkers. Retrospective studies are being conducted on a large-scale to identify biomarkers which can translate into targets for therapy or at least predict the risk.

Sutton et al have concluded that 91% of basal-like cancers are EGFR positive. Clinical trials using Cetuximab, a chimeric monoclonal anti-EGFR antibody is being tested in metastatic tumours along with Gefitinib.[52]

Sorlie et al revealed a panel for basal-like breast cancers; there was high expression of Cytokeratin 5, HER1, cKIT and absence of ER and HER2.[53] Korsching et al[54] and Bocker et al[55] emphasized that the basal-like stem cell expresses CK 5/6 preferentially.

Studies led by Lakhani et al have concluded saying that 88% of the patients with BRCA1 genotype expressed basal Cytokeratin 5/6. Both subsets have been associated with poorer prognosis. Since the cost of genetic testing is high, it cannot be executed in all patients of breast carcinoma with ease. Family history alone cannot be used as a valid indicator for genetic testing. A screening test to isolate women who are ER negative and CK 5/6 positive has been recommended.[56]

The basal Cytokeratin used in our study was CK 5/6. Basal cytokeratins show cytoplasmic positivity and were interpreted as positive when more than 1% of the cells took up the immunostain.

Based on studies conducted by Rakha et al[57], Laakso et al[58], Van de Rijn et al[59], an arbitrary scoring system was drawn up for quantifying the expression of CK 5/6.

Score	Description
0	Less than 1% positivity
1+	1-10% tumour cells are positive
2+	10 to 50% tumour cells are positive
3+	More than 50% tumour cells are positive

CLINICAL CONSEQUENCES OF CK 5/6 EXPRESSION STUDIES:

Apart from its use as a prognostic index, the various consequences of studying this marker's expression has been extensively discussed. The regular tri-panel consisting of ER, PR and HER2 has been deemed suboptimal in defining basal-like breast carcinomas. Even though Triple Negative Breast Cancers are the rough equivalents of Basal like breast carcinomas, the overlap is considered imperfect at best. [60]

Identification by immunohistochemical panels and molecular genetics aids in tailored therapeutics. A basal marker is necessary to differentiate between the two subgroups. TNBCs are mostly tumours with Grade 3 histology and nearly 48.6% of the patients require chemotherapy when compared with other subtypes.[61] Response has been noted in basal-like breast cancers treated with PARP inhibitors and Cisplatin. These platinum-based salts interfere with the BRCA1 pathway and induce response.[61,63]

MATERIALS AND METHODS

MATERIALS AND METHODS

Our study is a retrospective study. Cases of female breast carcinomas diagnosed between the years 2009 and 2014 were included. A few of the cases were rejected owing to the absence of sufficient clinical information or blocks were unavailable. Trucut biopsies were excluded.

The requisition form sent by the surgeon was used for deriving information such as age, site, nodal status and other gross findings. Retrieval of representative paraffin blocks and corresponding Haematoxylin & Eosin stained slides were done from the archives of the department of Pathology.

For the purpose of H&E staining, the representative blocks were utilized for making 4-micron thick sections and stained using a Leica Autostainer. These H&E stained slides were used for grading and assessing the histological type of tumour. Evidence of lymphovascular invasion, perineural invasion, skin involvement and admixed DCIS components were assessed and tabulated in the master chart.

HAEMATOXYLIN AND EOSIN STAINING

REAGENTS USED:

1. Harris haematoxylin

2. Eosin Y

PROCEDURE:

1. Deparaffinisation: Xylene is used.
2. Hydration: The cut sections are hydrated by immersing in graded alcohols and bringing to water.
3. Haematoxylin staining: Slides are flooded with Harris Haematoxylin for 10 minutes.
4. Running tap water is used to wash the haematoxylin-stained slide for 5 minutes (the sections should turn blue)
5. Differentiation: 1% Acid Alcohol (1% hydrochloric acid in 70% alcohol) is used to flood the slides for 10 seconds.
6. Tap water is used to wash the slides for 10 minutes.
7. The slide is immersed in ammonia water and then washed with tap water for 5 minutes.
8. Eosin: The slides are stained with Eosin Y for 10 to 15 minutes.
9. The stained slide is washed in running tap water for three minutes.

10. Dehydration: The sections are dehydrated through graded alcohols.
11. Clearing is executed with Xylene.
12. DPX is used for mounting.

Once H and E staining was completed, a review of the slides was executed to confirm adequacy and sections were cut from the representative blocks for the purpose of immunohistochemistry.

Assessment was done on 4-5 micron-thick H& E stained sections. Typing and grading was done according to Modified Scarff-Bloom-Richardson grading system.[8] The tumour was also evaluated for cytological pattern, skin involvement, perineural, and lymphovascular invasion.

IMMUNOHISTOCHEMICAL STAINING.

Blocks of normal skin and prostatic tissue were taken as control blocks. These blocks were cut at 5-micron thickness and the sections were taken onto a Poly-L-lysine coated slide and utilized as positive control sections for Cytokeratin 5/6. Blocks were also cut and stained with ER, PR and Her2/neu, with positive controls for all three cases.

The reagents used are as follows:

Antibody Reagent	Clone
ER	Clone EP1 by DAKO
PR	Clone PgR 636 by DAKO
HER2/neu	Anti-c-erbB-2 Clone CB11 by Biogenix
CK 5/6	FLEX Monoclonal Mouse Anti-Human Cytokeratin 5/6 (Clone D5/16 B4)

TEST PRINCIPLE:

The test is two-stage process. The first step involves the binding of the primary antibody to the targeted epitope in the tissue tested. The second step is to identify the bound primary antibody by using a secondary antibody by a

colorimetric reaction. The secondary antibody is bound to a dextran polymer with the help of horseradish peroxidase enzyme and an attached chromogen is responsible for the colour.

Retrieval of the antigen[9]

Formalin fixation leads to masking of specific antigens by cross-linkage. In order to expose these epitopes and optimize them for binding, antigenic retrieval has to be done.

The various methods used for this are:

1. Pressure cooked method.
2. Proteolytic digestion method.
3. Microwave method.

For the purposes of this study, the pressure cooker method was used. The epitopes are unmasked by the action of both heat and pressure. Before this process is initiated, the slides were dewaxed and hydrated through graded alcohols. Antigenic retrieval was done in the pressure cooker for 10 minutes in EDTA buffer at an alkaline pH (pH 9).

Reagents used:

- Ethylene Diamine Tetra-acetic Acid (EDTA) buffer at ph 9.

- 3% Hydrogen peroxide in distilled water – this blocks the action of endogenous peroxidase and therefore prevents nonspecific background staining.
- A solution of 0.01M Phosphate Buffered Saline (PBS) is prepared with a pH value of 7.6. The preparation is by combining the following substances in a litre of distilled water.

  1) Dibasic sodium phosphate, anhydrate 17.5 g

  2) Monobasic potassium phosphate, anhydrous 2.5 g

  3) Sodium chloride 17.0 g

- Blocking: The reagent used is casein in BS with 15mM sodium azide. It blocks non-specific protein binding.
- Primary antibodies against Cytokeratin 5/6 antigen.
- Poly HRP reagent –Horse radish peroxidase enzyme.
- DAB (3,3' Diamino Benzidine tetra hydrochloride) is used as the chromogen. This is responsible for the permanent brown precipitate.
- Harris Hematoxylin
- Distrene dibutyl phthalate Xylene) – Mountant.

PROCEDURE

1. Dewaxing of slides.
2. Hydration through graded alcohol.
3. Antigen retrieval: EDTA buffer is used at a pH of 9.0 for 10 minutes in a pressure cooker.
4. Washing with running tap water.
5. Washing in PBS buffer at pH 7.6 for 5 minutes.
6. The excess buffer is wiped off and the slides are immersed in 0.3% hydrogen peroxide for 20 minutes in order to block the endogenous peroxidase enzyme.
7. The slides are washed with PBS buffer three times for 5 minutes.
8. Slides are incubated in blocking solution for ten minutes.
9. The slides are washed with PBS buffer three times for 5 minutes.
10. Slides are incubated with CK5/6 primary antibody for one hour.
11. To improve the intensity, the sections are put in superenhancer for 30 minutes.
12. Slides are washed with PBS buffer three times for 5 minutes.
13. Diamino Benzidine is used for eight minutes.
14. Slides are washed with PBS buffer three times, 5 minutes each time.
15. Counterstaining is done with Harris Hematoxylin, for 1 minute.

16. Slides are washed in running tap water.
17. Sections are cleared with Xylene and mounted with DPX.

The stained slides were then screened to analyze the expression of CK 5/6. External control was provided by skin and prostate sections whereas internal control was provided by nipple areolar complex and associated ductal hyperplastic cells. An arbitrary scoring system was drawn up, based on many studies, as there is no established scoring system for CK 5/6 in breast carcinomas. Any invasive tumour cell showing positive staining is taken as positive.

DATA ANALYSIS:

The various parameters entered into the masterchart were:

i. Age of the patient.
ii. Histological type of tumour
iii. Size of the tumour
iv. Grade
v. Lymphovascular invasion
vi. Perineural invasion
vii. No. of lymph nodes showing evidence of metastasis
viii. TNM staging
ix. Involvement of surgical margins.

x. Staining properties of ER, PR, HER2/neu and CK 5/6.

The information was entered into a Microsoft Excel Worksheet and extrapolated into statistical package SPSS, version 11.0.

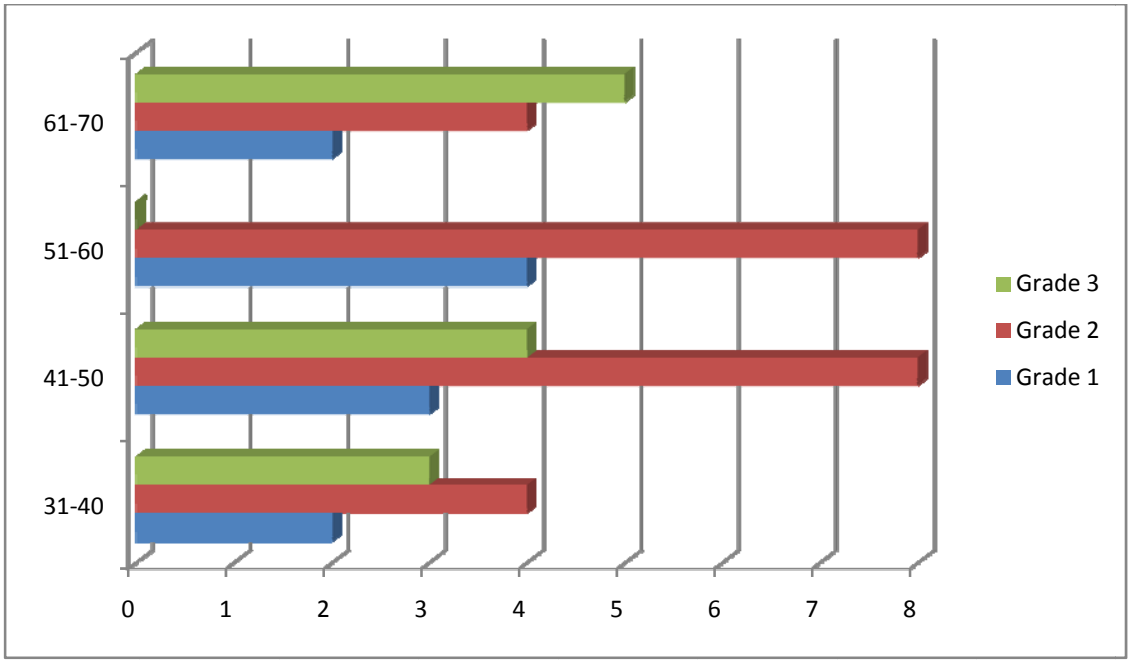
The Fisher's Exact Test, Pearson's Chi Square test, Likelihood ratio, Levene's Test for Equality of Variances and T-test for Quality of means were the various statitistical tests used.

RESULTS

RESULTS AND OBSERVATIONS

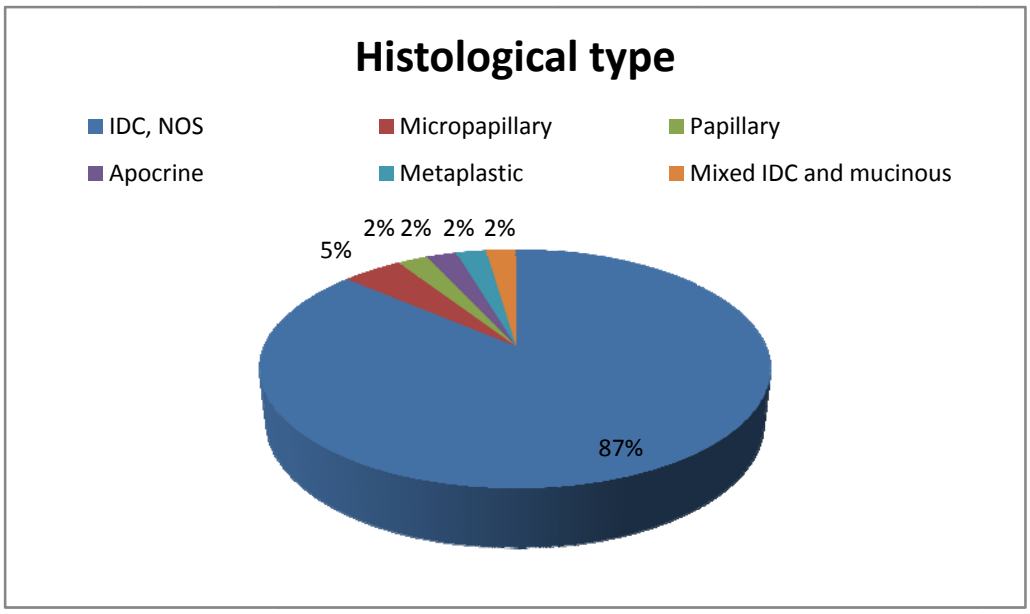
44 cases of female breast cancer were under study. The ages ranged between 33 and 67 years. The age group that had the most number of cases was the one with 41 to 50 year-old women. The commonest histological grade in our study was Grade 2, with 24 cases.

Age group	Grade I	Grade II	Grade III	Total
31-40	2	4	3	9
41-50	3	8	4	15
51-60	4	8	0	12
61-70	2	4	2	8
	11	24	9	44



Comparison of age and grade.

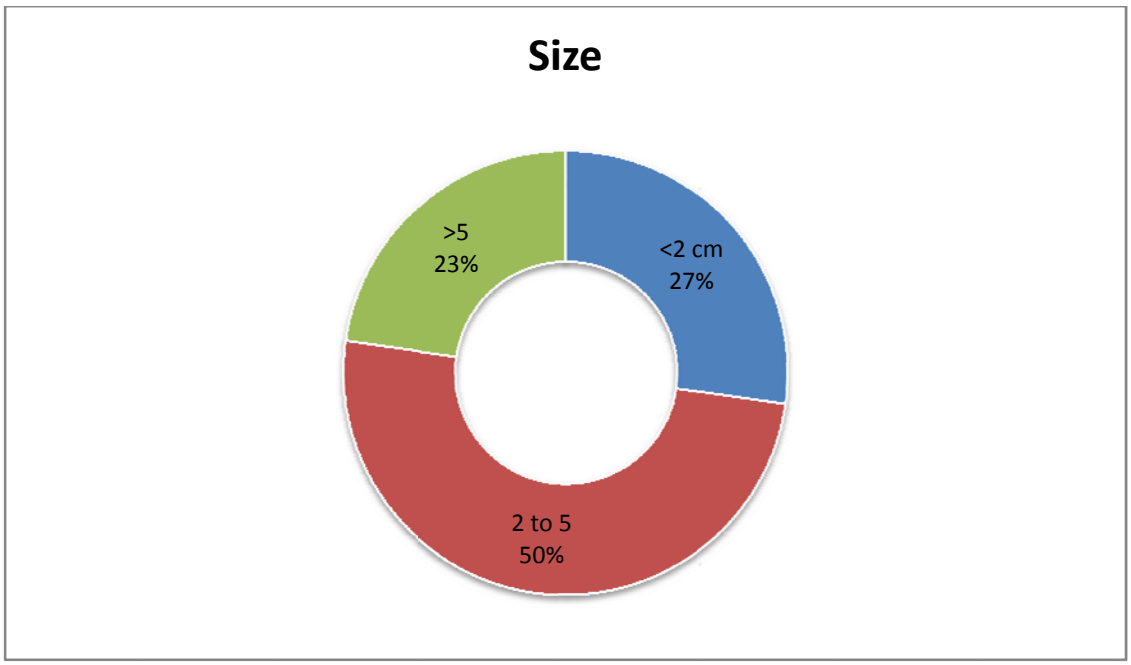
2. Histological type:



88% of the tumours were of the IDC of NST. A case of a 38-year old with apocrine carcinoma was included, who was negative for ER, PR, HER2neu and CK 5/6. Two cases of micropapillary and a case of papillary carcinoma were included which were also negative for ER, PR and CK 5/6. A case of metaplastic carcinoma was also part of the study which triple-negative and also CK 5/6 negative.

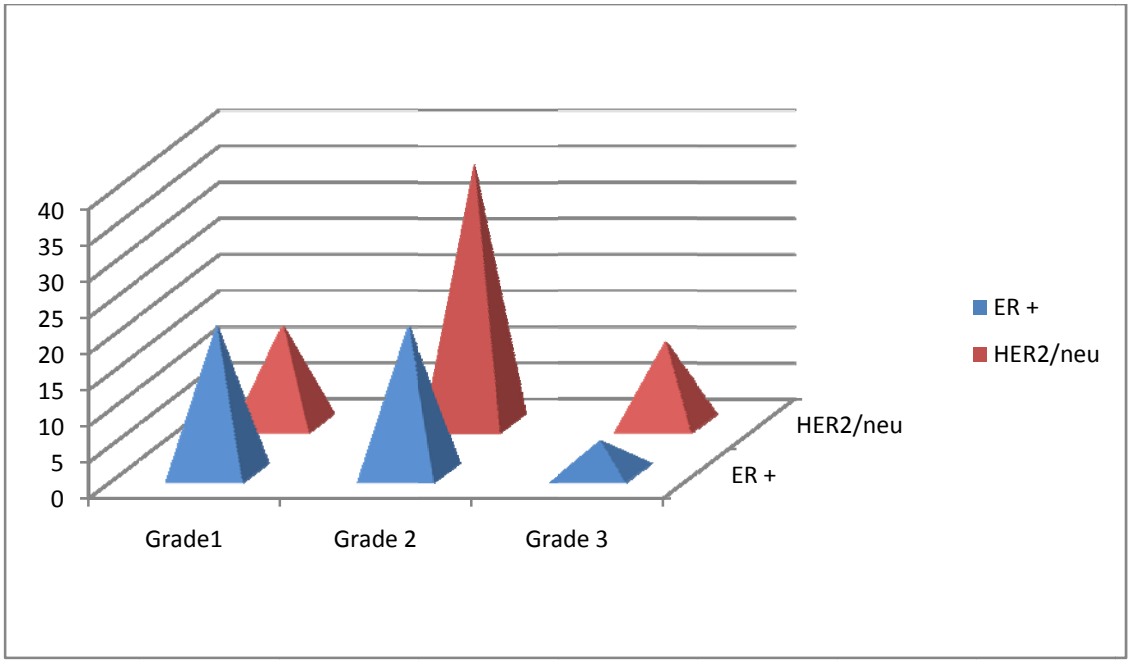
3. Size and number:

Size	No. of cases
<2	12
2-5	22
>5	10



The expression of ER and HER2:

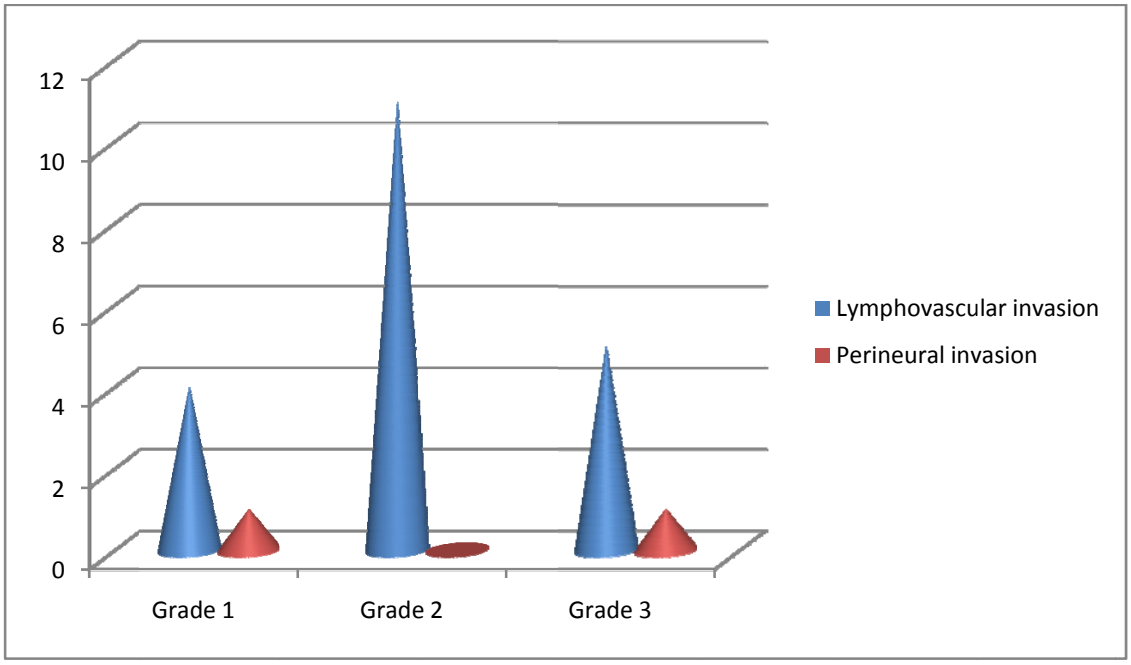
Grades	ER Positive	HER2/neu Positive
1	20.45%	13.6%
2	20.45%	36%
3	4.5%	11.36%



Invasive properties:

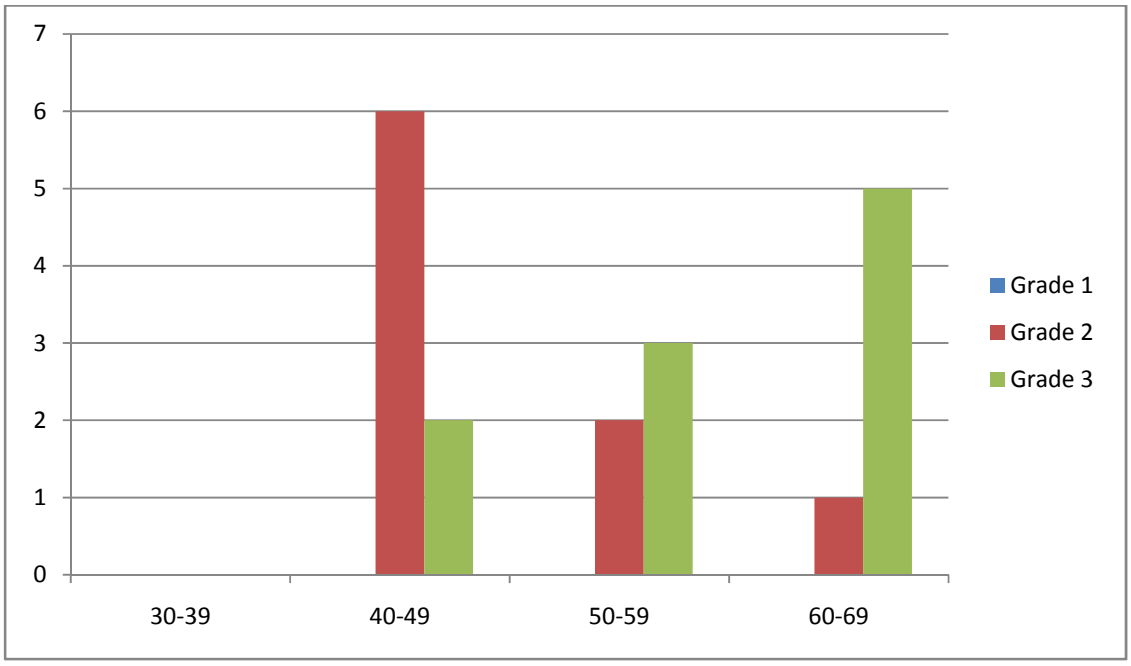
Lymophovascular invasion was seen in 20 out of 44 cases, with most cases belonging to Grade 2. Perineural invasion was seen in only two cases.

Grade	Lymphovascular	Perineural invasion
1	4	1
2	11	0
3	5	1
	20	2



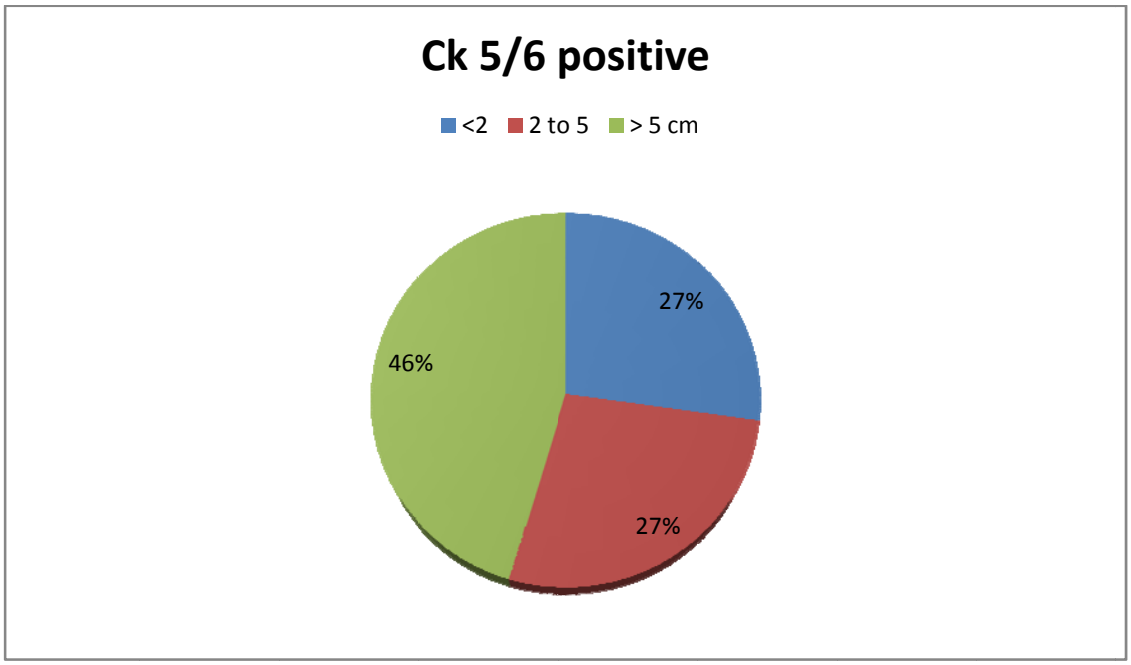
CK 5/6 positivity in age and grade:

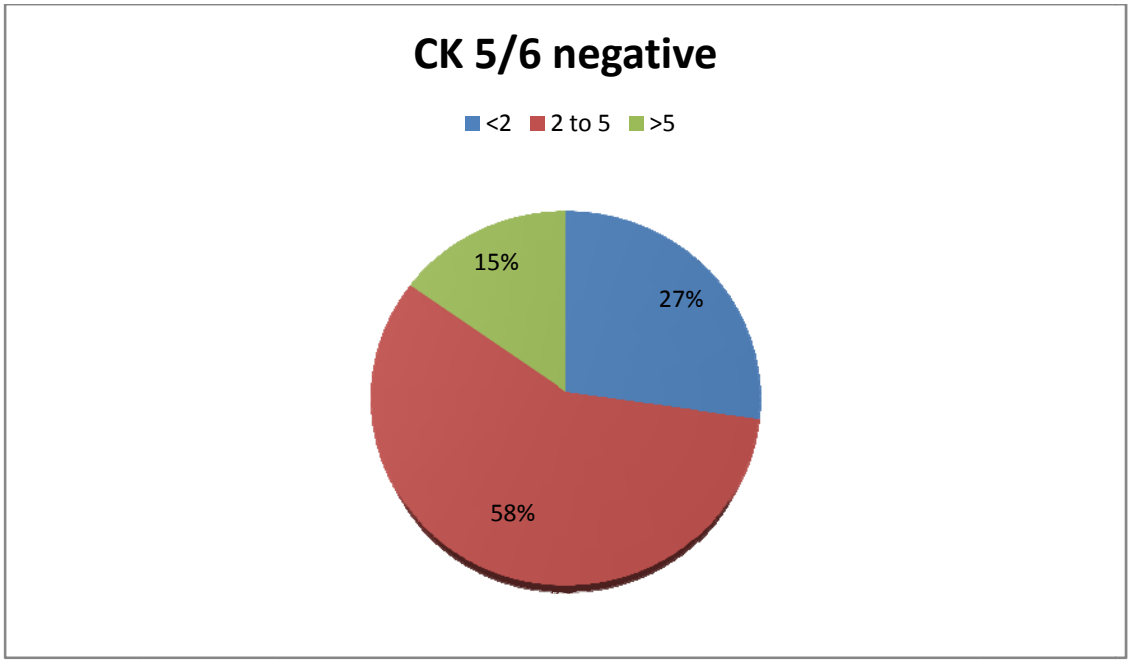
Age	Grade 1	Grade 2	Grade 3
30-39	0	0	0
40-49	0	6	0
50-59	0	2	1
60-69	0	1	0



Size and CK 5/6 expression

Size	CK 5/6 +	CK 5/6-
<2	3	9
2-5	3	19
>5	5	5





Size of the tumour statistically correlated with CK 5/6 positivity. Larger tumours had a greater incidence of CK 5/6 positivity with the largest tumour being 13.5 cm in size.

Group Statistics

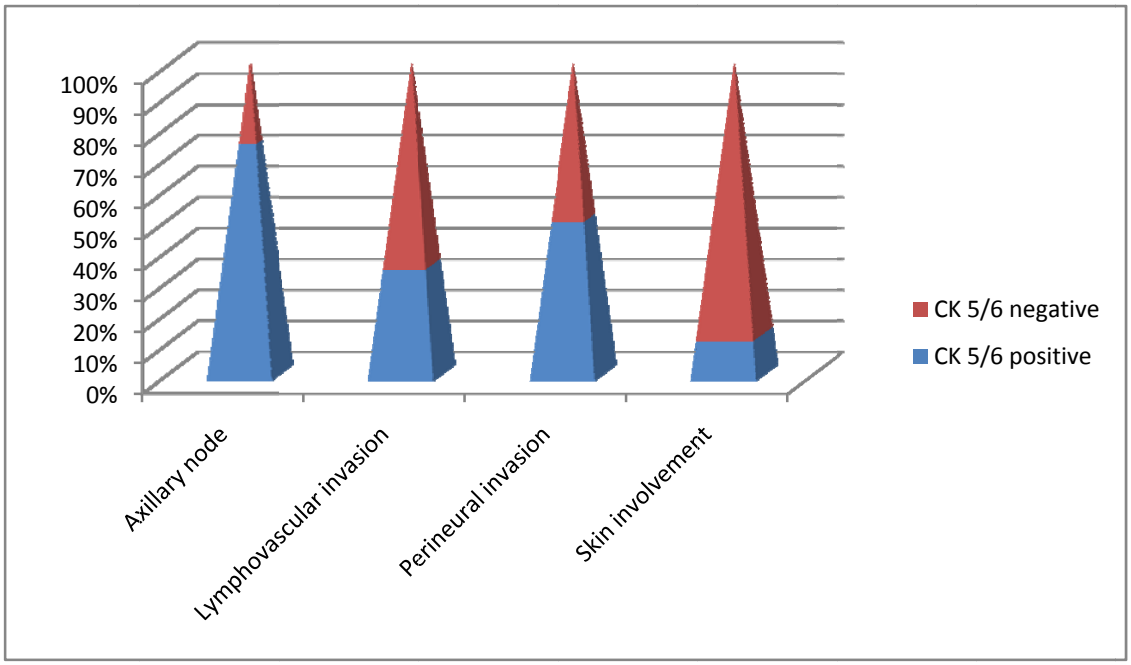
	CK	N	Mean	Std. Deviation	Std. Error Mean
AGE	.00	34	49.8235	9.64356	1.65386
	1.00	11	50.0909	8.76875	2.64388
SIZE	.00	34	3.2794	1.62570	.27881
	1.00	11	5.0455	3.78454	1.14108
LYMPH	.00	31	1.6774	2.45475	.44089
	1.00	9	3.0000	3.84057	1.28019

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
AGE	Equal variances assumed	.777	.383
	Equal variances not assumed		
SIZE	Equal variances assumed	15.699	.000
	Equal variances not assumed		
LYMPH	Equal variances assumed	3.924	.055
	Equal variances not assumed		

Correlation between CK 5/6 with invasive and prognostic features

Site of involvement	No. of cases that are CK 5/6 positive	No. of cases that are CK 5/6 negative	Total no. of cases
Axillary node	7/23	16/23	23
Lymphovascular invasion	7/20	13/20	20
Perineural invasion	1 / 2	1 / 2	2
Skin involvement	1/8	7/8	8



23 out of 44 cases showed evidence of nodal metastasis (52%). Perineural invasion was seen in only two cases. Eight out of 44 cases showed involvement of the skin. Of these, one involved the nipple and one clear-cut case of Paget's disease was observed. 20 out of 44 cases showed tumour emboli within the lymphatics and vascular spaces.

Statistical correlation was seen between the size of the tumour and Cytokeratin 5/6 positivity by using Levene's Test for Equality of Variances. Even though statistical correlation was not significant, we observed that higher grades were associated with a basal-like nature.

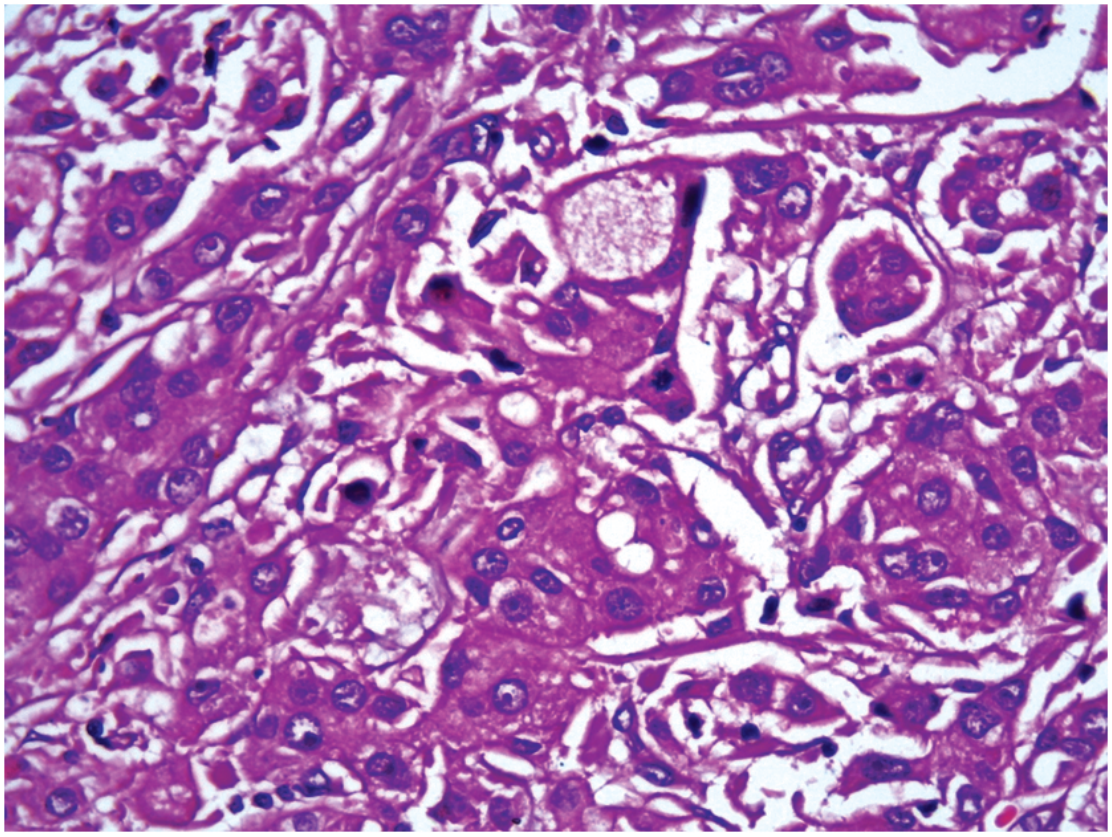


Fig 1: IDC, Lipid-rich variant (H&E, 400 x)

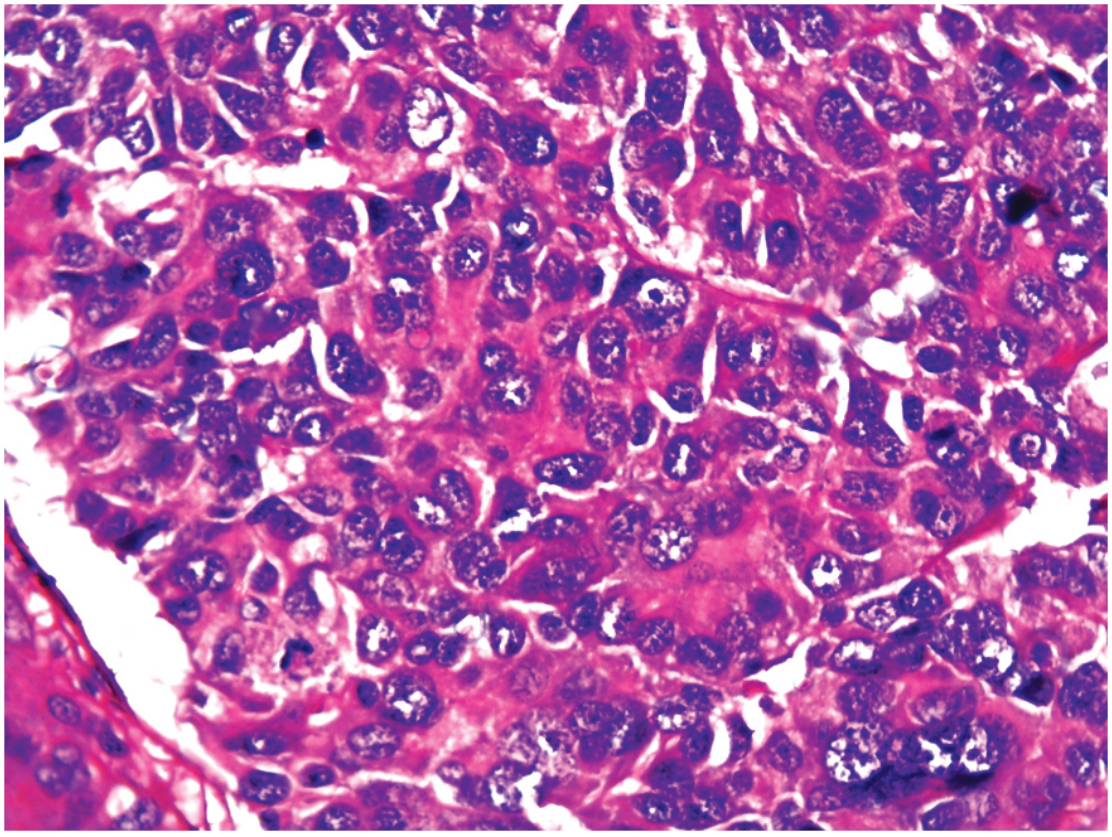


Fig 2: Grade 3 IDC (H&E, 400 x)

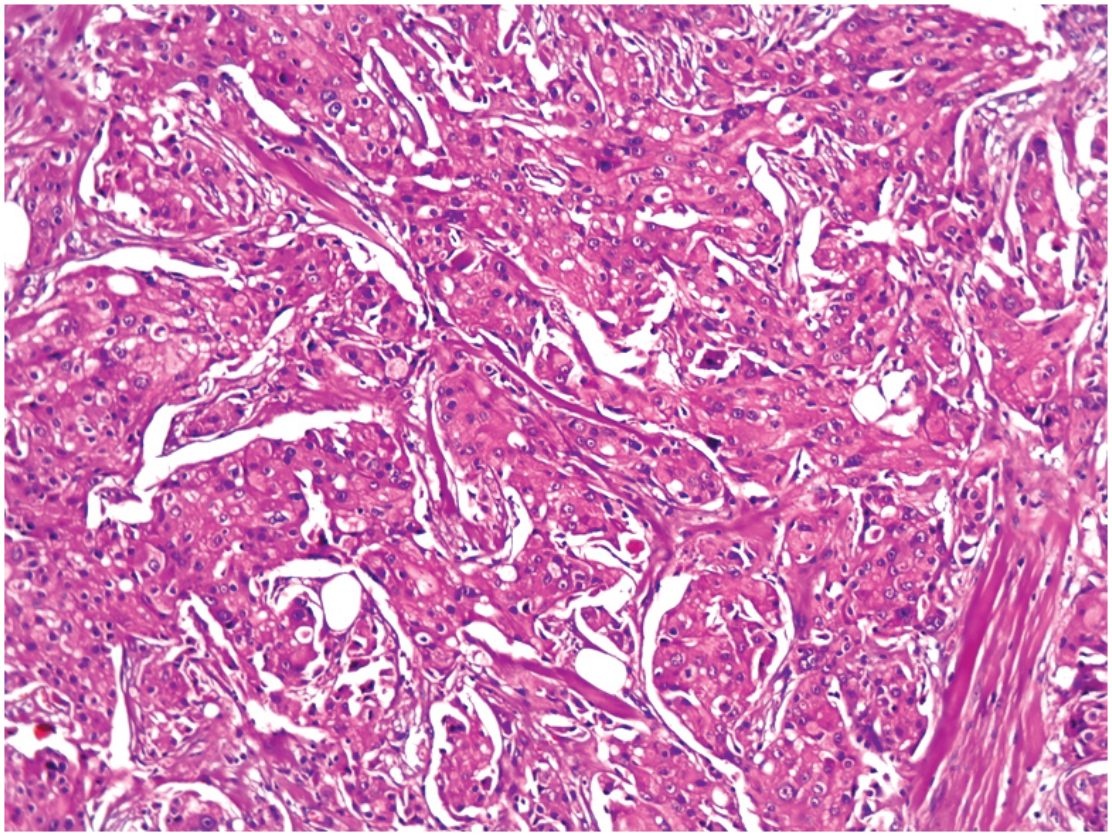


Fig 3: Apocrine carcinoma of breast (H & E,100 x)

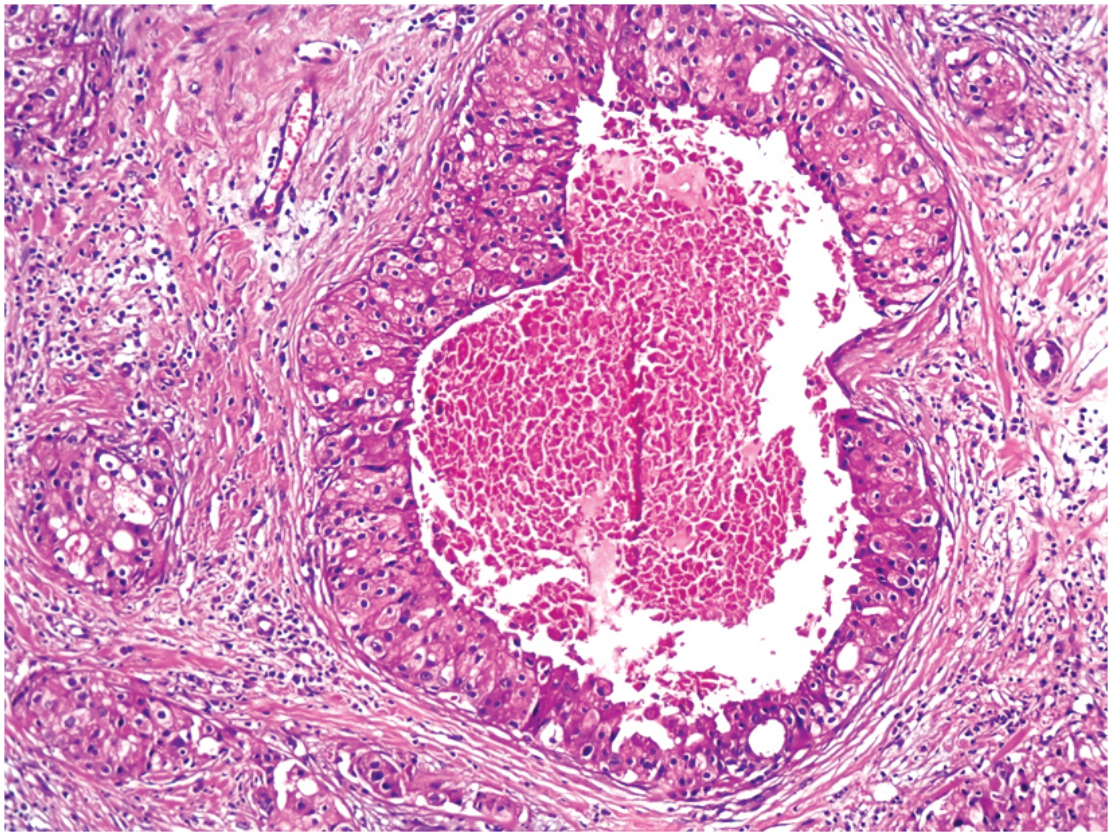


Fig 4: IDC with comedonecrosis. (H & E, 100 x)

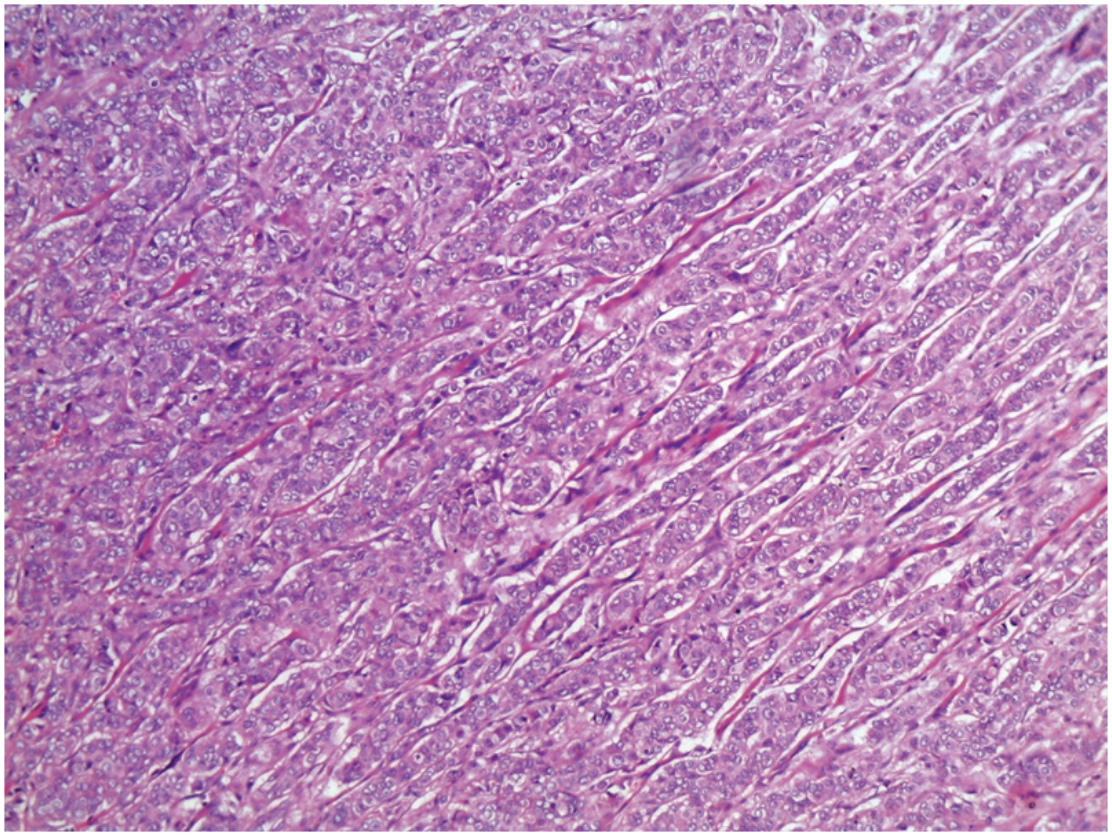


Fig 5: Tubule formation < 10% (H & E, 100 x)

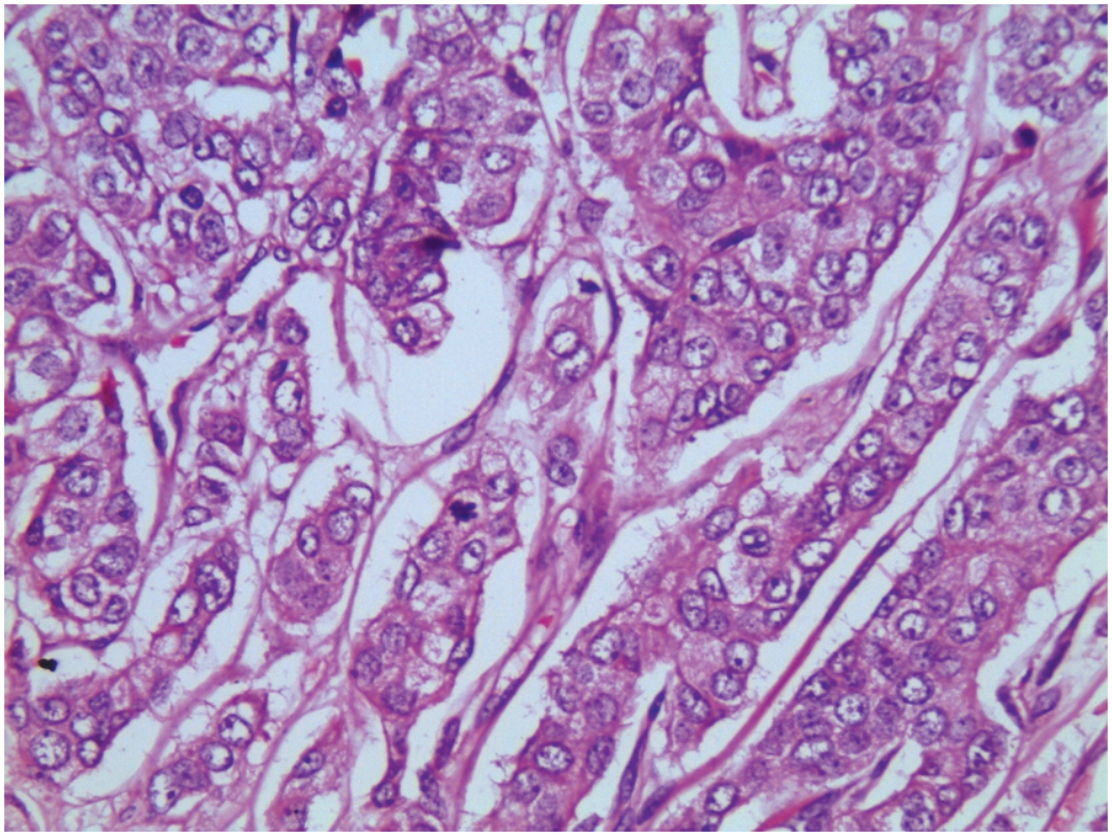


Fig 6: Grade 3 IDC with increased mitoses (H&E, 400 x)

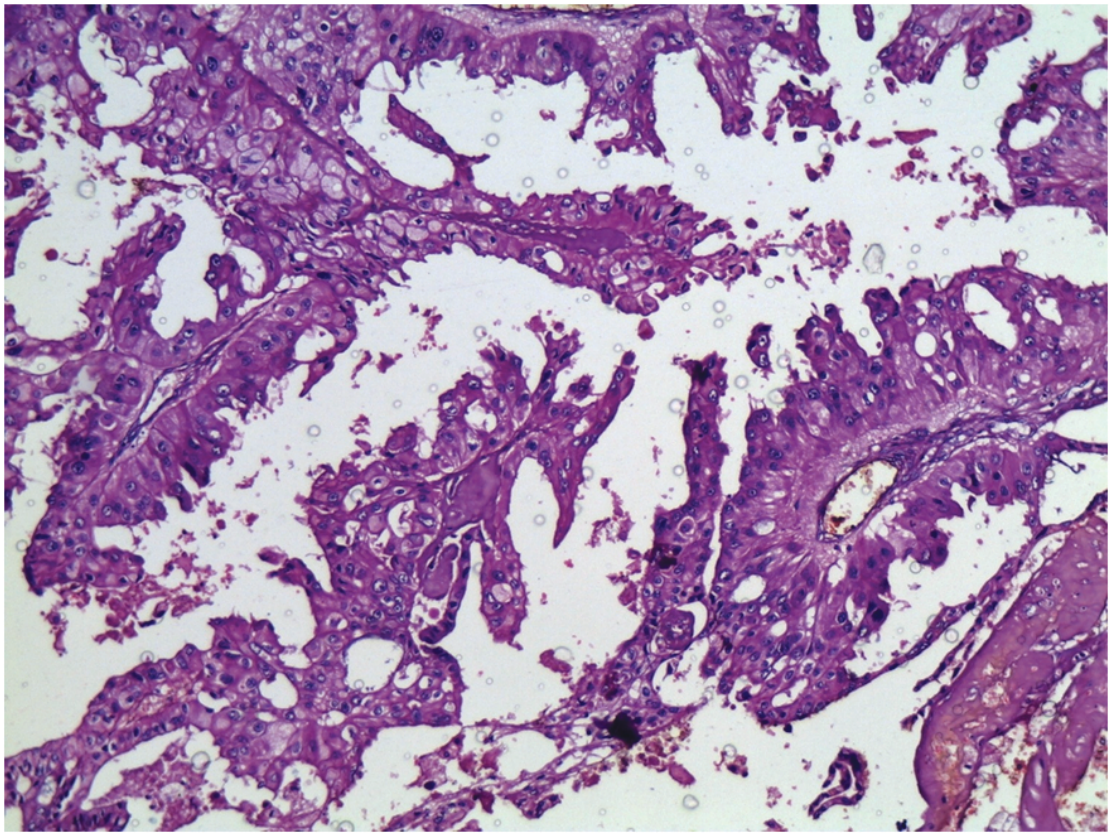


Fig 7: Papillary Carcinoma of breast (H & E, 100 x)

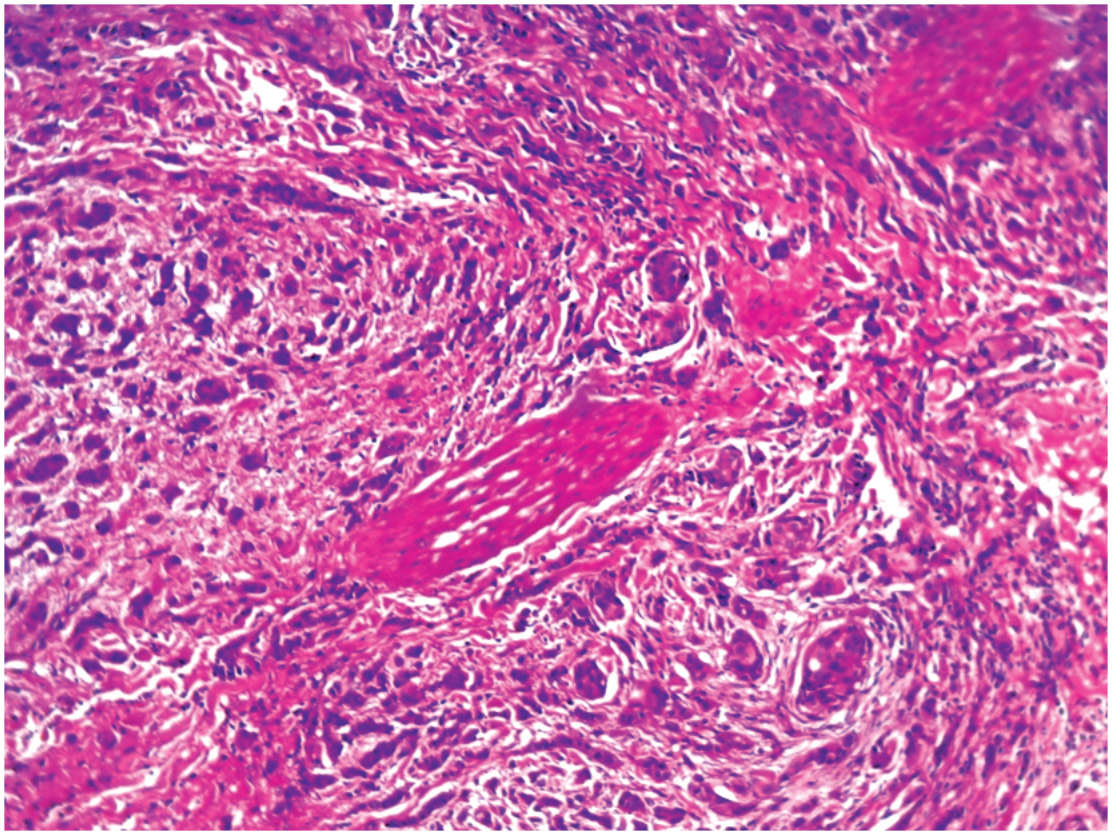


Fig 8: IDC with perineural invasion (H & E, 100 x)

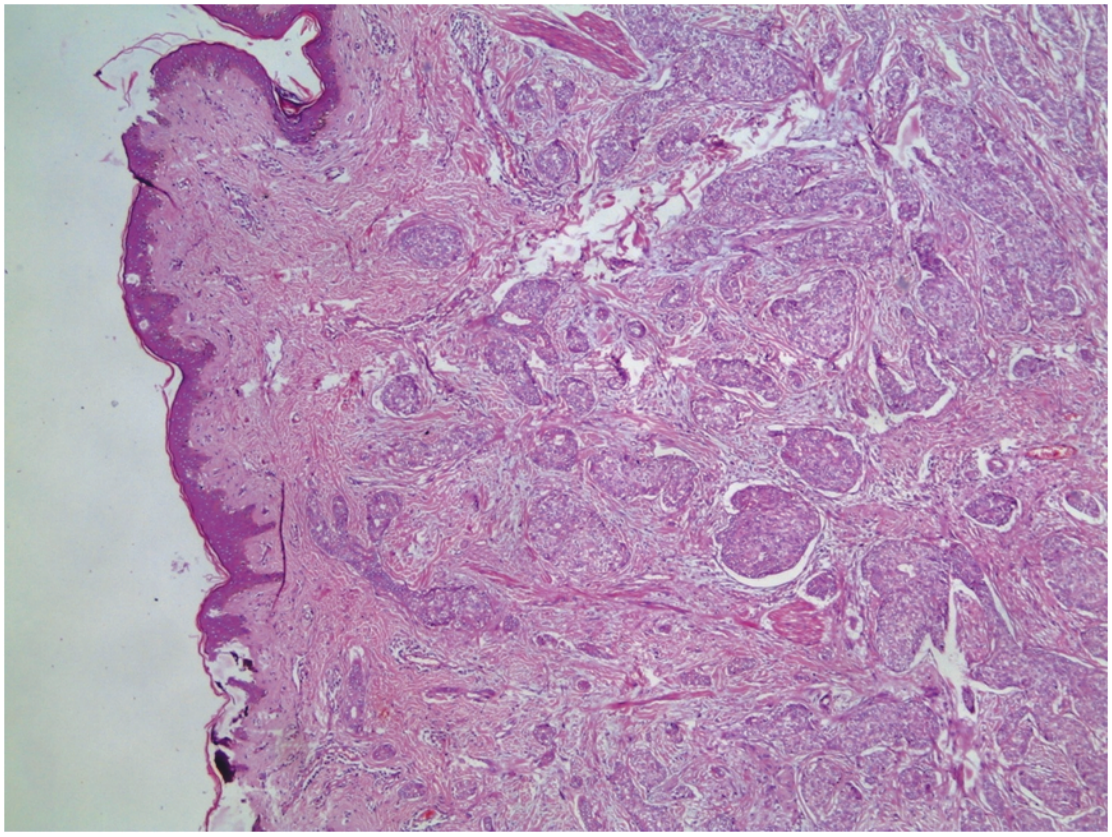


Fig 9: Subdermal lymphatic tumour emboli (H & E, 100 x)

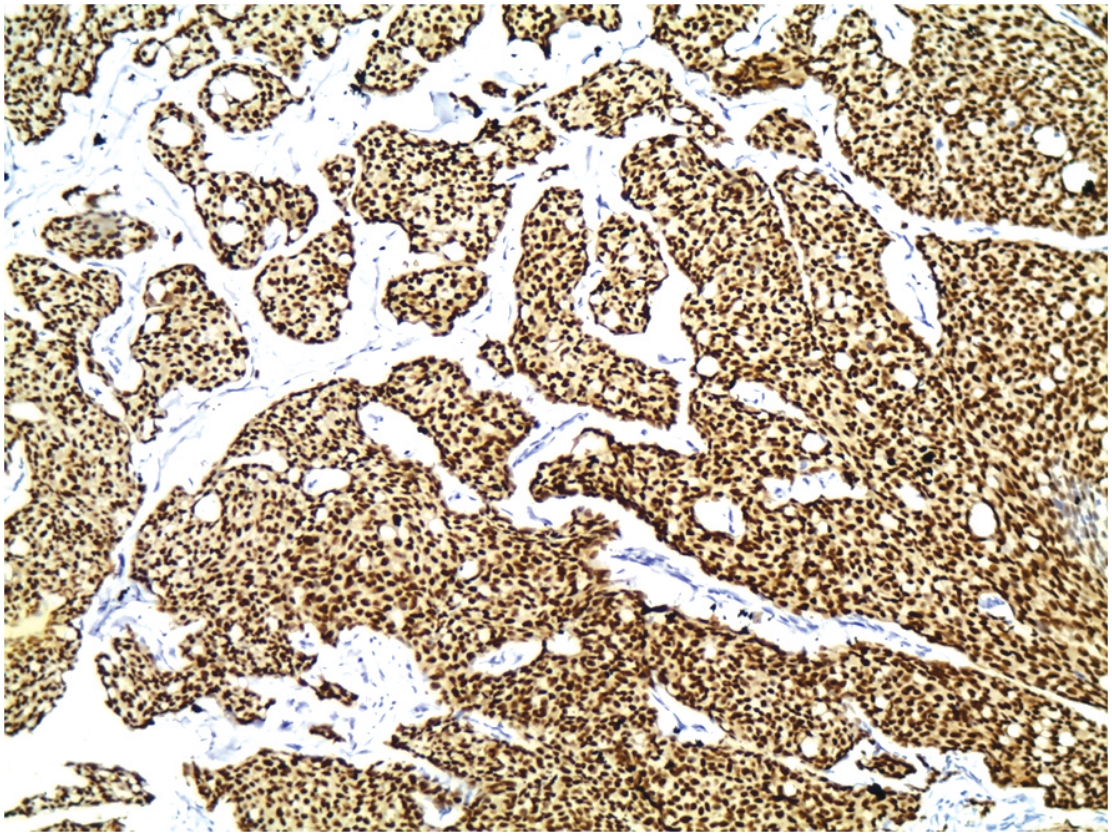


Fig 10: IDC with ER nuclear positivity (IHC, 100 x)

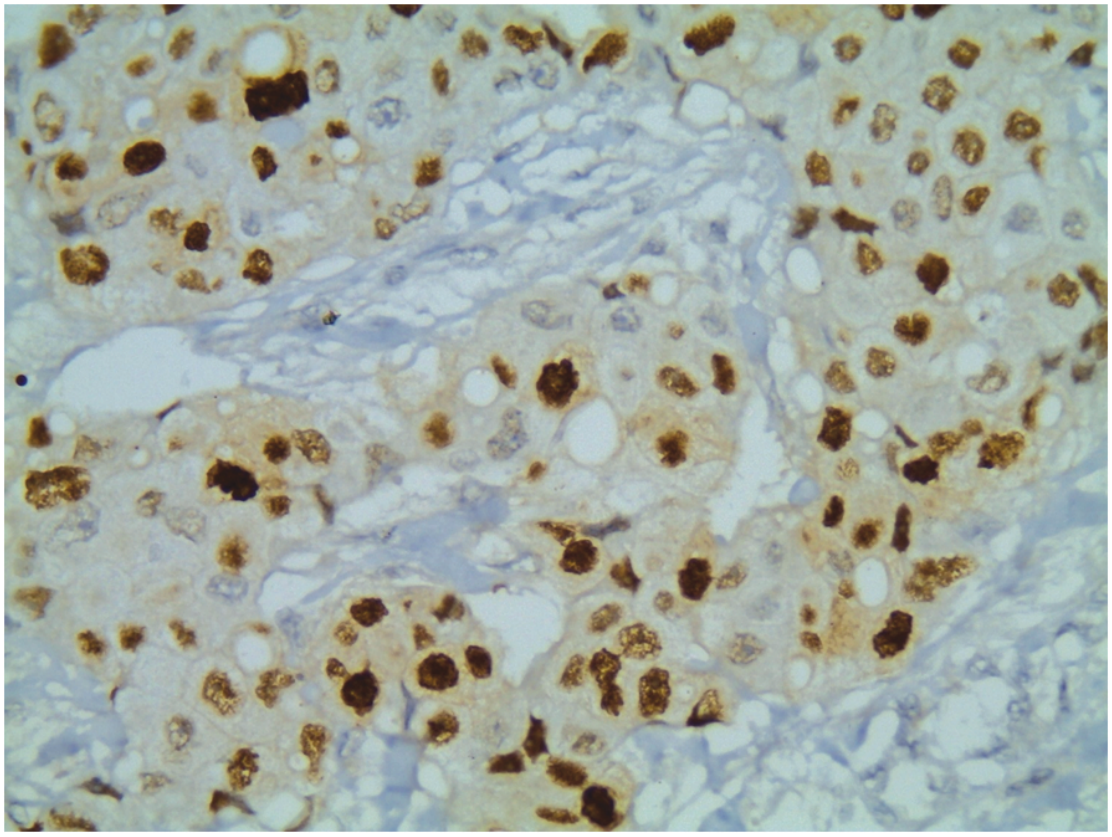


Fig 11: IDC with nuclear positivity for PR (IHC, 400 x)

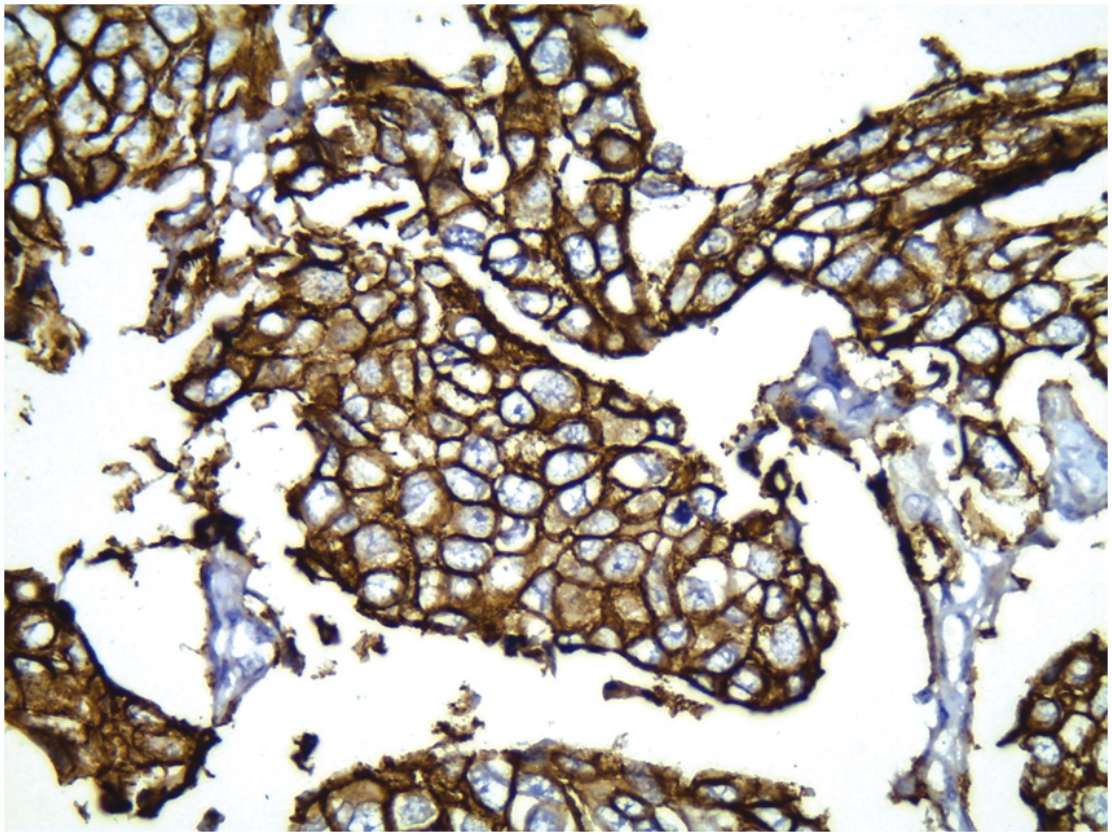


Fig 12: IDC with complete strong membranous staining for HER 2 (IHC, 400 x)

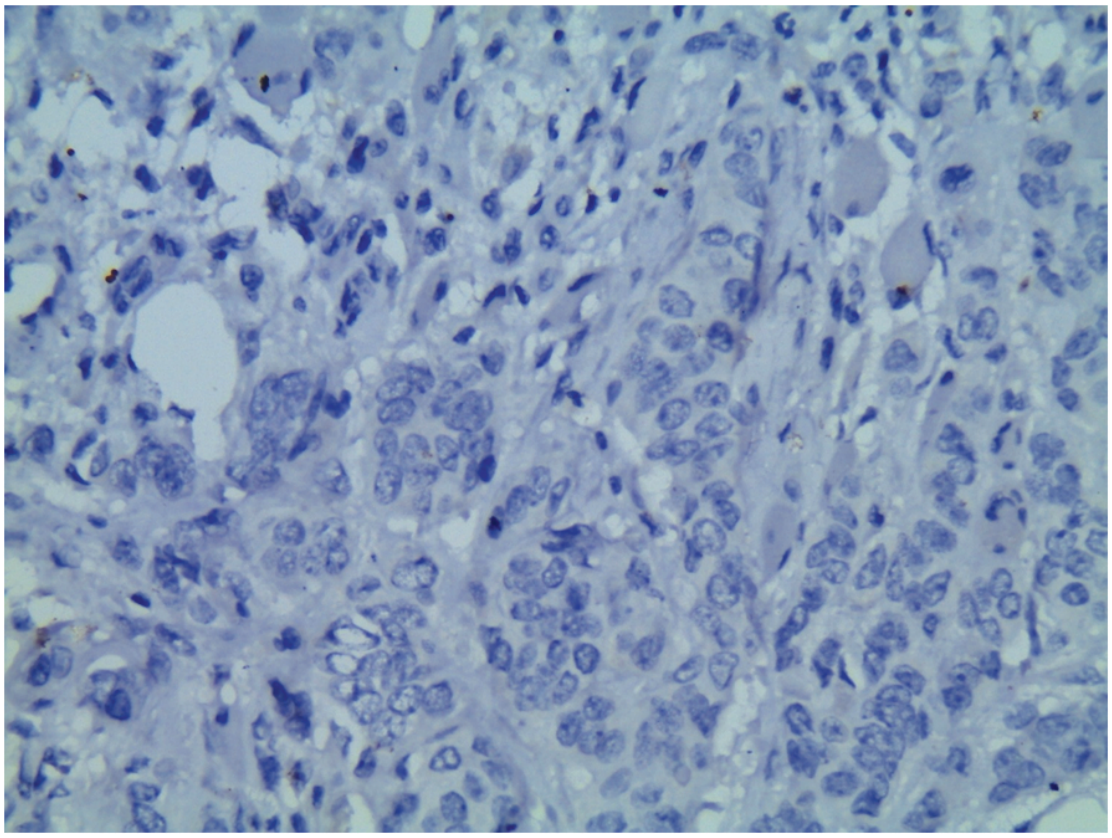


Fig 13: IDC with negative staining for CK 5/6 (IHC, 400 x)

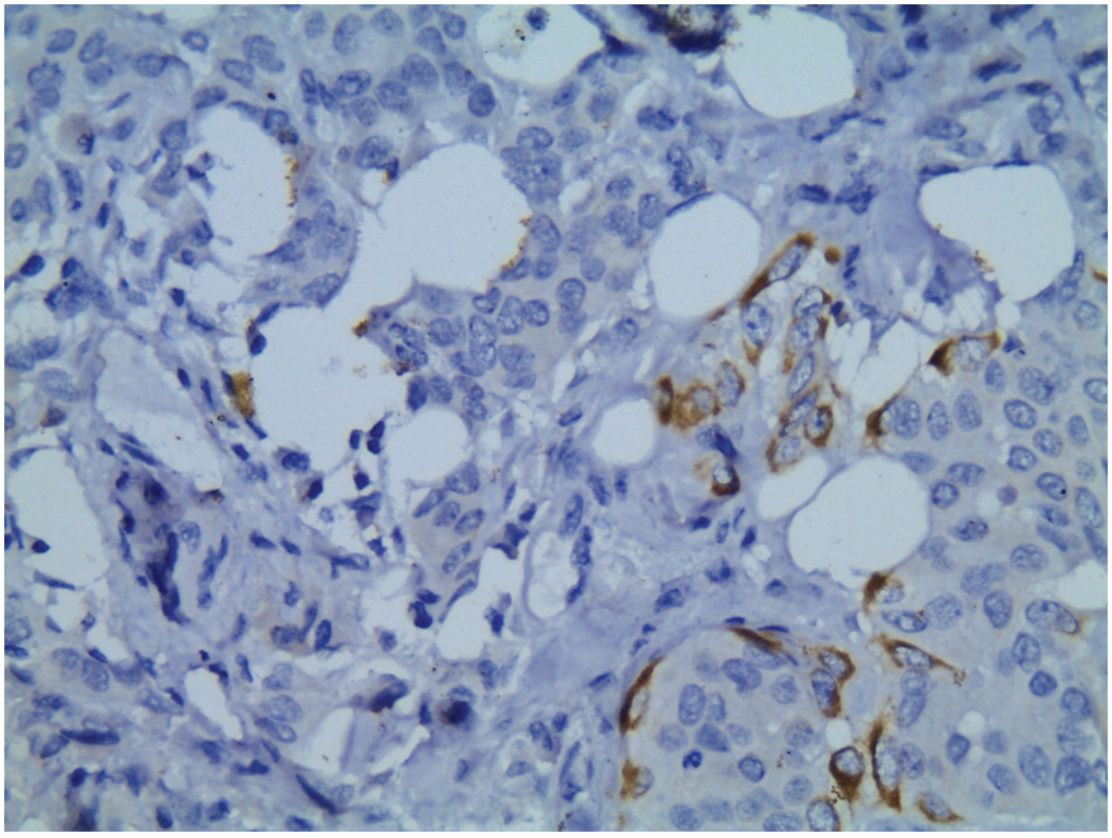


Fig 14: IDC with less than 10% staining for CK 5/6 . DCIS component shows positivity (IHC, 400 x)

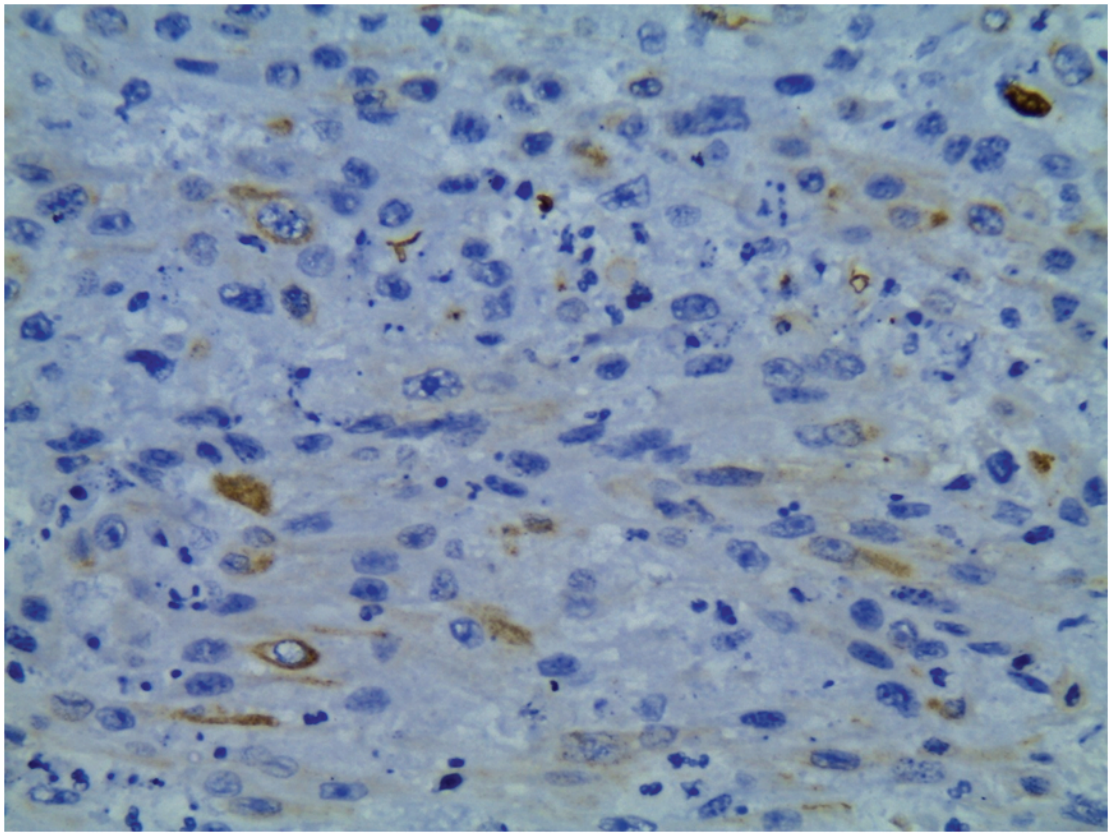


Fig 15: IDC, with 10-50% of the cells show cytoplasmic positivity for CK 5/6 (IHC, 400x)

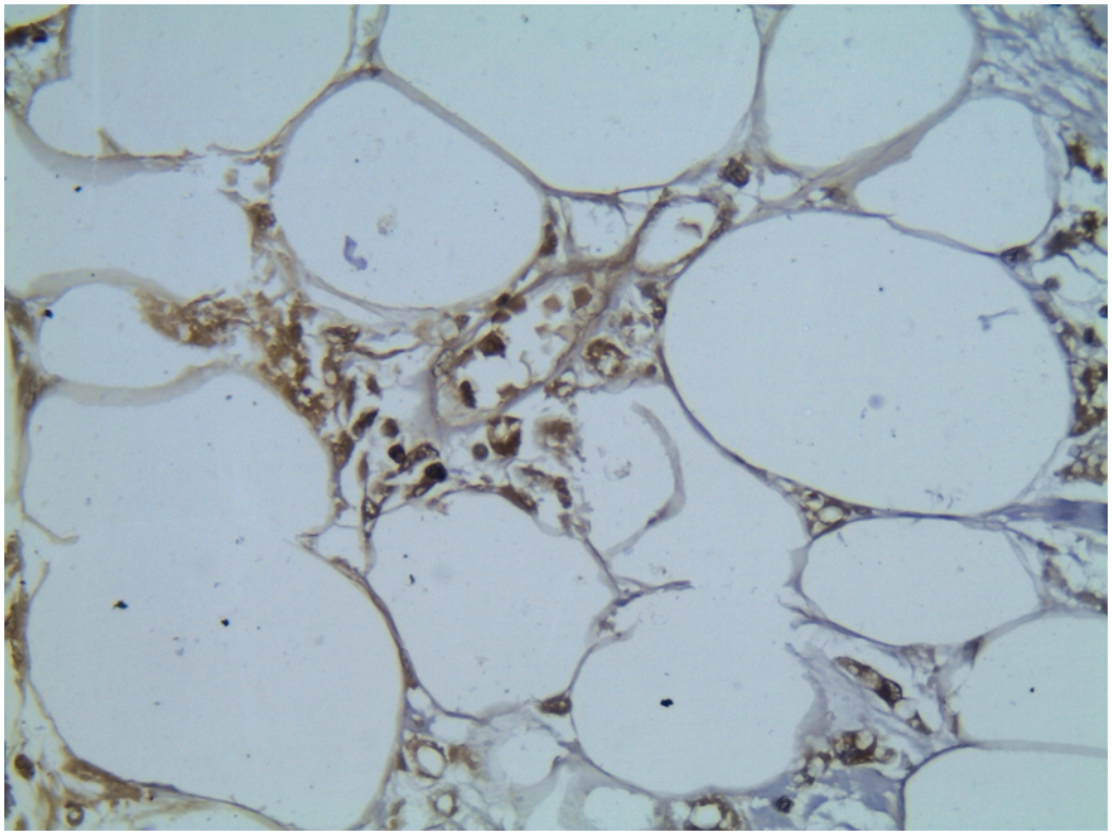


Fig 16: IDC with >50% of the cells showing cytoplasmic positivity for CK 5/6 (IHC, 400x)

DISCUSSION

DISCUSSION

Breast carcinomas have emerged as the most common malignancy in Indian women. Similarly high incidence is seen in South Indian population also, where they form one-third of all female cancers.[64] Not only is their incidence high, but the fatality rate of these cases exceeds those of western population. The cure rates, quality and the length of life have improved in these women, after the development of targeted therapy.

Glass et al observed quantitative and qualitative trends in breast cancer incidence. There has been a tremendous increase, particularly in ER positive tumours.[6] The reason for the exponential rise has been attributed to the use of post-menopausal hormone replacement therapy and widespread utilization of mammography.

The overall percentage of ER positive cases was 45%, lesser when compared with western literature. This is consistent with findings in a study conducted by Ambroise et al, which concluded saying that hormonal expression is lesser in the south Indian population.[64]

This stresses the need to look into other markers and their routine use in South Indian cohorts.

There are subsets of breast carcinoma which are not susceptible to conventional therapy and have a paradigm shift in molecular genetics and immunohistochemical expression. These are the Basal-like breast carcinomas and the Triple Negative Breast Cancers.

Overall, the mean age of patients with breast cancer was studied by Sarrio et al and found to be 53 years.[65] The mean age of the patients in our study being 52 years, this parameter also showed close correlation.

Oral contraceptive usage for more than a year was also associated with a 2.5 fold increase in incidence but there is no significantly increased risk for non-Triple Negative Breast Cancers. In our study, history of OCP use and parity status was not available.

Sarrio et al concurred that 53% of the tumours in their study showed lymph node invasion. 92.6% showed histological features of IDC. Grade 2 tumours formed 39% of the cohort while Grade 3 was 46%.

In our study, similar results were seen. Around 88% of the cases were of the IDC histological type. 45% showed evidence of lymphovascular invasion. More than half the cases belonged to Grade 2.

Barnes et al, in their study, determined that the receptor status in metastatic foci is similar to that of the primary tumour. This rule, however, does not

apply to cases which have been treated with adjuvant chemotherapy. ER positive tumours can generate ER negative metastasis.[5]

Gown et al observed that HER2 oncogene protein is expressed at low levels in a variety of normal epithelia including the ductal epithelium of breast. It is the amplification of the gene and overexpression of the corresponding gene product that is relevant in determining HER2/neu status.[43]

American Society of Clinical Oncologists and the College of American Pathology have recently released guidelines for laboratory testing of HER2 status. Normalization technique was introduced for standardization of results and to avoid discordance between immunohistochemistry and FISH results. There is improved accuracy of HER2 studies using a subtraction scoring system in which a signal score of non-neoplastic breast epithelium is subtracted from that of the tumour. Using this system, the proportion of HER2 positive tumours in our study is 63%

Comparative analysis of results in studies conducted by Harvey et al[42] with ours

Factor	Harvey et al % of ER positive tumours	Our study % of ER positive tumours
Nodal status		
*Node negative	73	27.2%
*Node positive	68	54%
Tumour size, cm		
<2	78	58.3%
>2	67	37.5%
Patient age, in years		
<35	46	100
35-65	65	0
>65	82	48%

From the above statistics, we inferred that due to the large size of the tumours in our study, there was increased nodal metastasis. Almost all the women with ER positive cases belonged to the 35 to 65 year age group.

Dolle et al inferred that Triple Negative Breast Cancers (TNBCs) are breast cancer subtypes associated with high mortality rate and resistance to hormonal manipulation and Herceptin.[66] Since these tumours are negative

for ER, PR and HER2, newer markers are to be identified for this subtype. These tumours have been seen with increased incidence in younger women (aged 45 years or younger). Our study showed close correlation, with the mean age of women with TNBCs being 46 years and nearly a third of the women were 40 or younger. These malignancies have also been associated with increased incidence in Black and Asian women, irrespective of the socio-economic status.[67]

The purpose of our study was to identify a newer basal marker and observe the expression and clinicopathological in cases of breast cancer at our centre. A study led by Heatley showed that CK 7, CK8 and CK 19 were absent in carcinomas and the usage of these antibodies should serve as means to distinguish benign from malignant.[4] CK 14 expression with absence of other myoepithelial markers has been reported.

The basal marker that we selected for our study was CK 5/6. Clark et al suggested that CK5 is positive in breast progenitor cells[68], which are believed to be the cell of origin in basal-like breast cancers[1]. Studies led by Sarrio et al, 15.24% of the patients in their study were of the basal-like subtype, with most of these tumours belonging to Grade 3. In our study, 25% of the cases were basal-like, with all of these tumours falling into either

Grade 2 or Grade 3. In a large-scale research by Nielson and Choo, 14% of the cases were found to be positive for basal cytokeratins.[51]

67% of the carcinosarcomas were basal-like. In our study, the only case of metaplastic tumour was negative for basal markers.

Studies conducted by Beyer and Yeh have shown that not all cases of Triple Negative Breast Carcinomas (TNBCs) are basal-like[70]. In a study conducted by Sutton et al, CK 5/6 was expressed in 62% of TNBCs. In our study, CK 5/6 was positive 50% of TNBCs.

In a study led by Lakhani, cytokeratin 5/6 was positive in 7.83% of the cases.[69] Their results compared with ours, is as follows:

Grade of the tumour	Ck 5/6 positivity rate Statistics by Lakhani et al	CK 5/6 positivity rate- our study
1	0	0%
2	0	37%
3	12%	22%

The high rates in our study are explained by the considerable overlap between Grade 2 and Grade 3 tumours. As our cohort was entirely of the Asian population, we observed higher rates of basal marker positivity.

In a study conducted by Nielson et al, nodal positivity in CK 5/6 was 69.2%[71] whereas the nodal positivity in basal cytokeratin positive cases in our study was nearly 80%. Vascular emboli were also prominent in these tumours, consistent with their highly invasive nature.

Rakha et al conducted large-scale studies and found that 75 to 100% of basal-like tumours belonged to Grade 3. They inferred that these tumours are heterogeneous entities with distinctive morphological, histological and genetic characteristics. These malignancies are associated with poor prognosis and have a distinctive response to chemotherapy. They concluded their study by recommending a panel of four biomarkers for basal-like breast cancers: Ck5/6, CK 14, CK 17 and EGFR.

However, Nielson et al observed that a panel consisting of ER, HER2 and CK 5/6 to identify the basal-like subset was useful as this immunnohistochemical combination had a 76% sensitivity and 100% specificity rate when compared with genetic microarray analysis.[71]

Cheang et al conducted the largest study as of date with 4046 patients and their results were similar to our study. 70% of tumours were of 50-62 year age group. 90.5% were of ductal. 5.2% were of Grade 1, 39% by Grade 2 and 51% were Grade 3.[72] The results are compared with our study and also with a study led by Laakso et al. The latter study used a cocktail of antibodies comprising of CK5/14, CK8/18 and p63. The parameters used in the comparative table below are those of the subset stained with antibodies to CK 5.

Characteristics	Cheang et al. statistics	Our study statistics
Age at diagnosis		
<40	9.4%	15.9%
41-49	19%	34%
50-65	35.5%	50%
>65	36.2%	4%
Histology		
Ductal	90.5%	88.7%
Others	9.5%	11.3%
Grade		
1	5.2%	25%
2	39.1%	54.5%
3	51.1%	20.5%
Tumour size		

<2 2-5 >5	51.7% 41.9% 5.4%	27% 50% 20.4%
Lymphovascular invasion Positive Negative	 43.3% 52.%	 45% 55%

As early as three decades back, the basal subset was described by Dairkee et al as tumours arising from progenitor/stem cells in the female breast.[74] A series of studies ensued, each with its own threshold for basal Cytokeratin expression. Fulford et al[75], Jones et al[76], Banerjee et al[77] and Abd El-Rehim et al[78] deemed cases as positive if even a single cell showed staining. The various cytokeratins used were mostly CK 5/6, CK14, CK17 and antibody cocktails.

The conclusions drawn from the landmark studies are as follows:

Authours of the study	Conclusion
Rakha et al	CK 14 and/or CK 5/6 expression suffices to define basal-like breast cancer
Jumpannen et al[79]	Expression has no impact on long-term survival if the tumours are ER-negative.
Laakso et al	True basal breast carcinomas are HER2 negative; CK5/6 expression has no prognostic use in high-risk patients.
Van de Rijn et al	Expression of basal cytokeratins was an independent prognostic factor in breast cancers with no axillary nodal metastasis
Nielson et al	A panel of four antibodies (ER, CK 5/6, HER2 and EGFR) can identify breast carcinomas with accuracy.
Jones et al	breast carcinomas have an overall low disease-free survival and demonstrate a distinctive chromosomal pattern
Fulford et al	Overall survival was better in basal-like Grade 3 carcinomas than their non-basal counterparts. The study also stressed that the risk of liver and bone metastases was lower in basal-like breast carcinomas.
Our study	CK5/6 positivity was seen in tumours of larger size and higher grades

LIMITATIONS OF THE STUDY

1. A more accurate diagnosis of basal-like breast carcinoma can be made using a panel of immunohistochemical markers namely CK5/6, CK14 and EGFR. However, data regarding ER, PR and HER2neu used in conjunction with CK 5/6 improves the specificity of CK 5/6 in detecting basal-like breast carcinomas.
2. There is no uniform scoring system for CK 5/6 expression in breast carcinomas. An arbitrary scoring system was drawn up based on various studies.
3. Details regarding therapeutic response and disease-free survival of the patients in our study were not available to us.

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

- 18% of the breast carcinomas in our study were negative for ER, PR, and HER2neu and hence, come under the category of Triple Negative Breast Cancer (TNBC).
- Out of these TNBCs, 50% were positive for CK 5/6.
- CK 5/6 positivity showed significant statistical correlation with the size of the tumour.
- All cases that were positive for CK 5/6 were of higher histological grade.

Based on our study, we recommend the routine use of CK 5/6 in all cases of Triple Negative Breast Carcinomas as they help in identifying the basal-like subtype without relying on expensive tests based on molecular genetics. Despite poor prognosis, this cohort has good response to platinum and anthracycline based chemotherapy. More research is necessary to identify specific therapies for this subset.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Rakha E, Reis-Filho JS. Basal-like breast carcinoma-From expression profiling to routine practice. *Archives Of Pathology & Laboratory Medicine* 2009; 133(): 860-868
2. Kumar, Abbas, Faustor, Aster. *Robbins and Cotran Pathologic Basis of Disease*, 8th ed. Philadelphia PA: Elsevier-Saunders; 2009.
3. http://globocan.iarc.fr/old/factsheet.asp
4. Heatley M, Maxwell P, Whiteside C, Toner. Cytokeratin intermediate filament expression in benign and malignant breast disease. *Journal of Clinical Pathology* 1994; 48(): 26-32.
5. Barnes D M, Hanby A M. Oestrogen and progesterone receptors in breast cancer: past, present and future.. *Histopathology* 2001; 38(): 271-274.
6. Glass A G, Lacey J V, Carreon J D, Hoover R N. Breast cancer incidence, 1980-2006: Combined roles of menopausal hormone therapy, screening mammography, and Estrogen Receptor status. *Journal Of The National Cancer Institute* 2007; 99(15): 1152-61.
7. Suvarna K, Layton C, Bancroft J. *Bancroft's Theory and Practice of Histological Techniques*, 6th ed. : Churchill-Livingstone; 2007

8. Galea MH, Blamey RW, Elston CE, Ellis IO. The Nottingham Prognostic Index in primary breast cancer. Breast Cancer Research & Treatment 1992;22(3):207-19
9. Taylor RC, Chanrong, BJ Naney, Wu N. Techniques of immunohistochemistry: principles, pitfalls and standardization. In: David J Dabbs (ed). Diagnostic Immunohistochemistry. 1st edition. Philadlphia, Churchill Livingstone 2002; 3-34.
10. Rosen. *Rosen's Breast Pathology*, 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2008.
11. Hassiotou F, Geddes D. Anatomy of the human mammary gland: current status of knowledge. *Clinical Anatomy* 2013; 26(): 29-48.
12. Rosai. *Rosai and Ackermann's Surgical Pathology*, 10th ed. Edinburgh: Mosby; 2011.
13. I.O. Ellis, C.J. Cornelisse, S.J. Schnitt, A.J. Sasco, X. Sastre-Garau, R. Kaaks et al. *Pathology and Genetics of Tumours of the Breast and Female Genital Organs*, 3rd ed. Lyons, France: IARC Press; 2003.
14. Dotto et al. Frequency of Clinically Occult Intraepithelial and Invasive Neoplasia in Reduction Mammoplasty Specimens: A Study of 516 Cases. *International Journal Of Surgical Pathology* 2008; 16(1): 25-30

15. http://www.ncrpindia.org/PBCR_2006_2008/Chennai.pdf
16. Miller. Estrogen and DNA Damage: The Silent Source of Breast Cancer?. *Journal of National Cancer Institute* 2003; 95(2): 100-102.
17. Schnitt SJ: Benign breast disease and breast cancer risk: morphology and beyond. Am J Surg Pathol 2003; 27:836.
18. Clemons M, Loijens L, Goss P: Breast cancer reisk following irradiation for Hodgkin's disease. Cancer Treat Rev; 2000. 26: 291.
19. A J Sasco. Tamoxifen, a human carcinogen or the share of common risk factors?. *European Journal of Cancer* 1998; 34(4): S43-S44
20. Collaborative Group on Hormonal factors in breast cancer: familial breast cancer: collaborative reanalysis of individual data from 52 epidemiological studies including 58,209 women with breast cancer and 101,986 women without the disease. Lancet 2001. 358: 1389.
21. Adem C, Reynolds C, Soderberg CL et al. Pathologic characteristics of breast parenchyma in patients with hereditary breast carcinoma, including BRCA1 and BRCA2 mutation carriers. Cancer 2003, 97: 1-11.
22. Ashton Ma, Lefkowitz M, Tavassoli FA. Epitheliod stromal cells in lymphocytic mastitis. A source of confusion with invasive carcinoma. Mod Pathol 1994, 7: 49-54.

23. Greenwalt DE, Johnson VG, Kuhajda FP et al. Localization of a membrane glycoprotein in benign fibrocystic disease and infiltrating duct carcinoma of the human breast with the use of a monoclonal antibody to guinea pig milk fat globule membrane. Am J Pathol 1985, 118: 351-359.
24. Ellis IO, Elston CW, Goulding H et al (1998). Miscellaneous benign lesions. The Breast CW. Elston and IO Ellis (Ed) 1st edition. Churchill Livingstone, Edinburgh. Pg: 224
25. Nobukawa B, Fujii H, Hirai S, Kumasaka T, Shimizu H, Matsumoto T et al. Breast carcinoma diverging to aberrant melanocytic differentiation: a case report with histopathologic and loss of heterozygosity analyses.. *American Journal of Surgical Pathology* 1999; 23(10): 1280-7
26. Gamallo C, Palacios J, Suarez A, Pizarro A, Navarro. Correlation of E-cadherin expression with differentiation grade and histological type in breast carcinoma. *American Journal of Pathology* 1993; 23(10): 1280-7.
27. Venable AJ, Schwartz AM, Silverberg SG. Infiltrating cribriform carcinoma of the breast: a distinct clinicopathologic entity. *Human Pathology* 1990; 21(): 333-338.

28. Sapino A, Frigerio A, Peterse JL, Arisio R, Coluccia C, Bussolati G. Expression of apocrine differentiation in neuroendocrine breast carcinomas of aged women. *Modern Pathology* 2001; 14(): 768-776.
29. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th Edition of the AJCC Cancer Staging Manual and the Future of TNM. Ann Surg Oncol (2010) 17:1471–1474
30. Kang Y: New tricks against an old foe: molecular dissection of metastasis tissue troism in breast cancer. Breast Dis 26: 129, 2006.
31. Luck AA et al: The influence of basal phenotype on the metastatic pattern of breast cancer. Clin Oncol 2008. 20: 40.
32. Fitzgibbons L, Page DL, Weaver D, Thor AD, Allred DC, Clark GM et al. Prognostic factors in breast cancer cancer. College of American Pathologists Consensus Statement 1999. *Archives of Pathology and Laboratory Medicine* 1999; 124(966-978): 768-76.
33. Cserni G. Evaluation of sentinel lymph nodes in breast cancer. Histopathology 2005, 46: 697-663.
34. Diaz LK, Hunt K, Ames F et al. Histological localization of sentinel lymph node metastases in breast cancer. Am J Surg Pathol 2003, 27: 385-389.

35. Levine PH, Veneroso C: The epidemiology of inflammatory breast cancer. Semin Oncol 2008. 26: 814.
36. Pinder SE, Murray S, Ellis IO et al. The importance of histologic grade of invasive breast carcinomas and response to chemotherapy.*Cancer* 1998; 83(): 1529-39.
37. Harris L, Fritsche H, Mennel R. American society of clinical oncology 2007 update of recommendations for the use of tumour markers in breast cancer. *Journal Of Clinical Oncology* 2007; 25(33): 5287-312.
38. Allred DC, Harvey JM, Berardo M, et al. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol 1998; 11: 155-68.
39. Rhodes A, Jasani B, Balaton AJ, et al. Frequency of oestrogen and progesterone receptor positivity by immunohistochemical analysis in 7016 breast carcinomas: correlation with patient age, assay sensitivity, threshold value, and mammographic screening. J Clin pathol 2000; 53:688-96.
40. Goldstein NS, Ferkowicz M, Odish E, et al. Minimum formalin fixation time for consistent estrogen receptor immunohistochemical

staining of invasive breast carcinoma. American Journal of Clinical Pathology 2003; 120: 86-92.

41. Cheang MC, Treaba DO, Speers CH, et al. Immunohistochemical detection using the new rabbit monoclonal antibody SP1 of estrogen receptor in breast cancer is superior to mouse monoclonal antibody ID5 in predicting survival. Journal of Clinical Oncology 2006; 24: 5637-5644

42. Harvey JM, Clark GM, Osbourne CK and Allred DC. Estrogen receptor status by immunohistochemistry is superior to ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. Journal Of Clinical Oncology 1999; 17(5): 1474-81.

43. Gown AM. Current issues in ER and HER2 testing by IHC in breast cancer. Modern Pathology 2008; 21: S8-S15.

44. Jensen EV, Block GE, Smith S et al. Estrogen receptors and breast cancer response to adrenalectomy. National Cancer Institute Monograph. 1971: 34; 55-70.

45. Elkin KB, Weinstein MC, Winer EP, et al. HER-2 testing and trastuzumab therapy for metastatic breast cancer: a cost-effectiveness analysis. J Clin Oncol 2004; 22: 854-863

46. Leake R, Barnes D, Pinder S et al. Immunohistochemical detection of steroid receptors in breast cancer: a working protocol. J Clin Pathol 2000; 53: 634-635.
47. Wolff AC, Hammond MEH, Schwartz IN et al. ASCO/CAP guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol 2007, 25: 118-145.
48. Gusterson BA, Ross DT, Heath VJ et al. Basal cytokeratins and their relationship to the cellular origin and functional classification of breast cancer. Breast Cancer Research 2005, 7: 143-148.
49. Moll R, Franke WW, Schiller DL et al. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell. 1982 Nov;31(1):11-24.
50. Allison KH. Molecular Pathology of Breast Cancer: What a pathologist needs to know. American Journal of Clinical Pathology. 2012; 138: 770-780
51. Choo JR and Nielson TO. Biomarkers for Basal-like breast cancer. Cancers 2010; 2: 1040-1065.
52. Sutton LM, Han JS, Molberg KH et al. Intratumoral expression level of epidermal growth factor receptor and cytokeratin 5/6 is significantly associated with nodal and distant metastases in patients

with basal-like triple-negative breast carcinoma. American Journal of Clinical Pathology 2010; 134: 782-787.

53. Sorlie T, Tibshirani R, Parker J et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc National Academy of Science USA 2003; 100: 8418-23

54. Korsching E, Packeisen J, Agelopoulos K, et al. Cytogenetic alterations and cytokeratin expression patterns in breast cancer: integrating a new model of breast differentiation into cytogenetic pathways of breast carcinogenesis. Lab Investig 2002; 82: 1525-33.

55. Bocker W, Bier B, Freytag G, et al. An immunohistochemical study of the breast using antibodies to basal and luminal keratins, alpha-smooth muscle actin, vimentin, collagen IV and laminin. Part I: normal breast and benign proliferative lesions. Virchows Arch A Pathol Anat Histopathol 1992; 421: 315-22.

56. Lakhani SR, Reis-Filho JS, Fulford L et al. Prediction of BRCA1 status in patients with breast cancer using estrogen receptor and basal phenotype. Clin Cancer Res 2005; 11: 5175-5180.

57. Rakha EA, El-Sayed M, Green AR, et al. Breast cancer with basal differentiation: a proposal for pathology definition based on cytokeratin expression. Histopathology 2007; 50:434-8.

58. Laakso M, Tanner M, Nilsson J et al. Basoluminal carcinoma: a new biologically and prognostically distinct entity between basal and luminal breast cancer. Clin Cancer Res 2006; 12: 4185-91.
59. Van de RM, Perou CM, Tibshirani R et al. Expression of cytokeratins 17 and 5 identifies a group of breast carcinomas with poor clinical outcome. Am J Pathology 2002; 161: 1991-6.
60. De Ruijter TC, veeck J, de Hoon JPJ et al. Characteristics of triple-negative breast cancer. J Cancer Res Clin Oncol. Feb 2011; 137(2): 183–192.
61. Dent R, Trudeau M, Pritichard KI et al. Triple-negative breast cancer: Clinical features and patterns of recurrence. Clin Cancer Res 2007; 13: 4429-4434.
62. Carey LA, Dees EC, Sawyer L et al. The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes. Clin Cancer Res 2007; 13: 2329-2334.
63. Liedtke C, Mazouni C, Hess KR et al. Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. Journal of Clinical Oncology. 2008; 26 (8): 1275-1281.

64. Ambroise M, Ghosh M, VS Mallikarjuna, et al. Immunohistochemical Profile of Breast Cancer Patients at a Tertiary Care Hospital in South India. Asian Pacific J Cancer Prev, 12, 625-629
65. Sarrio D, Rodriguez-Pinilla SM, Hardisson D, et al. Epithelial Mesenchymal Transition in Breast Cancer relates to the Basal-like phenotype. Cancer Res 2008; 68: 989-997.
66. Dolle JM, Daling JR, White E, et al. Risk factors for triple-negative breast cancer in women under the age of 45 years. Cancer Epidemiol Biomarkers Prev 2009; 18:1157-1166.
67. HM, Gaudet M, Ward EM et al. Association of race/ethnicity, socioeconomic status, and breast cancer subtypes in the National Cancer Data Base (2010-2011). Breast Cancer Research and Treatment 2014; 5
68. Clark *et al*. Molecular subtyping of DCIS: heterogeneity of breast cancer reflected in pre-invasive disease. *Br J Cancer* 2011; 104: 120-7.
69. Fulford LG, Reis-Filho JS, Ryder K et al. Basal-like grade II invasive ductal carcinoma of the breast patterns of metastasis and long term survival. Breast Cancer Res 2007; 9: R4

70. Beyer G, Yeh IT. Basal-like breast cancers: More than just triple negatives. T***he FASEB Journal.*** 2008;22:898.18.

71. Nielson TO, Hsu FD, Jensen K et al. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res 2004; 10: 5367-74.

72. Ross JS, Linette GP, Stec J et al. Breast cancer biomarkers and molecular medicine: part II. Expert Rev Mol Diagn 2004; 4: 169-188.

73. Cheang MCU, Voduc D, Bajdik et al. Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-nagtaive phenotype. Clin Cancer Res 2008; 14: 1368-1376.

74. Dairkee SH, Puett L, Hackett AJ. Expression of Basal and Luminal Epithelium-specific keratins in normal, benign and malignant breast tissue. J National Cancer Inst 988; 80: 691-695.

75. Fulford LG, Easton DF, Reis Filho JS et al. Specific morphological features predictive for the basal phenotype in grade 3 invasive ductal carcinoma of the breast. Histopathology 2006. 49: 22-34.

76. Jones C, Ford E, Gillett C et al. Molecular cytogenetic identification of subgroups of grade III invasive breast carcinomas with different clinical outcomes. Clin Cancer Res 2004; 10: 5988-97.

77. Banerjee D, Reis-Filho JS, Ashley S et al. Basal-like breast carcinomas: clinical outcome and response to chemotherapy. J Clin Pathol 2006; 59: 729-35.

78. Abd El-Rehim DM, Ball G, Pinder SL et al. High-throughput protein expression analysis using tissue microarray technology of a large well-characterised series identifies biologically distinct classes of breast cancer confirming recent cDNA expression analyses. Int J Cancer 2005; 116: 340-50.

79. Jumpannen M, Gruvberger-Saal S, Kauraniemi P et al. Basal-like phenotype is not associated with patient survival in estrogen-receptor-negative breast cancers. Clin Cancer Res 2007; 9: R4.

MASTER CHART

Age	Slide no	Histological type of tumour	Size	Grade	LV invasion	Perineural	No of LN	TNM staging	Staging	Margins	ER	PR	Her2NEU	CK 5/6
67	674/09	Infiltrating ductal, NOS	2 cm	2 or 3	No	No	0/11	T1N0M0	IA	No	Negative	Negative	2+	Positive
35	1677/10	Infiltrating ductal, NOS	3	3	No	No	0/10	T2N0M0	IB	No	Negative	Negative	3+	Negative
60	1702/10	Infiltrating ductal, NOS	3	1	Vascular	No	3 out of 8	T2N1M0	IIB	No	Positive	Negative	Strong	Negative
65	988/10	IDC,IPC,Micropapillary	2	2	No	No	1 out of 8	T1N1M0	IA	No	Positive	Positive	3+	Negative
59	4258/10	IDC, Paget's	3.5	1	Vascular	No	o/13	T2N0M0	IIA	No	Positive	Positive	Strong	Negative
49	2909/10	IDC, intraductal papilloma	2.5	2	No	No	7 out of 18	T2N2M0	IIIA	No	Negative	Negative	Strong	Positive
40	4576/10	IDC, skin +	2	1	No	No	1 out of 8	T4b N1M0	IIIB	No	Positive	Positive	Strong	Negative
55	700/10	Micropapillary	4	2	No	No	1 out of 11	T2N1M0	IIB	No	Negative	Negative	3+	Negative
43	1407/10	IDC	6.5	3	No	No	3 out of 8	T2N1M0	IIB	No	Negative	Negative	N/a	Negative
38	254/11	IDC	4	3	No	No	0/17	T2N0M0	IIA	No	Negative	Negative	100%	Negative
43	694/11	IDC w/ DCIS, UDH	1.5	1	No	No	0/18	T1N0M0	IA	No	Positive	Positive	Strong	Negative
56	438/11	IDC	1.8	2	Vascular	No	3 out of 17	T1N1M0	IIA	No	Mod.	Negative	Strong	Negative
55	2600/11	IDC	3.2	2	No	No	2 out of 12	T3N1M0	IIIA	No	Negative	Negative	Strong	Negative
52	2900/11	IDC, skin+	4.5	2	Lymphatic	No	8 out of 21	T4bN2M0	IIIB	No	Negative	Negative	Strong	Negative
53	4116/12	IDC	5	1	No	No	1 out of 17	T3N1M0	IIIA	No	Positive	Positive	Positive	Negative
44	2708/12	IDC	2	2	No	No	0/14	T1N0M0	IA	No	Negative	Negative	Strong	Negative
49	4049/12	IDC	3.5	2	No	No	0/14	T2N0M0	IIA	No	Negative	Negative	Strong	Negative
40	2577/12	IDC, NOS	2.3	2	No	No	8 out of 25	T2N2M0	IIIA	No	Positive	Positive	Strong	Negative
62	4170/12	IDC, nipple+ Skeletal m+	4.5	2	Vascular	No	N/A			Yes	Negative	Negative	Moderate	Negative
66	3279/12	Invasive Papillary Ca	5	1	No	No	0/32	T2N0M0	IIA	No	Negative	Negative	Moderate	Negative
48	807/12	IDC	2.5	2	No	No	N/A	T2N0M0	IIA	No	Weak	Weak	Negative	Negative
44	4265/12	IDC	3.5	2	Vascular	No	0/21	T2N0M0	IIA	No	<10	Negative	Strong	Positive 2
47	3840/13	IDC, NOS	3	2 or 3	Lymphovascular	No	1 out of 19	T2N1M0	IIB	No	Positive	Positive	Strong	Negative

Age	Slide no	Histological type of tumour	Size	Grade	LV invasion	Perineural	No of LN	TNM staging	Staging	Margins	ER	PR	Her2NEU	CK 5/6
64	3675/13	IDC	1	3	Lymphovascular	Yes	5 out of 10	T1N1M0	IA	No	Negative	Negative	Strong	Positive 2
51	1833/13	Metaplastic Ca	5	2	No	No	0/25	T2N0M0	IIA	No	Negative	Negative	Negative	Negative
58	4310/13	IDC	1.9	1	No	No	0/9	T1N0M0	IA	No	Negative	Negative	Positive	Negative
52	3600/13	IDC w/ keratin pearl	9		Yes	No	11 out of 19	T3N2M0	IIIA	No	Negative	Negative	Positive	Positive
40	135/14	IDC, skin involvement +	7.5	2	No	No	N/A	T4b	IIIB	No	Strong	Strong	Positive	Positive 1
42	393/14	ILC	3.5	1	No	No	0/16	T2N0M0	IIA	No	Strong	Strong	Weak	Negative
53	533/14	IDC, Lipid-rich variant	7.5	2	Yes	No	1 out of 19	T3N1M0	IIIA	No	Negative	Negative	Negative	Positive 2
50	1037/14	IDC	1	3	Yes	No	5 out of 5	T1N2M0	IIIA	No, tumor deposits +	Negative	Negative	Positive	Negative
38	1159/14	Apocrine carcinoma	3.5	2	No	No	0/26	T2N0M0	IIA	No	Negative	Negative	Negative	Negative
45	4926/12	IDC, NOS	4.5	2	Yes	No	1 out of 23	T2N1M0	IIB	No	Positive	Positive	Positive	Positive
47	4797/10	IDC w/ lobular component	1	2	Yes	No	1 out of 13	T1N1M0	IIA	No	Positive	Positive	Negative	Positive
59	3646/09	IDC, NOS	6	2	No	No	0/19	T3N0M0	IIIA	No	Negative	Negative	Positive	Negative
55	3739/12	IDC, NOS	2.5	2	No	No	0/40	T2N0M0	IIA	No	Positive	Negative	Positive	Negative
37	653/10	IDC, NOS	6.5	3	Yes	No	2 out of 15	T3N1M0	IIIA	No	Negative	Negative	Negative	Negative
63	4634/10	IDC, skin+	3.5	1	Yes	No	7 out of 12	T2N2M0	IIIA	No	Positive	Positive	Negative	Negative
62	2362/13	IDC, NOS, Skin +	8	2	Yes	No	N/A	T3N0M0	IIIA	No	Positive	Positive	Negative	Negative
63	2958/14	Mixed IDC and mucinous	4	2	Yes	No	1 out of 11	T2N1M0	IIB	No	Positive	Positive	Negative	Negative
50	1037/14	IDC, NOS	1	3	Yes	No	5 out of 5	T1N1M0	IIB	No	Positive	Positive	Negative	Negative
50	770/11	IDC, skin +	12.5	2	No	No	N/A	T4NxM1		Yes	Negative	Negative	Negative	Positive 3+
40	3704/13	IDC	4	2	Yes	No	1 out of 11	T3N1M0	IIIA	No	Negative	Negative	Negative	Positive 1
48	4509/13	IDC, NOS	1.5	1	No	No	0/16	T1N0M0	IA	No	Positive	Positive	Negative	Negative
33	325/14	IDC	1.8	1	Yes	Yes	0/18	T1N0M0	IA	No	Positive	Positive	Positive	Negative