COMPARISON OF CLINICOPATHOLOGICAL PARAMETERS AND MOLECULAR CLASSIFICATION OF BREAST CANCERS.

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

M.D. (PATHOLOGY)

BRANCH - III

INSTITUTE OF PATHOLOGY,

MADRAS MEDICAL COLLEGE,

CHENNAI – 600 003.

THE TAMIL NADU

DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI

APRIL 2015

CERTIFICATE

This is to certify that this Dissertation entitled **"COMPARISON OF CLINICOPATHOLOGICAL PARAMETERS AND MOLECULAR CLASSIFICATION OF BREAST CANCERS"** is the bonafide original work of **Dr.K.INDUMATHI,** in partial fulfillment of the requirement for M.D., (Branch III) in Pathology examination of the Tamilnadu Dr.M.G.R Medical University to be held in April 2015.

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INSTITUTIONAL ETHICS COMMITTEE
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CERTIFICATE OF APPROVAL

TO
Dr. Indumathi.K,
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Institute of Pathology,
Madras Medical college Chennai-3

Dear Dr.Indumathi. K,

The Institutional ethics committee of Madras Medical college,reviewed and discussed your application for approval of the proposal entitled **"Comparison of clinicopathological parameters and molecular classification of breast cancers"** No.24092013

The following members of Ethics Committee were present in the meeting held on 10.09.2013 conducted at madras medical college, Chennai-3

1. Dr. G. Sivakumar, MS FICS FAIS	-Chairperson
2. Prof. R.Nandhini, MD Director, Instt. of Pharmacology MMC, Ch-3	-Member secretary
3. Prof. Shyamraj,MD Director i/c, Instt. of Biochemistry MMC, Ch-3	-Member
4. Prof. P.Karkuzhali,MD Director, Instt. of Pathology MMC, Ch-3	-Member
5. Prof. R.Kalai Selvi , MD Prof of Pharmacology MMC, Ch-3	-Member
6. Prof. Siva Subramanian, MD Director, Instt. of Internal medicine MMC, Ch-3	-Member
7. Thiru. S. Govindasamy, BABL	-Lawyer
8. Tmt. Arnold saulina, MA MSW	-Social Scientist

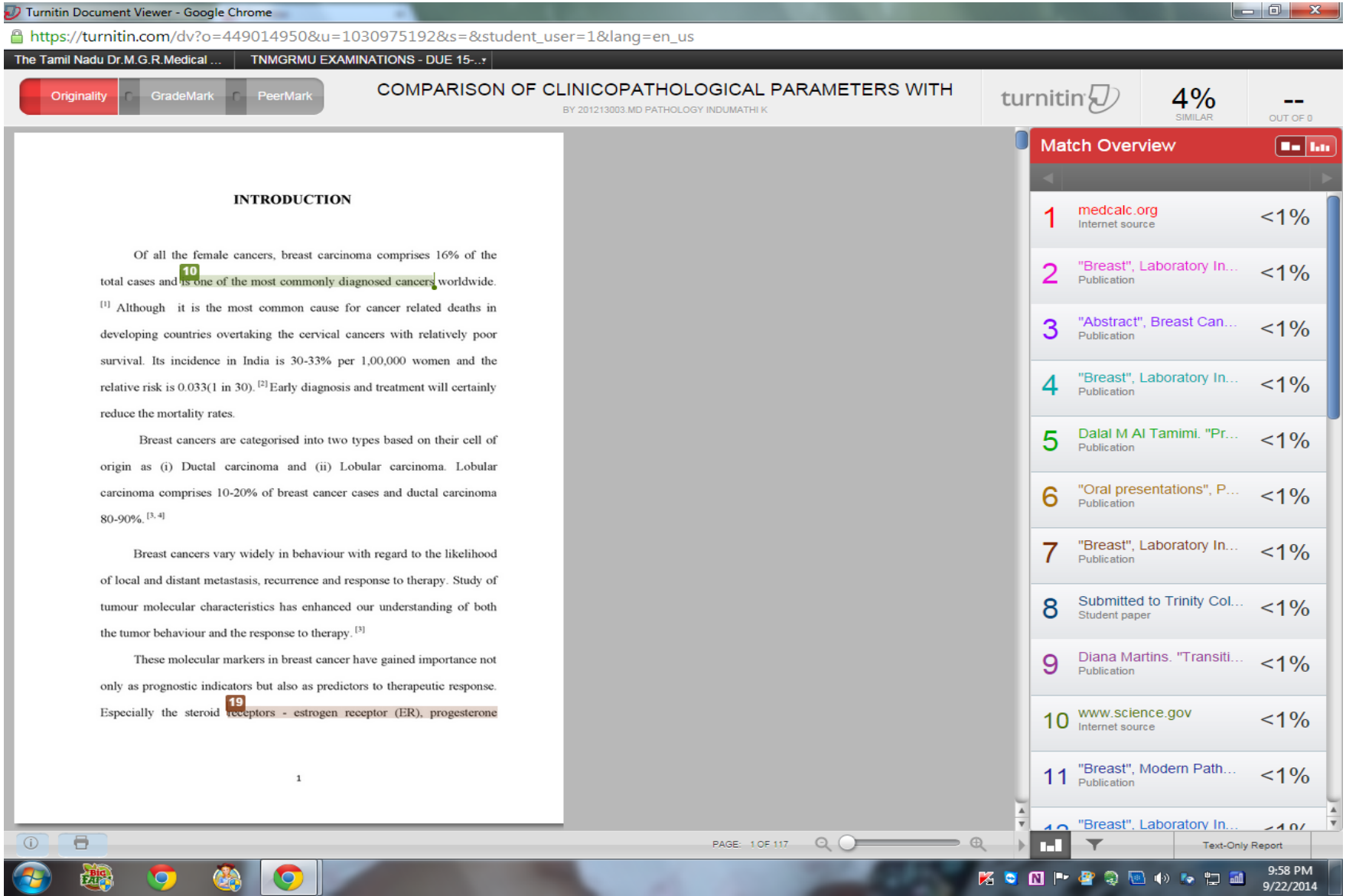
We approve the proposal to be conducted in its presented form.

Sd/ Chairman & Other Members

The Institutional Ethics Commttee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and the patients information/ informed consent and asks to be provided a copy of the final report.

R Nandhini

Member Secretary, Ethics Committee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INTRODUCTION
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Breast cancers are categorised into two types based on their cell of origin as (i) Ductal carcinoma and (ii) Lobular carcinoma. Lobular carcinoma comprises 10-20% of breast cancer cases and ductal carcinoma 80-90%. [3, 4]
Breast cancers vary widely in behaviour with regard to the likelihood of local and distant metastasis, recurrence and response to therapy. Study of tumour molecular characteristics has enhanced our understanding of both the tumor behaviour and the response to therapy. [3]
These molecular markers in breast cancer have gained importance not only as prognostic indicators but also as predictors to therapeutic response. Especially the steroid receptors - estrogen receptor (ER), progesterone
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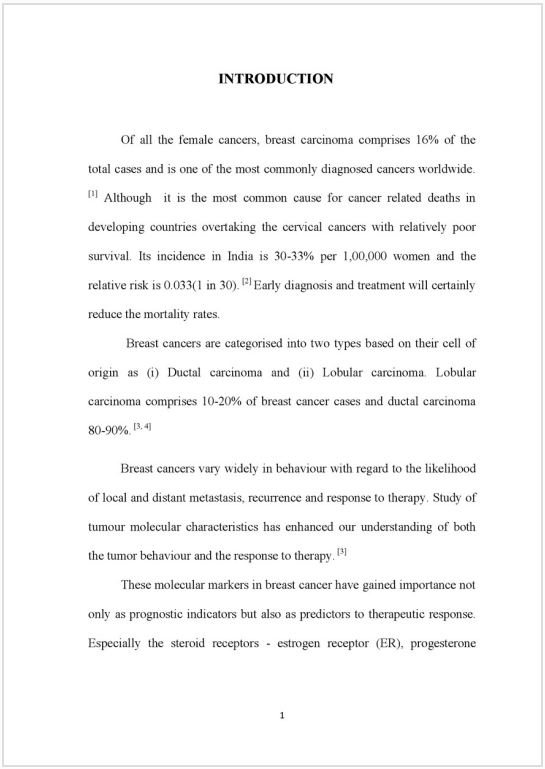

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1

DECLARATION

I **Dr.K.INDUMATHI,** solemnly declare that the dissertation titled **"COMPARISON OF CLINICOPATHOLOGICAL PARAMETERS AND MOLECULAR CLASSIFICATION OF BREAST CANCERS"** is the bonafide work done by me at Institute of Pathology, Madras Medical College under the expert guidance and supervision of **Prof. Dr. M. SARASWATHI, M.D.,** Professor and Director of Institute of Pathology, Madras Medical College. The dissertation is submitted to the Tamilnadu Dr.M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch III) in Pathology.

Place : Chennai

Date : **Dr. K. INDUMATHI**

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Prof. Dr. S. PAPPATHI M.D., D.C.H and all my **Assistant Professors**, my colleagues, technicians and staffs of Institute of Pathology, Madras Medical College for their co-operation and encouragements during my study period.

I sincerely express my heart felt gratitude to my most lovable parents Mr.M. Karnan and Mrs.Rani Karnan, my dear husband Dr. D. Thennarasu and my dear most sons Ezhilamudhan and Adavdeepan, for all their sacrifices, for creating adequate time for me to do my work and balanced my absence with love throughout the study period.

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Above all I thank the ALL MIGHTY, for everything that he has given me.

ABBREVIATIONS

ER	: Estrogen receptor
PR	: Progesterone receptor.
RNA	: Ribonucleic acid
DNA	: Deoxy ribonucleic acid
HER 2	: Human epidermal growth factor receptor 2
CK 5/6	: Cytokeratin 5/6
ICMR	: Indian council of medical research
EGFR	: Epidermal growth factor receptor.
GCDFP	: Gross cystic disease fluid protein.
DCIS	: Ductal carcinoma in situ.
P53	: Protein 53
RT PCR	: Reverse transcriptase polymerase chain Reaction
FISH	: Fluorescent in situ hybridisation
BRCA	: Breast cancer antigen
LHRH	: Luteinizing hormone releasing hormone

CONTENTS

ABSTRACT

COMPARISON OF CLINICOPATHOLOGICAL PARAMETERS AND MOLECULAR CLASSIFICATION OF BREAST CANCERS.

AIM:

This is a retrospective study of 60 cases, to detect the expression of ER, PR, HER2neu, CK5/6 and Ki67 proliferation index in breast carcinomas by immunohistochemical method and to determine the newer molecular classification. Few patients have recurrence inspite of being diagnosed under the category of low risk and few do well in the high risk group which can be attributed to the molecular level differentiation. The aim of this study to classify the patients under molecular classification, to compare the clinicopathological parameters with it and to denote the significance of targeted therapy.

MATERIALS AND METHODS:

This is a retrospective study of detecting the expression of the above said markers in modified radical mastectomy specimens received at the Institute of Pathology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai during the period from January 2011 to June 2013. 4 microns thick sections of the paraffin tissue blocks were Hematoxylin and eosin stained and reported as Infiltrating ductal carcinoma no special type (IDC NST) and its special variants like medullary, papillary, metaplastic, lobular, mucinous and apocrine carcinoma. A total of 60 cases which included 30 of IDC NST and 30 cases of special variants were selected for immunohistochemical analysis.

RESULTS:

Out of the 60 cases studied, the most common was found to be the luminal A type comprising 37% and the least common was the luminal B and hybrid types each comprising 8%. The most common grade for HER2 was Grade III (50%). The association of histological grade with the molecular classification was statistically significant with the p value of 0.01. Basal

type (56%) had the highest incidence of N3 stage.ER, PR, HER2 neu, CK5/6 expression and proliferation index with Ki67 had a statistically significant association with the molecular classification. High proliferation index (>14%) with Ki67 was noted in Luminal B, Basal and Hybrid types. 78% of the total 60 cases were alive and healthy. One death was reported in HER2, Hybrid and Basal types. The negative kappa value obtained while studying the agreement between the histopathological and molecular classification, indicates that the agreement is worse than chance and hence the importance of molecular classification is substantiated for the targeted therapy.

CONCLUSION:

To conclude, breast cancers are heterogenous and having diverse clinical outcomes, these researches on molecular subgroups would pave way towards the "personalisation" of treatment for breast cancers with the more feasible and economic tool of immunohistochemistry.

KEY WORDS: Molecular Classification, Histopathological Classification, immunohistochemistry with ER, PR, HER2 neu, CK 5/6 and Ki67, Targeted Therapy.

INTRODUCTION

Of all the female cancers, breast carcinoma comprises 16% of the total cases and is one of the most commonly diagnosed cancers worldwide. [1] Although it is the most common cause for cancer related deaths in developing countries overtaking the cervical cancers with relatively poor survival. Its incidence in India is 30-33% per 1,00,000 women and the relative risk is 0.033(1 in 30). [2] Early diagnosis and treatment will certainly reduce the mortality rates.

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Breast cancers vary widely in behaviour with regard to thelikelihood of local and distant metastasis, recurrence and response to therapy. Study of tumour molecular characteristics has enhanced our understanding of both the tumor behaviour and the response to therapy. [3]

These molecular markers in breast cancer have gained importance not only as prognostic indicators but also as predictors to therapeutic response.Especially the steroid receptors -estrogen receptor (ER),

progesterone receptor (PR), HER2 neu, CK5/6 and Ki67 have gained increasing interest. [5]

Recent advances in breast pathology that examines the RNA, DNA and proteins of malignant cells have provided an algorithm for the new molecular classification of breast cancers. [5, 6] Based on the gene expression profiling, five major patterns of gene expression has been identified: Luminal A, Luminal B, HER2 type, Basal and unclassified types. [5, 6]

This classification correlates wellwith the prognosis and response to treatment. The most favourable long term disease free survival is seen with Luminal A type tumors. However the tumors most sensitive to chemotherapy are the basal type and HER2 subtypes but the overall prognosis of these tumors worst. [7, 8]

In this study of 60 cases which included invasive ductal carcinoma no special type(IDC NOS) and its special variants, an attempt has been made to evaluate the hormonal status and proliferation index by immunohistochemistry. Further the histological grade and other prognostic factors were correlated.

AIMS AND OBJECTIVES

1. To identify the relative frequency and distribution of breastcarcinoma in the population.
2. To study the histomorphological features of breast carcinomaincluding grade, lymph node status, lymphovascular invasion,lymphocytic response and necrosis.
3. To assess the expression of ER, PR, HER 2 neu, CK5/6 and Ki67 in invasive breast carcinomas.
4. To subtype the breast cancers based on their expression of these markers as Luminal type A, Luminal type B, HER2 neu type and Basal type.
5. To compare the clinicopathological parameters and molecular classification of breast carcinoma.
6. To assess the correlation between histopathological classification and molecular classification.
7. To denote the significance of molecular classification in the treatment of the patients.

REVIEW OF LITERATURE

Invasive breast carcinomas aremalignant duct epithelial tumours which exhibit invasion of the adjacent tissues, with an increased tendency for distant metastasis. [9] Breast carcinoma is one of the cancers commonly described in ancient documents due to its visibility. [10]

The oldest description of breast cancers was given in 1600 BC, by Edwin Smith Papyrus. [10, 11]The first documented case of breast cancer was described by Imhotep in 2650 BC. [12]

Leonides (30 AD) compared cancers to crabs, due to the tenacious adherence to the surrounding tissues. [13] In 1874, Paget described the changes in the nipple that preceded breast cancer and it continues to bear his name. [14]

Radical mastectomy was first performed by William Stewart Halstedin 1882. [15] X-rays were discovered by Wilhelm Conrad Röntgen in1895 and it forms the basis for mammogram and radiotherapy. [16, 17]

In 1925, Greenhough was the first to evaluate grading system for breast cancer.[18] In 1928, Scarff et al proposed tubule formation, nuclear pleomorphism and hyperchromasia as criteria to grade breast cancers. [19] In 1957, Bloom and Richardson proposed the numeric scoring system based on tubule formation, nuclear pleomorphism and mitosis for gradingadapted by WHO.[20,21]In 1983, Bloodgood et al recognized ductal carcinoma in situ

where neoplastic cells are limited within the terminal duct lobular unit.[22,23] Early 1990, Nottingham modification of Bloom Richardson grading system was adapted by WHO.[24, 25]

EPIDEMIOLOGY

According to the 2001-03 ICMR report, breast cancer constitutes about 25% of the total cancers among Indian women. [26, 27, 28]Breast cancers are the second most common in Indian rural women after cervical cancers and it is the most common cancer in metropolitan cities. [29]

In India, the crude incidence rate of breast carcinoma is 85/1,00,000 women/year. [30] The death per incident ratio is highest in India, with 50% compared to 30% in China and 18% in the United States. [31]

The annual age-adjusted rate is 30 to 33 per 1,00,000 in urban women and 8.6 per 1,00,000 in rural women. [32]

India is rapidly stepping towards industrialization resulting in lifestyle changes. This probably contributes to the increase in breast cancer incidence in our country.

The presenting symptoms include breast lump, nipple discharge, retraction or eczema. Screening for breast abnormalities are done by the triple assessment which includes clinical examination, imaging and tissue sampling.

RISK FACTORS:

Many risk factors are implicated in the development of breast cancers.Some studies have proposed that the common denominator for most of these factors is strong and prolonged estrogen stimulation that takes place on a genetically susceptible background. [33] The strongly associated risk factors are family history of first degree relative[34] with breast carcinoma, early menarche, nulliparity, late age at first child birth, [35] late menopause, sedentary life style with high calorie diet, obesity, long term exposure to hormone replacement therapy with estrogen alone, [36,37] oral contraceptive pills[38] and ionising radiation. [39, 40]

ETIOLOGY:

The two main etiological factors involved in breast carcinoma are hormone excess and genetic predisposition.

Estrogen and breast cancer

The main function of estrogen is stimulation of cell growth and proliferation by acting via estrogen receptor (ER) as a transcriptional activator. [41] However, this process is slow. Recently, a non genomic pathway has been demonstrated which does not involve ER, but acts through a G-protein coupled receptor, GPR30. These result in activation of metalloproteinases and cleavage of heparin - bound epidermal growth

factor. The released EGF then acts on its receptor, EGFR and stimulates cell proliferation.[42] The existence of this pathway indicates that drugs acting only through ER may not be enough to inhibit tumour growth.

Genes involved in Breast Cancer

Hereditary breast cancers are about 5% to 10%. BRCA1 and BRCA2 are the major genes involved in hereditarybreast cancer. The BRCA1 gene is present on chromosome 17q and itsproduct is responsible for DNA repair. The increased risk of occurrences of breast cancer, ovarian cancer and pancreatic cancer are associated with these mutations. [43, 44]Patients with BRCA2 gene mutations present on chromosome 13q have increased risk of male breast cancers, prostatic cancers,pancreatic cancers and cutaneous melanomas.[45, 46] Invasive ductal carcinoma is a group of breast carcinoma in which the stromal invasion of malignant cells is evident beyond the epithelial component. Current histomorphological subtyping of breast carcinoma is based on World Health Organisation classification (Annexure II).

BREAST CANCER CLASSIFICATION.

Histopathological classification:

The breast is composed of groups of lobules and divided into 12 to 25 lobes. Acini are grape like clusters of glands which comprise the lobule and secrete the breast milk. Milk is delivered to nipple by thin tubular structures connecting the lobules. (Fig.1). Fatty and connective tissue occupies the remaining space. Ductal carcinoma arises from the ductal epithelial cells and lobular carcinoma from the lobes and lobules.

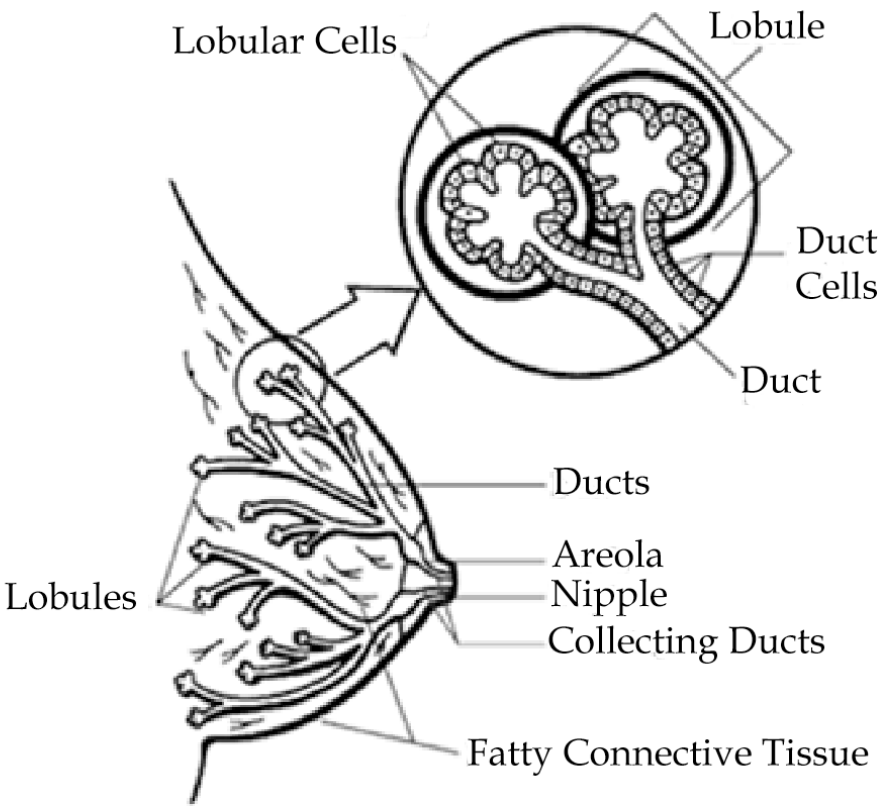


Fig 1: Anatomy of female breast.

Rarely, breast cancers can occurin fat, muscles and blood vessels which form the connective tissue stroma. Those cancers are called sarcoma and they comprise less than 1% of breast cancer and less than 5% of all soft tissue sarcomas. [47] Breast cancers are also classified based on the invasiveness as (i)invasive (infiltrating) cancers and (ii) non-invasive (in situ) carcinomas. Breast cancers with generalised inflammation of the breast are called the inflammatory breast cancer which is another rare type. The frequency and 10 year survival of various histological types of invasive ductal carcinomas.(Table 1).

Table 1: The frequency and 10 year survival of histopathologicalsubtypes of invasive ductal carcinoma.

S.NO	HISTOPATHOLOGICAL TYPE OF INVASIVE BREAST CANCER	FREQUENCY (%)	10 YEAR SURVIVAL (%)
1	IDC NOS	50-60	35-50
2	Inflammatory carcinoma	1-6	30-40
3	Apocrine carcinoma	1-4	LIKE IDC NOS
4	Medullary carcinoma	5-7	50-90
5	Metaplastic carcinoma	<5	Unknown
6	Mucinous carcinoma	<3	85-95
7	Papillary carcinoma	1-2	Unknown
8	Tubular carcinoma	1-2	90-100
9	Invasive lobular carcinoma	5-15	35-50
10	Adenoid cystic carcinoma	0.1	85-100
11	Neuroendocrine carcinoma	2-5	Unknown

INVASIVE DUCTAL CARCINOMA NOSPECIAL TYPE (IDC NST)

IDC NST accounts for 75-80% of breast cancers and is the most common type. [48] These tumors lack sufficient characteristics to be classified as a specific histological type as tubular or mucinous carcinoma. These tumours elicit a marked fibroblastic stromal reaction and produce a firm palpable mass. It may produce dimpling of the skin due to traction on the suspensory ligaments.

Grossly, the tumour is ill defined, firm, with a yellow grey cut surface, with infiltrating borders as radiating trabeculae into the surrounding parenchyma, resulting in a stellate appearance. Histologically, the tumour cells are arranged in a variety of patterns such as acinar configurations, cords and broad sheets of cells, with surrounding dense stroma. The tumours show a wide range of differentiation with poorly differentiated tumours showing solid sheets of pleomorphic cells. These tumours are graded using Nottingham modification of Scarff Bloom Richardson system (Annexure III).

MEDULLARY CARCINOMA

It is common in patients under 50 years of age, particularly associated with BRCA1 mutations carriers. [49] Grossly, the tumour is well circumscribed and becomes larger and resembles fibroadenoma clinically and grossly. Its cut surface is homogeneous, solid and grey with occasional foci of necrosis. Rarely do they present as cystic masses. Microscopicallythe

tumor borders are always of pushing margins. They show a diffuse pattern of growth with minimal or no glandular differentiation or intraductal growth component and absence of mucin production. The cells are arranged in solid sheets of large pleomorphic cells with prominent nucleoli, forming a syncytium. The tumour has scant fibrous stroma and frequent mitotic figures. Numerous lymphocytes surround the sheets of tumour cells with most of them being cytotoxic T lymphocytes.[50] They typically express CK7, often express vimentin , S-100 and P53, but not CK20.[51] They are almost invariably negative for hormone receptors as well as HER2 neu ('triple negative' phenotype).Axillary nodal metastasis are common and overall prognosis is better than the invasive ductal carcinoma especially for tumors less than 3 cm in size despite the nodal metastasis.

MUCINOUS CARCINOMA

It commonly occurs in postmenopausal women. It is also known as colloid, mucoidor gelatinous carcinoma. Grossly, it is well circumscribed with a glistening jelly like mass held together by delicate septa. Microscopically, the tumour cells form small clusters and appear to float in a sea of mucin. These clusters may be solid, exhibit acinar formations or form micropapillary structures.[52]Mucin is almost always extracellular and it may be of acid or neutral type.[53]Histochemically, the mucins secreted by this tumour are distinct O-acylated forms of sialomucins.[54]

Immunohistochemically, there is strong MUC2 cytoplasmic

immunoreactivity and decreased MUC1 immunoreactivity compared with ductal carcinoma NOS.[55] Hormone receptors are always positive, while HER2 neu is almost always negative.[56]Nearly half of the cases of mucinous carcinoma show neuroendocrine differentiation light microscopy showing nests of cells with salt and pepper chromatin expressing neuroendocrine markers as neuron specific enolase, chromogranin and synaptophysin and presence of dense core granules by ultrastructural examination. Pure mucinous carcinomas are those in which the mucin occupies more than 90% of the tumor component and mixed mucinous carcinomas show 50% of mucin with 50% of tumor cells.

APOCRINE CARCINOMA

These tumors comprise 1- 4% of the breast cancers and is an uncommon type. More than 90% of the tumour is composed of apocrine cells.[57]There are two types of apocrine cells – Type A cells have abundant acidophilic granular cytoplasm which contains golden brown granules which are strongly Periodic acid schiff positive and Type B cells have clear, foamy cytoplasm. The nuclei are vesicular and nucleoli are prominent. Glandular differentiation is often found and associated with the characteristic bulbous expansion in the luminal side (Apocrine snouts). Ultra structurally the cells of apocrine carcinoma are rich in mitochondria and membrane bound vesicles with dense homogenous osmophilic cores. It is negative for bcl2, PR and ER. It expresses GCDFP 15.

METAPLASTIC CARCINOMA

This is an uncommon variant of ductal carcinoma, in which the predominant component of the tumor has an appearance other than epithelial and glandular type and more in keeping with another cell type. [58]

It is a rare neoplasm. [59] Tumors that have overt carcinomas with direct transition to osseous or cartilaginous matrix are referred to as "matrix producing carcinomas". [60, 61] The neoplasm is heterogeneous showing areas of spindle, squamous, osteoclast type of giant cells, choriocarcinomatous elements, melanoma like or mesenchymal differentiation ranging from osseous and chondroid differentiation to frank sarcoma. Grossly, they present as well delineated, firm, pearly white sometimes bluish and glistening mass representing cartilaginous areas. Overall, they are more aggressive than IDC NOS.

TUBULAR CARCINOMA

It commonly occurs in patients around 50 years of age. Grossly, it is characteristically small, measuring about 1cm with poorly circumscribed margins and hard consistency. Microscopically, it is characterised by the haphazard arrangement of well differentiated, irregular and angulated glands in a desmoplastic stroma with the lining cells being small and regular. Periphery often shows invasion of fat. Necrosis, mitosis and pleomorphism are characteristically absent. Low-grade DCIS and flat epithelial atypia are thought to be precursor lesions of tubular carcinoma.[62, 63]

CRIBRIFORM CARCINOMA

These tumours accounts for 0.8 to 3.5% of breast carcinomas. Histologically, more than 90% of tumour shows cells arranged resulting in a cribriform- sieve like pattern similar to that seen in the in situ counterpart but with stromal invasion. This pattern is often seen in association with tubule formation, the relative proportion of the two elements determines the terminology used. [64]

INVASIVE PAPILLARY CARCINOMA

These tumours accounts for less than 1 to 2 % of breast carcinoma. Frequently occurs in post-menopausal women. Most papillary carcinomas of breast are predominantly or entirely in situ lesions. Fischer et al first reported that invasive papillary carcinoma is grossly circumscribed. Microscopically, the invasive papillary carcinomas may be of papillary type or ordinary ductal type.The cells are arranged as delicate or blunt papillae with amphophilic cytoplasm. Many a times the intra cystic papillary carcinomas are invasive ones with the cystic component and pushing margins. Absence of myoepithelial cells helps in arriving at the diagnosis. Prognosis is better than IDC NOS.

INVASIVE MICROPAPILLARY CARCINOMA

They account for less than 2% of the breast cancers and are highly invasive tumours. They are characterised by formation of papillary and pseudo papillary structures lacking a fibro vascular core. They show a high nuclear grade and half of the cases show psammoma bodies. [65]

NEUROENDOCRINE CARCINOMA

They constitute about 5% of all breast carcinomas. It comprises carcinoid tumours, large cell neuroendocrine carcinomas and small cell carcinomas. The term "carcinoid" was proposed for those invasive tumors that exhibit neuroendocrine differentiation. [66]Multicentricity and bilaterality can occur.[66]Microscopically, the neoplastic cells are small, arranged in solid nests separated by fibrous stroma. Ribbons and rosette like formations may be seen. Mitoses are generally rare. They express neuroendocrine markers in more than 50% of the tumour cell and this feature helps to distinguish them from breast carcinoma with focal endocrine differentiation.[67]

INVASIVE LOBULAR CARCINOMA

Lobular carcinoma is the second commonest type of breast cancer accounting for 10% of the cases. The tumour is more often bilateral and multicentric. The amount of stromal reaction varies from scanty to dense desmoplasia and therefore it may present as a discrete mass or diffuse indurated area. The stroma often shows periductal and

perivenouselastosis.The tumour cells are small, uniform and bland looking often arranged in Indian file pattern or may form concentric arrays around ducts resulting in targetoid pattern. 10% of cases show mixed features of invasive ductal and lobular carcinomas. It is characterised by the presence of High molecular weight keratin, lack of accumulation of p53, and most importantly, decrease or absence of E-Cadherin. [68, 69, 70]

INFLAMMATORY CARCINOMA

In this type of breast cancer, the entire breast is reddened, warm with widespread oedema of the skin. Pathologically, it revealed an undifferentiated carcinoma with widespread lymphatic permeation. Presence of dermal lymphatic invasion that is confirmed by a skin biopsy, even though clinically not apparent, is an ominous sign. Such cases are called occult inflammatory carcinoma.[72]

MOLECULAR CLASSIFICATION

Breast cancer is a pathologically and clinically heterogeneous disease. It has been a tradition that the details regarding the status of the patient which includes the tumor size, histological grade, histological type and nodal status were provided to the oncologists by the pathologists. Later on along with this the hormonal status were also evaluated and the breast tumors were broadly categorised as low risk (ER positive,nodes negative,

tumor size<1 cm and low grade) and high risk (ER negative, nodes positive, tumor size>1 cm and high grade) tumors.

Approximately 15% of the patients end up with recurrence or die due to metastasis even among the low risk category, despite the best treatment and paradoxically 15% of the patients of high risk category have a favourable prognosis. So eventually approximately 15% of patients are put under mistreatment because of this system of classification of high and low risk group.

Morphologically, similar tumours may show difference in prognosis and response to treatment. It is proposed that these molecular differences between the tumors of similar histology exhibits different clinical behaviour. Modern techniques like Immunohistochemistry (IHC), DNA microarray technology, Quantitative RT-PCR and FISH are ideally suitable to reveal the molecular differences between the same or different groups of histopathological specimens. [1]

IHC was discovered 30 years before and it is used as tool to classify tumors of breast into ER positive and negative tumors. FISH which was developed 20 years ago is used for the classification of breast tumors into HER2 amplified or HER2 non-amplified categories. [2, 3]

Breast tumor cells generally overexpress hormone receptors as well as human epidermal growth factor-2 (HER2 neu) receptor for breast carcinoma formation and its progression. Therefore, based on the hormone

receptor expression, three groups of breast carcinomas were identified as (i) ER/PR positive (ii) ER/PR negative andHER2 positive and (iii) triple negative (ER, PR and HER2 negative).

The classification of breast carcinoma on the basis of hormone receptor status improves the clinical outcome and prognosis of ER positive tumours as ER positive cancer cells depends on estrogen for its growth and the treatment of those patients with anti-estrogenic agents (e.g. Tamoxifen) will inhibit its proliferation and control its progression. Generally, HER-2 positive tumors had a worse prognosis, in spite of HER-2 positivetumors showing a good response to the monoclonal antibody Transtuzumab. When conventional chemotherapy is combined with transtuzumabthe clinical outcome and pathological complete response to therapy has improved significantly. [2]

Sorlie et al and Perou et al in the earlier parts of the decade demonstrated the "heat maps" generated by the microarray technique in which the "clustering analysis" technique was used to find the patterns of expression of 426 genes. [7]. The study of expression of these genes, lead to the subclassification of breast tumors which are similar to those of histologically and immunohistochemically classified tumor. For example luminal A category corresponds to the low risk group and luminal B with those to the HER 2 positive group. By demonstrating the positivity for ckit,

p63, EGFR, and CK 5/6, basal like tumors are distinguished from other subtypes. [76].

Immunohistochemistry has become surrogate simplified advancement for DNA microarray gene expression classification. This tool has become a reliable method to identify the major molecular subtypes. This technique is an easy, reproducible and an economically feasible alternative for the analysis of prognostic factors. [72]

Based on the gene expression profiling the latest molecular classification segregates breast cancer into four types (i) luminal, (ii) basal, (iii) HER2 and (iv) normal /unclassified type[7, 74, 75] (Fig. 2). Breast cancer patients are broadly classified into two main categories based on the ER status as analysed by the hierarchical cluster analysis generated by using gene profile data. ER positive tumours (luminal type) are further classified into Luminal A which does not express HER2 and has a low proliferation index whereas Luminal B express HER2 along with ER and low molecular weight keratins. ER negative tumours are further classified into HER2 type which shows strong positivity for HER2 whereas Basal type which are negative for all three markers. High proliferation is noted in Luminal B, HER2 and Basal types. [74, 75]

Luminal A tumors are those which are ER strong positive, PR variable positive, and negative for HER2, EGFR and CK5/6. It is the most frequent subtype and itshows a good prognosis and responds well to

hormonetherapy. Several studies have reported that ER+ tumors show little response to conventional chemotherapy.

Identification of luminal B tumors is a point of controversy when they are identified at the protein level. Some authors have found that the HER 2 associated genes are expressed in 35-50% of luminal tumors. However, tumors under this subtype have a worse prognosis than Luminal A tumors. This tumor requires deprivation of estrogen in combination of transtuzumab that blocks HER2.

The HER2+ subtypes are tumors which express only HER2 neu, and these tumors often has high proliferation index. When tumors express strong copositivity of hormone receptors and HER2 neu they are categorised into the separate hybrid type called the "luminal–HER2 hybrids." [72]

Cheang *et al*. stated that the hallmark of luminal B tumors was a Ki67 proliferation index of more than 13.25%. Ki67 is demonstrated in the proliferating cell population, and its expression with nuclear positivity is directly proportional to the aggressive nature of the tumor. [72]

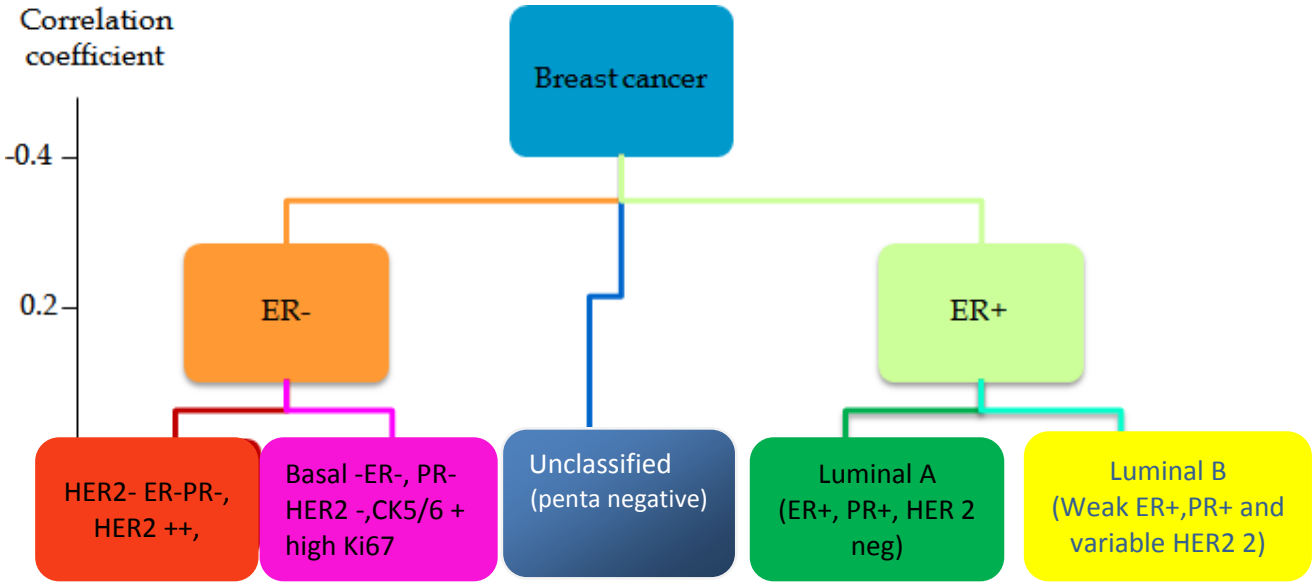
HER2+ tumors are those which are ER and PR negative, HER2 positive, and CK5/6 negative with high proliferation index. Overexpression of HER2 implies a poor prognosis. It demonstrates the highest sensitivity to neoadjuvantchemotherapy based on taxanes and anthracyclins.The poor prognosis of HER2 is due to its high riskof early relapse.[72]

Basal- like tumors aretriple negative with CK5/6 and/orEGFR positive, and with high proliferation index.The basal type is so named because of its pattern of expression that is similar to basal epithelial and myoepithelial cells of breast tissue.Using IHC, this class has also been called “triple negative”for not expressing ER, PR, or HER2. It has been associated with the BRCA1mutation [8, 4]. Ribeiro *et al*. demonstrated that normal luminal cells express CK5/6 and these cells originate as the basal phenotype of breast cancer and undergo malignant transformation. Low regulation of BRCA1leads to an abnormal proliferationof these cells by stimulation of the p53 expression. [76] These tumors are very aggressive, with high grade, and p53 mutation. [76]Several studies have demonstrated the poor outcome of this class. And it is still not clear if this poor outcome is due to a lack of therapeutic options or due to an inherent aggressiveness of the tumor. For being triple negative, they do not benefit the conventional targeted therapy. However, they respond with high sensitivity to chemotherapy.With regard to the targetedtherapeutic options, some trials suggest that basal class tumors can be managed with the monoclonal antibodies against the epidermal growth factor. [72, 76]

Unclassified (pentanegative) tumors are hormone receptors negative,HER2 negative, and CK5/6 and EGFR negative. Those triple negative tumors which lack the expression of the basal markers correspond to the unclassified tumors. Normal like tumors were earlier considered

synonyms to this subtype but currently the "normal- like" subtype is different from the unclassified (penta −ve) "ER−, PR−, HER2−, and CK5/6 and EGFR−" group, as the concept of reduced expression of biomarkers is not a consistent finding of normal like cancers. [72] The unclassified type has a good prognosis and 6% of pathologic complete remission rate. [72, 74, 76]

Fig.2. Dendrogram of molecular classification of breast cancers based on immunohistochemistry:



Molecular subclasses show great difference in clinical outcome as per relapse free survival (RFS) and overall survival (OS) is concerned as shown

in Table 2. Among the subtypes, basal and HER2 are associated with worse outcome and shortest survival time [1].

Table 2: Molecular subtypes and its outcomes.

MOLECULAR TYPE OF BREAST CANCER	FREQUENCY (%)	5 YEAR OS (%)	5 YEAR RFS (%)	10 YEAR OS (%)	10 YEAR RFS (%)
LUMINAL A	50-60	85-95	80-90	75-85	75-85
LUMNAL B	5-10	70-80	65-75	55-65	54-64
BASAL	10-20	63-73	60-70	57-67	45-55
HER 2	10-20	55-65	15-20	45-55	15-30
NORMAL-LIKE	10-15	84-94	80-90	75-85	72-82

RFS: This is the percentage of patients who are symptom free during the period between the day of breast surgery and the date of second episode of breast carcinoma.
OS: This is the percentage of patients who survived during the period between the day of breast surgery and date of death related or unrelated to breast cancer.

PROGNOSTIC FACTORS

Patient's age:

Women younger than 50 years of age have the best prognosis. Relative survival declines after 50 years.

Size:

The tumour size shows a good correlation with the nodal status and survival rate.[78, 79]

Cytoarchitectural type:

There is no significant prognostic difference between ordinary invasive ductal and invasive lobular carcinoma.[80] Morphologic variants of invasive ductal carcinoma with a more favourable prognosis are tubular carcinoma, mucinous carcinoma, medullary carcinoma, cribriform carcinoma, adenoid cystic carcinoma, papillary carcinoma, and secretory carcinoma.Metaplastic carcinoma,Squamous cell carcinoma, invasive carcinoma with neuroendocrine features and signet ring cell carcinoma behave in an aggressive way. [81]

Microscopic grade:

Tumours are graded based on Nottingham modification of the Scarff Bloom–Richardson system (Annexure III). Ellis et al reported this grading system to have excellent correlation with patients' survival and rate of metastasis. [24]

Axillary node metastases:

Metastasis to axillary nodes has a significant impact on prognosis. There is a marked difference in survival between patients with positive and negative nodes and the survival rate also varies depending on the level of axillary node involved, their absolute number, the amount of tumour cells in the node, [82] the presence or absence of tumor cells in the efferent blood vessels and presence or absence of extranodal spread.

Other factors reported to have poor prognosis include tumour necrosis, lymphocytic infiltration and skin infiltration, association with pregnancy, lactation,[83] BRCA mutation, [84] vimentin and keratin expression. [85]

IMMUNOHISTOCHEMISTRY (IHC)

IHC is a molecular technique which was first described by Dr.Albert Coons in 1941. The original method consisted of an antibody developed in rabbits and then tagged with a fluorescent probe. It was mixed with tissue sections and examined using a fluorescent microscope after a period of incubation. Since then, numerous advancements have been made. [86]The most commonly used techniques are the peroxidase -antiperoxidase immune complex method developed by Sternberger in 1970 and the biotin-avidinimmunoenzymatic technique developed by Heitzman and Richards in 1974. [87, 88]

USES OF IMMUNOHISTOCHEMISTRY IN BREASTPATHOLOGY[89, 90]

1. The use of myoepithelial markers to assess stromal invasion.
2. E Catherin to differentiate between ductal and lobular carcinoma.
3. High molecular weight cytokeratins to distinguish between ductal carcinoma in situ and usual ductal hyperplasia
4. Cytokeratin stains to detect sentinel lymph nodes metastasis.
5. To find the site of origin in metastatic cancers.
6. Assessment of Estrogen and Progesterone receptor status &HER2neu

overexpression using specific antibodies to receptor proteins.

7. Evaluation of spindle cell lesions to distinguish metaplastic carcinomafrom mesenchymal lesions.

8. Assessment of proliferation index along with hormone receptor status and basal markers expression, and to classify as molecular subtypes.

ANTIGEN RETRIEVAL

Shi et al in 1991developed the antigen retrieval technique, in which he used a heating method at high temperatures to bring out the antigenicity of the tissue which had been masked by formalin fixation.

Antigen retrieval can be done by proteolytic induced epitope retrieval or heat induced epitope retrieval

HEAT INDUCED EPITOPE RETRIEVAL

In this technique, tissue sections are placed in the retrieval solution which is heated for varying period of time that leads to the breakdown of protein cross-links which are formed during fixation with formalin and recovers the tissue antigenicity. [91]

Commonly used heating devices are the pressure cooker, microwave oven, autoclave, steamer and water bath. Heating is usually done for about 20 minutes followed by 20 minutes of cooling. The retrieval solution commonly used is the Citrate buffer with pH 6.0.

PROTEOLYTIC INDUCED EPITOPE RETRIEVAL [92]

Proteases liketrypsin, proteinase K, chymotrypsin and pepsin are used for restoring the tissue antigenicity. However, the limitation of this technique is thatsome epitopes are destroyed during this process and therefore alter the tissue morphology.

TARGET ANTIGEN DETECTION METHODS

After addition of specific antibodies to the antigens, next step is to visualize the antigen antibody reaction complex. The methods employed are the direct and the indirect methods. The direct method is a one-step staining procedure in which a labelled antibody directly reacts with the antigen in the tissue sections. Most commonly used labels are fluorochrome, horse radish peroxidase and alkaline phosphatase. Although this method is simple, rapid, and uses only one antibody, the sensitivity is lower. This is because signal amplification is less, and therefore it is not as commonly used when compared to the indirect methods.

In the indirect method, first layer is formed by an unlabelled primary antibody which binds to the target antigen. Then, a second layer is formed by using a labelled secondary antibody that reacts with the primary antibody. This technique is more sensitive than the direct method because of better signal amplification. This is due to the binding of several secondary antibodies with conjugated fluorochrome to each primary antibody.

Anotheradvantage with this method is that it uses only a small number of secondary antibodies. [93]

HORMONE RECEPTORS

A significant milestone in the management of breast cancer is the realisation of the presence of hormone receptors in the tumors, which correlated well with the response to hormonal therapy and chemotherapy. [94, 95] Currently the estrogen receptor status is regarded as the most powerful predictive factor in the management of breast cancer. Though ER and PR are co dependant variables, PR is a weaker predictor of response to hormone therapy than ER. [96]

Hormone receptors are measured by the immunohistochemical method and attempts are made to semi quantitate them by standardizing the technical procedure and reporting by using appropriate controls. [97, 98, 99,100] Delay in fixation alters the results significantly. [101]The two parameters are evaluated by counting the number of tumour nuclei stained and given in percentage of the entire tumour cell nuclei and the intensity of the staining reaction. [102]

Generally ER negative tumours tend to have grade three histology, lymphoid stroma, pushing margins, comedo type of necrosis and central fibrosis. [103] Most medullary, metaplastic and apocrine carcinomas are negative whereas tubular, mucinous, lobular carcinomas show a high degree of positivity. [104,105,106]

For ER and PR, only nuclear reactivity is considered significant. When present in more than 5% of tumor cells they are regarded as positive and when in less than 5% of tumor cells they are regarded as negative. [107]

The guideline recommendations for immunohistochemistry testing of ER and PR receptors in breast cancer was jointly formulated by the American Society of Clinical Oncology and the College of American Pathologists (ASCO/CAP)[108,109] Some of the more salient points of this document are the following:

- The pathologist must report the percentage of cells that are immunoreactive
- Tumours having 1% or higher invasive cancer cells staining are regarded as positive.
- The average intensity of the stain must be included (weak, moderate or strong).
- The pathologist must give an interpretation as to whether the sample is positive or negative.
- The use of a composite score based on percentage plus intensity (Allred, H, or Quick scores) is optional.
- Specimens should be placed in 10% neutral buffered formalin no later than 1 hour (but ideally much sooner) after being removed from the

patient.

- Fixation time should be at least 6 hours and not longer than 72 hours.
- Normal breast cells in the sample can be used as internal positive controls.

HER 2 neu

It is a member of EGFR (epidermal growth factor receptor) family and is an oncogene that encodes a transmembrane glycoprotein. [110,111]The development and progression of certain aggressive breast cancer is associated with the amplification and overexpression of this oncogene. It can be measured by immunohistochemistry or FISH.[112,113,114] Theyare graded by immunohistochemistry according to the scheme in Annexure IV. Its overexpression is found in high grade DCIS, 20-30% of invasive ductal carcinomas and rarely in invasive lobular carcinoma.

Cytokeratin 5/6

CK 5/6 is a type II keratin which belongs to the high molecular weight category. Myoepithelial cells of the breast, glandular epithelium and the basal cells of the prostate some ovarian tumors show strong expression of this marker.[115] They are considered to be a very good indicator of squamous and transitional epithelium and a good discriminator of mesothelioma and adenocarcinoma of lung.[116]

CK 5/6 expression in breast carcinoma indicates a basal like molecular subtype and is associated with adverse prognosis. It is also used in differentiating benign from malignant breast lesions as staining reaction in malignant lesions are only cytoplasmic and the intensity is less compared to the benign ones.[117]

CK 5/6 scoring was done based on the criteria proposed by Smedts[118] and Ordonez [119] in their studies on gynaecological malignancies. The scoring was done by counting the positive cells and they are given in a percentage (Annexure V).

Cytokeratin 5/6 staining has a wide variety of staining patterns from identifying the myoepithelial cell layer which shows a strong cytosolic staining, whereas identifying a layer of ductal epithelial cells shows a variable positivity and that may represent committed stem cells.

Ki67

The rate of proliferation within the tumor cells can be detected with this molecule. The Ki67 labelling index (LI) has been used as an indication for evaluation and many reports have shown its clinical significance in a variety of tumors irrespective of their origin.

Proliferation activity of breast carcinomas has been studied by various methodologies. Investigators use either immunohistochemistry for studying the proliferating cell nuclear antigen (PCNA) expression or flow cytometry to measure the fraction of S-phase. [120,121]. In breast cancers a high

proliferation index correlates with a worse prognosis. Later gene expression profiling studies largely included the proliferation genes PCNA and Ki67 and revealed the "molecular portraits" [122]

Collosoet al have found the superior prognostic and predictive value of Ki67 than the other proliferation markers as cyclin D,cyclinE,p21,p27,topoisomerase II alpha.[123] Among breast cancers it has been found that the average Ki67 labelling index was highest for high grade tumors and low for HER 2 positive tumors.

For Ki67 nuclear reactivity is taken into account,which is recorded as continuous variables,based on the proportion of positive tumor cells (0%-100%) irrespective of the staining intensity.They are regarded as high when >14% and low when <14%.

STAGE WISE TREATMENT OF INFILTRATING DUCTAL CARCINOMA.

According to stage, for early breast cancer, the appropriate management would be breast conservative surgery. Mastectomy is done for large tumors. To shrink the bulky tumors before surgery, preoperative neoadjuvant chemotherapy is given. For tumors larger than 1 cm adjuvant systemic chemotherapy is advisable following surgery. Radiation therapy will be required for patients who had a breast conserving surgery, or some who have a modified radical mastectomy with margins involved, with

lymphovascular invasion and more than 4 nodes showing secondary carcinomatous deposits.

STAGE I

These tumors are relatively smaller in size less than 2 cm and either have no nodal involvement or have sentinel lymph node involvement.

Local therapy:

Lumpectomy or Modified radical mastectomy(MRM) is the mainstay of treatment for stage I tumors. Radiation therapy is given after breast conservative surgery and treated without radiation if the patient is more than 70 years old, tumor size less than 2 cm and when the tumor is completely removed with no nodal involvement. Hormonal therapy may be given for those who show hormone receptors positivity. [5, 6].

Adjuvant systemic therapy:

No matter how small the tumor is, all cases that express hormone receptors are treated with hormonal therapy. For HER 2 positive tumors, Herceptin is usually recommended.

STAGE II

These are tumors of large size that are spread to less than 4 lymph nodes.

Local therapy:

Surgery followed by radiation therapy.

Adjuvant systemic therapy:

For all tumors in this stage, adjuvant systemic chemotherapy is recommended. Depending on the age, hormone receptor status, HER 2 status hormonal therapy or Herceptin or chemotherapy or a combination of these is given.

Neoadjuvant therapy:

It is an option for patients who opt for a breast conservative surgery wherein they are subjected to systemic therapy pre operatively to shrink the tumor size. If the tumor size does not shrink to the expected size, the adjuvant chemotherapy would likely to be of different set of drugs. For HER 2 positive tumors Herceptin is also used as neoadjuvant therapy.

STAGE III

Stage III tumors are those which are of large size of more than 5 cm size or several nodal involvement or spreading into adjacent structures (skin over the breast or muscle underneath). Neoadjuvant systemic therapy followed by modified radical mastectomy with adjuvant therapy (hormonal therapy if hormone receptors positive, Herceptin if HER2 positive) and radiation therapy following surgery are recommended.

ADJUVANT DRUG THERAPY

Based on the prognostic factors like tumor size and lymph nodes involved, adjuvant therapy is considered valuable in the treatment of stage I

to III breast tumors. It can be chemo therapy, hormonal therapy, Herceptin or a combination of these.

HORMONAL THERAPY

Regardless of tumor size and lymph node involvement, it is the treatment for hormone receptor positive cancers. It is not likely to respond for hormone receptor negative tumor patients. Tamoxifen and LHRH analog drugs are used in hormone receptor positive tumors in women who have not attained menopause. Aromatase inhibitors are given to women who have become postmenoupausal within five years of tamoxifen treatment.

Chemotherapy:

For all hormone receptor negative and positive tumors, chemotherapy gives added benefit based on the stage and characteristics of their tumor by reducing the rate of recurrence.

Drug combinations most commonly used are:

- TAC: Taxotere, adriamycin, and cyclophosphamide
- FAC: 5 Flurouracil, adriamysin and cyclophosphamide.
- Herceptinis added to all for HER2/neu positive tumors along with the conventional chemotherapy.

STAGE IV

Bones, liver, lung and brain and lymph nodes are involved in this stage. Although surgery and radiation can help to some extent, the main stay

of treatment is systemic therapy. Combination of chemotherapy or targeted therapy or hormone therapy may be given. This will help to shrink the tumor size and improve the patients symptomatically. However all these therapies have possible side effects that should also be encountered while treating these patients.

Radiation therapy is given to treat small number of metastasis confined to one area, to relieve compression in spinal metastasis, to provide relief of pain and other symptoms and to treat brain metastasis [12].

RECURRENT BREAST CANCER

Recurrent cancers are those that come back after treatment either in the local or in the distant area. Regional recurrences are breast cancers that recur in lymph node. Involvement of opposite breast is considered as new cancer.

The recurred tumor is surgically removed, subjected to radiation therapy or given targeted chemo therapy. Regional recurrence and distal recurrence are also treated similarly with the change in the chemo therapy either by adding newer drugs to old regimen or substituting the previously given drugs.

TARGETED THERAPY FOR BREAST CANCER

These are recent drugs that specifically target the cells that cause cancer which are identified on the basis of gene expression profiling.

HORMONAL THERAPY

Tumors those are positive for ER and PR are treated with tamoxifen and aromatase inhibitors depending upon their postmenopausal status.

HER2 TARGETED DRUGS.

These includespertuzumab (Perjta), Transtuzumab (Herceptin), lapatinib (Tykerb), and ado tarnstuzumabemtansine.

ANTI ANGIOGENESIS DRUGS

For cancer cells to grow, they need adequate blood supply. This is achieved by neo angiogenesis as the VEGF and PDGF are stimulated by the cancer cells that leads to the proliferation of newer vessels. They show increased vascular invasion that ultimately affect the prognosis adversely.

OTHER TARGETED THERAPIES

Everolimus and exemestane are other newer drugs that help hormone therapy to work better. These drugs play a better role in shrinking the breast tumor size when given in combination with hormonal therapy drugs than when given alone. Bisphosphonates, denozumab and vitamin D are other drugs that reduce fractures and strengthen the bones.

ROLE OF MOLECULAR CLASSIFICATION IN THE TREATMENT OF BREAST CANCERS.

The gene expression signatures of the various genes expressed in breast cancer are used in this classification. Morphologically identical breast carcinomas can have varied responses to therapy. This is because,there exist molecular level difference between morphologically similar tumors [126]. Initial studies in molecular classification claimed that these signatures would provide an objective assessment of the risk of relapse and would be more reproducible than the currently used methods. As a result, the predictive factors for the different treatments, point that molecular classification is a more powerful tool.The on-going and upcoming researches may provide us with more precise prognostic and predictive information about breast cancer and perhaps serves as a breakthrough step towards "personalization" of breast cancer treatment. [124] New biological insights and targeted therapy towards the particular molecular subtypes are a result of better knowledge and understanding of the molecular classification.[125].This would result in the less frequent use of chemotherapy by choosing the appropriate drugs that would target the cancer cells and thereby it can provide a considerable advantage in reducing the drug related toxicities and costs. [126]

Molecular classification can also be used in the assessment of prognostic and predictive values. Gene expression differences are found to

exist between the cancers that recurred and those that did not recur. On evaluating these differentially expressed genes, scoring is obtained to predict the outcome.[125]

MOLECULAR CLASSIFICATION BY IMMUNOHISTOCHEMISTRY

Immunohistochemical analysis (IHC) can also be used to classify tumors based on molecular classification with a limited panel of markers (ER, PR, HER2 neu, Ki67 and CK5/6). Apart from having distinct clinical outcomes, these subgroups have distinct clinical and morphological features. Subsequent studies have proposed a standardised molecular classification based on IHC to facilitate its clinical application and promote more uniform large multicentric studies.[127].

MATERIALS AND METHODS

This study is a retrospective descriptive study ofinvasive breast cancers conducted in the Institute of Pathology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai during the period between Jan 2011 to Jun 2013.

Source of data

The invasive ductal carcinoma cases reported in mastectomy specimen received in the Institute of Pathology, Madras Medical College between Jan 2011 to Jun 2013from the Department of General Surgery, Surgical Oncology and Plastic surgery, Rajiv Gandhi Government General Hospital. A total of 369 mastectomy specimens (simple, modified radical or radical mastectomy) were received during this period.

Inclusion criteria:

- ✓ All modified radical mastectomy specimens of breast carcinomas.
- ✓ All invasive breast carcinomas no special type (ductal and lobular), medullary, mucinous, papillary, apocrine and metaplastic carcinomas irrespective of the age and sex were included for the study.

Exclusion criteria:

- ✓ All trucut biopsies.
- ✓ Phylloides tumors.
- ✓ Benign breast lesions.
- ✓ Tumors with preexisting premalignant conditions.

- ✓ Recurrent tumors.

METHOD OF DATA COLLECTION

Detailed history of the cases regarding age, sex, menstrual history,side of the breast, type of procedure, history of neo adjuvant therapy,details of gross characteristics such as tumour size, nodal status detailswere obtained for those 60 cases included in the study that was reported during the study period from surgical pathology, surgical oncology and medical oncology records. Formalin fixed tissue were cut, processed and paraffin embedded.

4 μm thick sections of the paraffin tissue blocks were cut and stained with eosin and hematoxylin. Slides were collected from slide filing and were reviewed and graded using the Nottingham modificationof the Scarff Bloom Richardson Grading system (Annexure III) and theywere further evaluated for the presence of necrosis, lymphocytic responseand lymphovascular invasion, skin infiltration by tumour. 10 cases of each grade fromInvasive ductal carcinoma NSTand 5 cases from special type as medullary, metaplastic, mucinous, apocrine, papillary and invasive lobular were randomly selected from thetotal cases and their representative formalin fixed paraffin embeddedtissue samples were subjected to immunohistochemical analysis of 5 markers which includes ER, PR, H2N, CK5/6 and Ki67. Slides were evaluated and scoring was given. The results were recorded with photographs.

IMMUNOHISTOCHEMICAL EVALUATION

Immunohistochemical analysis of ER, PR, H2N, CK5/6 and Ki67 was done in paraffin embedded tissue samples using supersensitive polymer HRP system based on non-biotin polymeric technology.

Table 3: Immunohistochemical markers used in the current study

Antigen	Vendor	Clone	Dilution	Positive control
ER	Dako	Rabbit Monoclonal EP1	Ready to use	Breast
PR	Dako	Mouse Monoclonal	Ready to use	Breast
H2n	Dako	Rabbit Monoclonal SP-3	Ready to use	Breast
CK5/6	Dako	Mouse Monoclonal	Ready to use	Squamous cell carcinoma of skin
Ki67	Dako	Mouse Monoclonal	Ready to use	High grade lymphoma

4 μ thick sections from selected formalin fixed paraffin embedded tissue samples were transferred onto gelatin coated slides. Heat induced antigen retrieval was done using microwave method. The ER, PR, CK5/6 and Ki67 antigens are bound with mouse monoclonal antibodies (dako) and HER2neu antigen is bound with rabbit monoclonal antibody(dako). Laterantigen antibody complex are detected by the addition of secondary antibody conjugated with horse radish peroxidase-polymer and Diaminobenzidine substrate. The step by step procedure of Immunohistochemistry is given in Annexure IV.

INTERPRETATION & SCORING SYSTEM

ER and PR

Hormone receptors like estrogen and progesterone receptor, when expressed show a nuclear positivity. The number of cells expressing and their intensity of staining is scored as two values and a composite score based on percentage plus intensity of more than 2 is considered to be positive.(Annexure V).

H2N:

HER2neu expression is demonstrated in tumor cells as cytoplasmic membrane positivity and its intensity and number of tumor cells expressing is graded as 1+, 2+ and 3+. (Annexure V)

CK 5/6

CK 5/6 scoring was done based on the criteria proposed by Smedts et al[118] and Ordonez et al[119] in their studies on gynaecological malignancies. The scoring was based on the percentage of positive cells (Annexure V).

Ki67

For Ki67 nuclear reactivity is taken into account,which is recorded as continuous variables,based on the proportion of positive tumor cells (0%-100%) irrespective of the staining intensity.They are regarded as high when >14% and low when <14%.

Molecular subtypes were derived and compared with the clinicopathological parameters with the SPSS version 17 software.

McNemar test

Description:

The Mcnemar's test is done by a 2x2 classification table. This is done to test the difference between paired proportions. Here in this study it is done on the same set of patients who serve as their own control, based on the "before and after" design. Two discrete dichotomous variables are used in the classification system.

In the current study 60 cases were classified on the basis of histopathology and the same set of patients were subjected to immunohistochemistry and classified under molecular classification. The difference between the two classification systems and 95% confidence interval were determined. The p value was derived and its significance was studied.

INTER RATER AGREEMENT KAPPA

DESCRIPTION:

Inter rater agreement is used to evaluate the strength of agreement between two classification systems. The agreement is quantified by the KAPPA statistic.

- KAPPA is 1; when the agreement between the two classification systems is perfect.
- KAPPA is 0; when there is no agreement better than chance.
- KAPPA is negative; when agreement is worse than chance.

The Standard errors reported by MedCalc are the appropriate standard errors for testing the hypothesis that the underlying value of weighted kappa is equal to a prespecified value other than zero.

Table 4: Kappa value and its related strength of agreement

K value	Strength of agreement
<0.2	Poor
0.21-0.4	Fair
0.41-0.6	Moderate
0.61-0.8	Good
0.81-1.0	Very good

The value of KAPPA, with its standard error and 95% confidence interval was derived. The agreement between the two classification systems was analysed.

OBSERVATION AND RESULTS

In the study period of 29 months from January 2011 to June 2013, a total of 26,536 specimens were received in the Institute of Pathology, Madras Medical College for histological examination.

Total numbers of breast specimens received were 1412 cases, ofthese breast tumours accounted for 1023 cases with a percentage of 3.85% of all cases (including both incisional and excisional biopsies).

The total number of non neoplastic, benign and malignant cases was 289, 472 and 651 respectively. Thus the distribution of non neoplastic breast lesions were 20.46%, benign tumours were 33.42% and of malignant tumours were 46.11% is shown below in Table 5 and chart 1

Table 5: Distribution of breast cases.

	Non neoplastic	**Benign**	**Malignant**
Breast	289	472	651

Out of a total of 651 breast cancer cases, 369 cases constituted radical mastectomy specimens. Among these 369 cases a total of 60 cases were included in this study which comprised of 30 cases of Infiltrating ductal carcinoma NST and 30 cases of special variants which included apocrine, medullary, mucinous, metaplastic, lobular and papillary carcinomas each constituting 5 cases.(Table 6, Chart 2).

Table 6: Distribution of cases included in the study

HISTOPATHOLOGICAL CLASSIFICATION	No of cases (%)
Infiltrating ductal carcinoma no special type (IDC NST)	30 (50)
Metaplastic carcinoma	10 (8.3)
Papillary carcinoma	10 (8.3)
Lobular carcinoma	10 (8.3)
Apocrine carcinoma	10 (8.3)
Medullary carcinoma	10 (8.3)
Mucinous carcinoma	10 (8.3)

ER, PR, HER2, CK5/6 and Ki67 were done for all 60 cases, results interpreted and scoring was given. Based on which they were classified into luminal A, luminal B, HER 2, basal and unclassified as per molecular classification.

Among the 60 cases, 22 cases were luminal A and constituted the most common type (36.7%), followed by 14 cases of basal type(23.3%), 8 cases of HER2 (13.3%), 6 cases of unclassified (10%) and 5 cases of luminal B and hybrid types(8.3%). As seen in Table 7 and Chart 4.

Chart 1: Distribution of total breast cases.

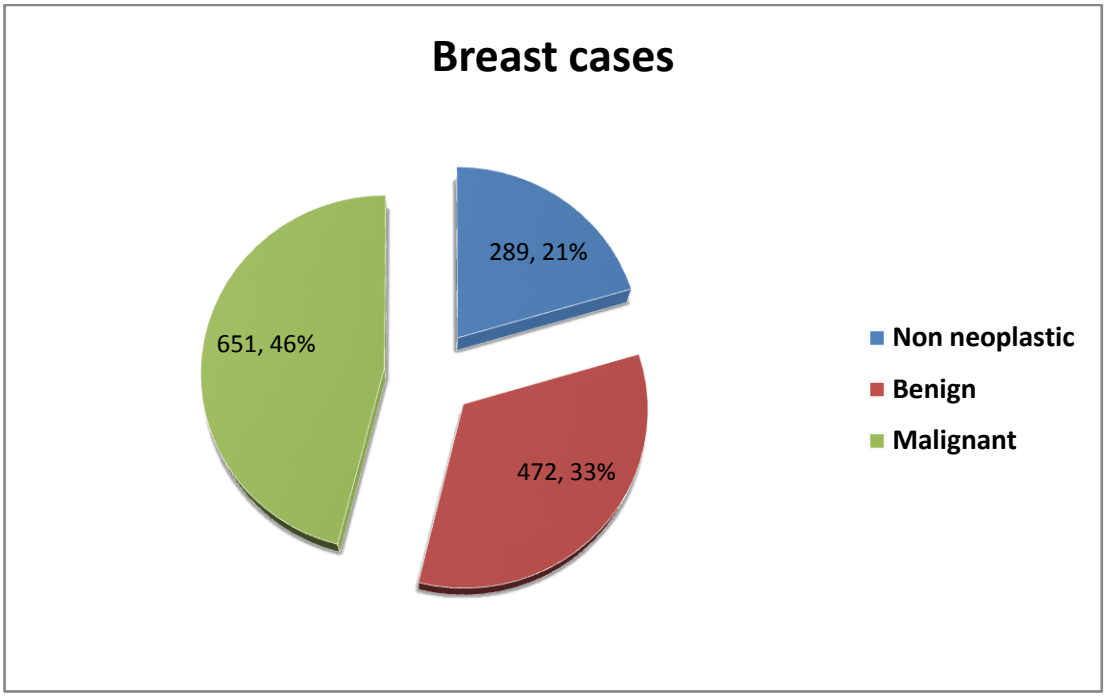


Chart 2: Distribution of cases included in the study

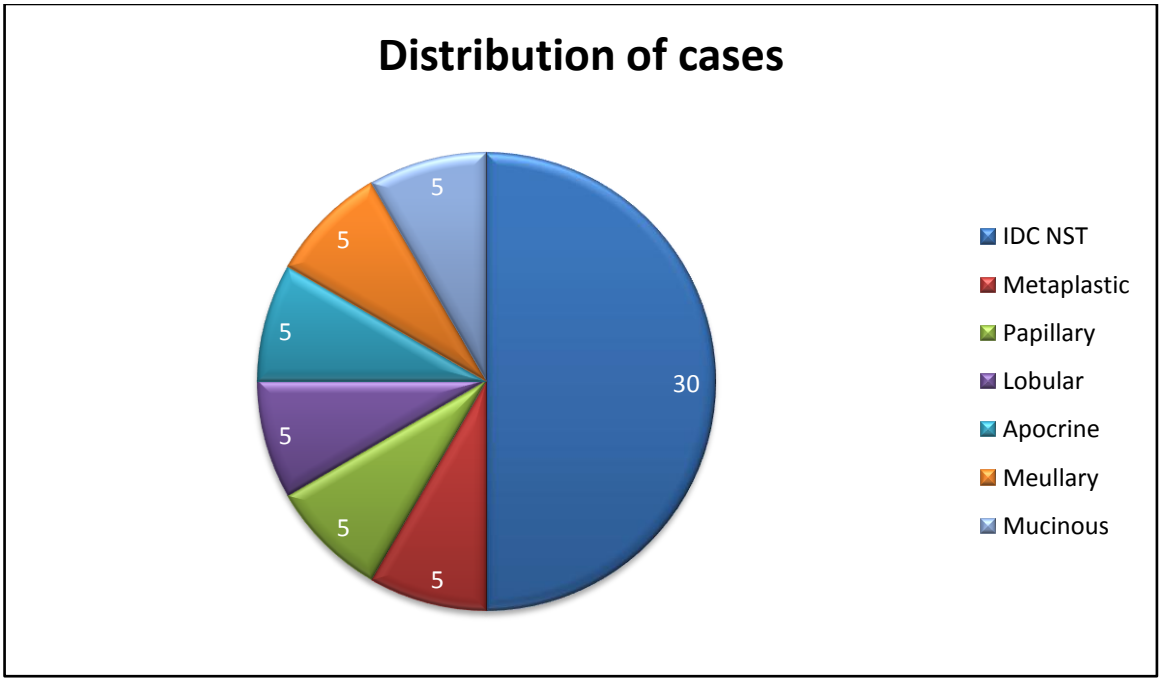


Table 7: Distribution of Molecular Subtypes in the current study

Molecular subtypes	Luminal A	Luminal B	HER 2	Hybrid	Basal	Unclassified
No of cases (%)	22(36.7)	5(8.3)	8(13.3)	5(8.3)	14(23.3)	6(10)

The clinicopathological parameters were compared with the molecular classification as follows.

The age wise distribution of the 60 cases is given below. Table 8 and chart 4

Table 8: Age wise distribution of breast cancers in molecular classification

	AGE (YEARS)						
Molecular Classification	**20-29 (% of MC)**	**30-39 (% of MC)**	**40-49 (% of MC)**	**50-59 (% of MC)**	**60-69 (% of MC)**	**70-79 (% of MC)**	**Total**
Luminal A	0	1(4.5)	5(23)	9(40)	5(23)	2(9)	22
Luminal B	0	1(20)	1(20)	2(40)	1(20)	0	5
HER2	0	2(25)	3(38)	1(13)	2(25)	0	8
Hybrid	0	0	2(40)	2(40)	1(20)	0	5
Basal	2(14)	1(7)	4(29)	3(22)	3(22)	1(7)	14
Unclassified	0	1(17)	2(33)	2(33)	1(17)	0	6
Total	2(3.3)	6(10)	17(28)	19(32)	13(22)	3(5)	60
Pearson chi square test	**0.923**						

The most common age group affected by breast cancers are 50-59 years. Among the molecular classification, the luminal A and luminal B showedhigherincidence of breast cancer in 50-59 age group with 40% incidence, whereas as HER 2(23%) and basal types (20%) had

ahigherincidence at an earlier age group of 40-49 years. Unclassified showed equal distribution of 33% among both age groups. The youngest age of presentation is at 26 years and oldest was 75 years old. There was no statistical significance associated with this comparison. (Table 8 and Cart 4)

Among the 60 cases entered in this study it was found that left side (58%) of the breast was predominantly affected than the right (42%) as shown in Table 9 and Chart 5.

Table 9: Distribution of side of involvement in molecular classification

	SIDE		
Molecular Classification (MC)	**Right (% of MC)**	**Left (% of MC)**	**Total**
Luminal A	10(46)	12(55)	22
Luminal B	2(40)	3(60)	5
HER2	1(13)	7(82)	8
Hybrid	3(60)	2(40)	5
Basal	6(43)	8(57)	14
Unclassified	3(50)	3(50)	6
Total	25(42)	35(58)	60
Pearsons chi square test	**0.578**		

On analysing the side of involvement it was found that left sided tumors were more than right in luminal A, luminal B, HER 2 and basal types with 55%, 60%, 82% and 57% respectively. Among 5 cases of Hybrid tumors 3(60%) were on right side and unclassified type showed equal distribution of cases on right and left side of the breast. This was not statistically significant.(Table 9 and Chart 5)

Chart 3: Distribution of Molecular Subtypes in the current study

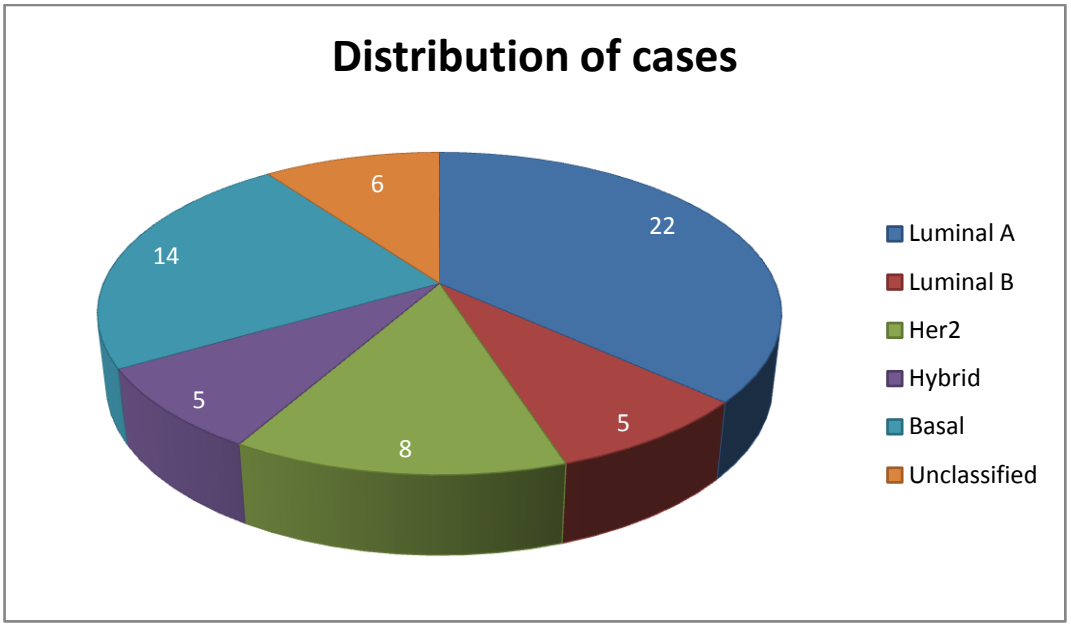
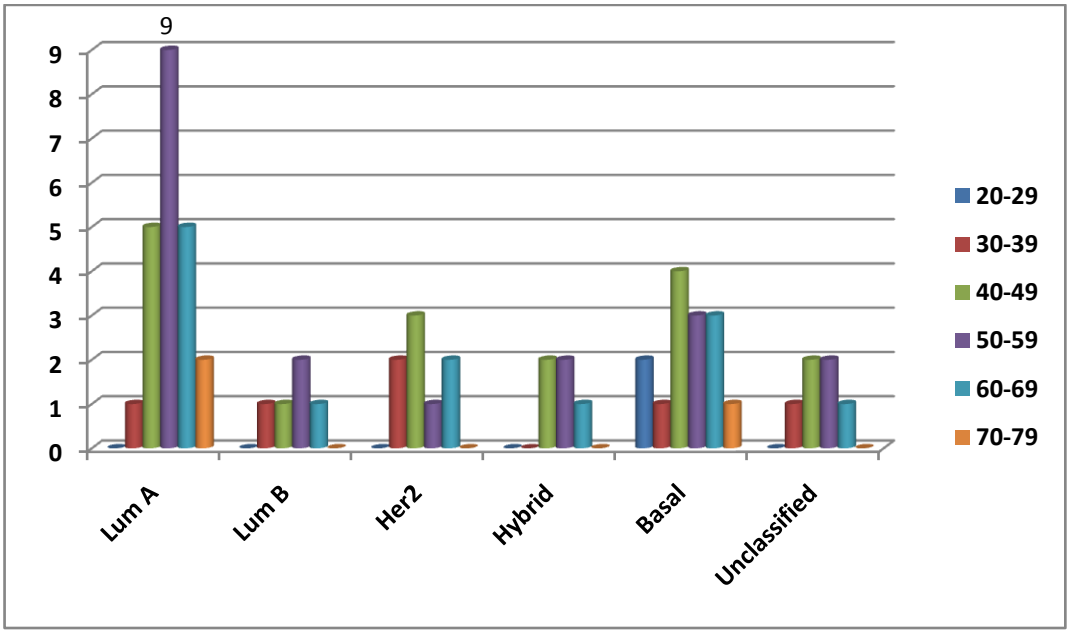


Chart 4: Age wise distribution of breast cancers in molecular classification



In this study the most common site of tumor location was found to be the upper outer quadrant (UOQ) corresponding to 60%, followed by central

quadrant with 21.7%, lower outer quadrant with 10.0% and the least common site was the upper inner quadrant with 8.3%, as shown in the Table 10 and Chart 6.

Table 10: Association of tumor location with molecular classification.

	TUMOR LOCATION				
Molecular Classification	**UOQ (% of MC)**	**LOQ (% of MC)**	**LIQ (% of MC)**	**CQ (% of MC)**	**Total**
Luminal A	11(50)	3(14)	4(18)	4(18)	22
Luminal B	4(80)	0	0	1(20)	5
HER2	5(63)	1(12)	0	2(25)	8
Hybrid	4(80)	0	1(20)	0	5
Basal	9(64)	0	0	5(36)	14
Unclassified	3(50)	2(33)	0	1(17)	6
Total	36(60)	6(10)	5(8)	13(22)	60
Pearsons chi square test	**0.388**				

Among the molecular classification, all the classes showed an increased incidence of the tumor to be located in the upper outer quadrant with luminal A constituting 50%, luminal B 80%, HER2 type 63%, hybrid cases 80%, basal type 64% and unclassified 50%, followed by the central quadrant. None of the hybrid cases were located in the central quadrant.Tumor location was not a statistically significant parameter.(Table 10 and Chart 6)

Chart 5: Distribution of side of involvement in molecular classification

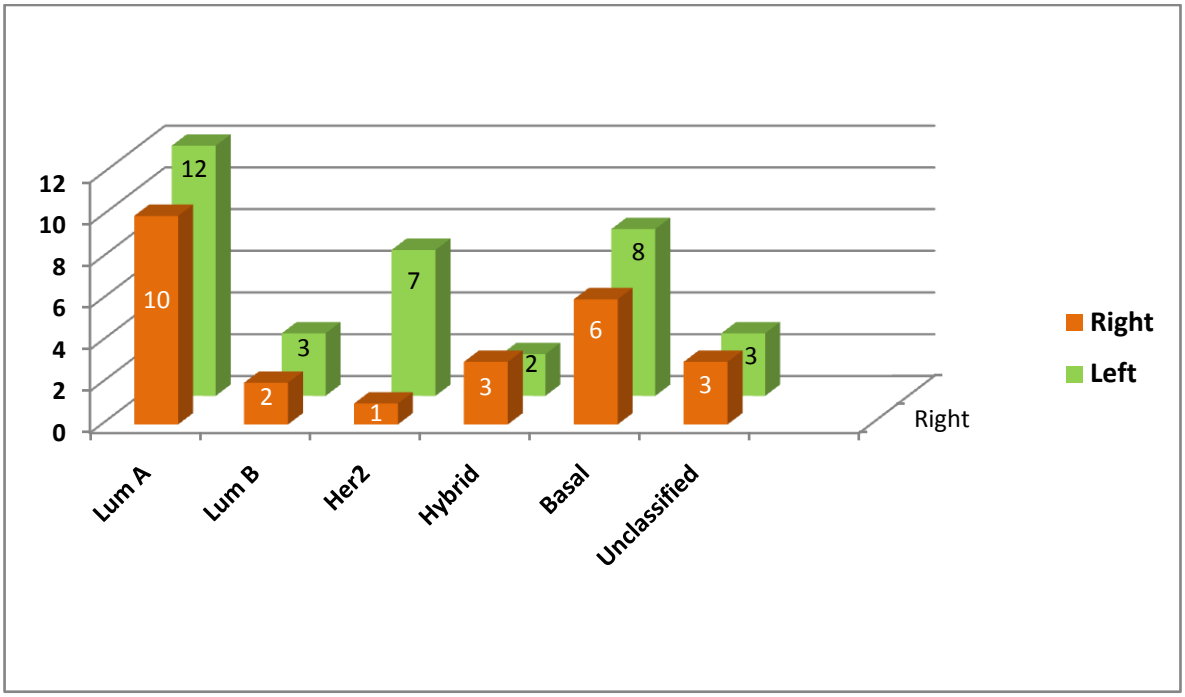
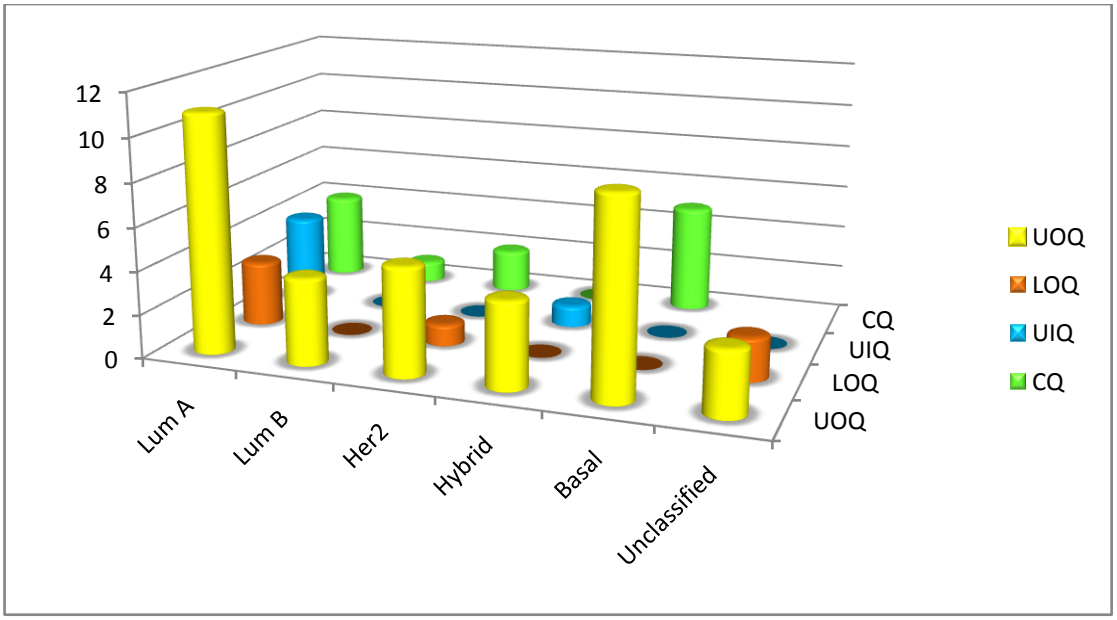


Chart 6: Association of tumor location with molecular classification



The size of the tumor was categorised on the basis of TNM staging. Majority of cases (68.3%) had tumor size of 2-5 cm, 25.4% had tumors more than 5 cm and only 6.7% of cases had less than 2 cm sized tumor. This is shown in Table 11 and chart 7.

Table11: Association of tumor size with molecular classification

	SIZE			
Molecular Classification	**<2 cm (% of MC)**	**2-5 cm (% of MC)**	**>5cm (% of MC)**	**Total**
Luminal A	2(9)	16(73)	4(18)	22
Luminal B	0	3(60)	2(40)	5
HER2	0	5(63)	3(37)	8
Hybrid	0	4(80)	1(20)	5
Basal	1(7)	10(72)	3(22)	14
Unclassified	1(17)	3(50)	2(33)	6
Total	4(7)	41(68)	15(25)	60
Pearsons chi square test	**0.921**			

All the 6 classes of molecular classification including the hybrid cases showed an increased incidence in tumors of size between 2cm and 5 cm. with 73% of luminal A, 72% of basal, 63% of HER 2, 18% of hybrid cases, 60% of luminal B and 50% of unclassified types. None of the luminal B, HER 2 type and basal type tumors was of small size. About 40% of luminal B, 37% of HER2 type and 33% of unclassified types had tumors of more than 5 cm size.(Table 11 and chart 7)

Among the 369 cases of radical mastectomy, 30(50%) cases of IDC NST and 30 (50%) cases of special variants which included medullary,

mucinous, metaplastic, apocrine, lobular and papillary each constituting 8.3% of cases, were taken in the study. This is shown in the Table 12 and chart 8.

Table 12: Association of histologic type with molecular classification.

	HISTOLOGICAL TYPE							
Molecular Classification	**IDC NST (% of MC)**	**Metaplastic (% of MC)**	**Papillary (% of MC)**	**Lobular (% of MC)**	**Apocrine (% of MC)**	**Medullary (% of MC)**	**Mucinous (% of MC)**	**Total**
Luminal A	6(27)	2(9)	5(23)	4(18)	0	1(5)	4(18)	22
Luminal B	3(60)	1(20)	0	0	1(20)	0	0	5
HER2	6(75)	1(13)	0	1(13)	0	0	0	8
Hybrid	2(40)	0	0	0	2(40)	1(20)	0	5
Basal	8(57)	0	0	0	2(14)	3(22)	1(7)	14
Unclassified	5(83)	1(17)	0	0	0	0	0	6
Total	30(50)	5(8)	5(8)	5(8)	5(8)	5(8)	5(8)	60
Pearson chi square test	**0.064**							

On analysing the molecular classification and its comparison with the histological types it was found that among the 30 cases of infiltrating ductal carcinoma NST, 6 were luminal A and HER2 type, 3 were luminal B, 8 were basal, 5 were unclassified. 2 cases showed strong positivity of both HER2 and luminal markers and were considered as hybrid cases. Among the 30 cases of special variants 16 cases were luminal A, 2 case were luminal B and HER2, 6 were basal and 1 unclassified. 3 cases were found to express hybrid markers.

This shows that 72% of luminal A tumors belongs to histological variants of special types rather than infiltrating ductal carcinoma NST. Where as 40%, 25%, 42% and 16% of luminal B, HER 2, basal and unclassified types respectively belongs to variants.

Among the 5 cases of luminal B, 3 cases (60%) of them were invasive ductal carcinoma no special type, one apocrine and one metaplastic (each 20%).

Among the 8 cases of HER2 type, 6 cases (75%) were IDC NST and the rest were metaplastic carcinoma and lobular carcinoma each one case.

2 cases of apocrine and 1 case of medullary carcinoma showed expression of hybrid markers and rest 2 cases were IDC NST.

Within the 14 cases of basal type, majority belongs to IDC NST (57%), followed by 3 cases (22%) of medullary, 2 cases (14%) of apocrine and 1 case (7%) of mucinous carcinoma. None of the metaplastic, lobular and papillary was basal.

None of the variants except 1 case of metaplastic carcinoma belongs to the unclassified type (penta negative) the rest (83%) were IDC NST. This comparison did not show any statistical significance.(Table 12 and Chart 8).

Chart 7: Association of tumor size with molecular classification

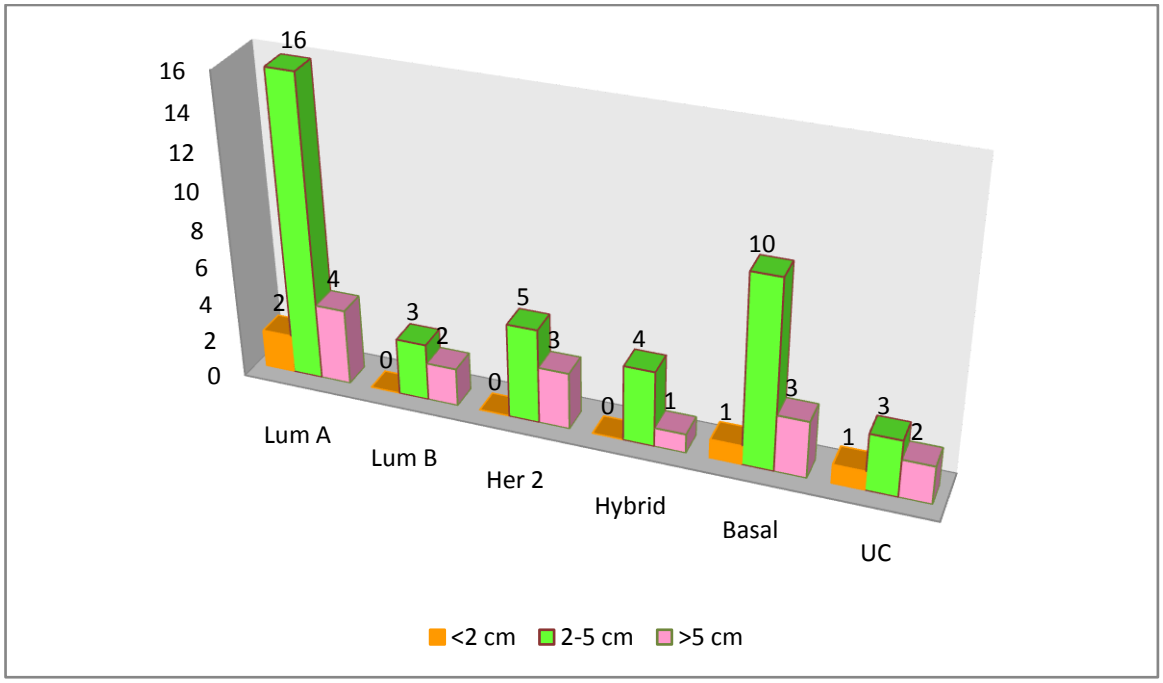
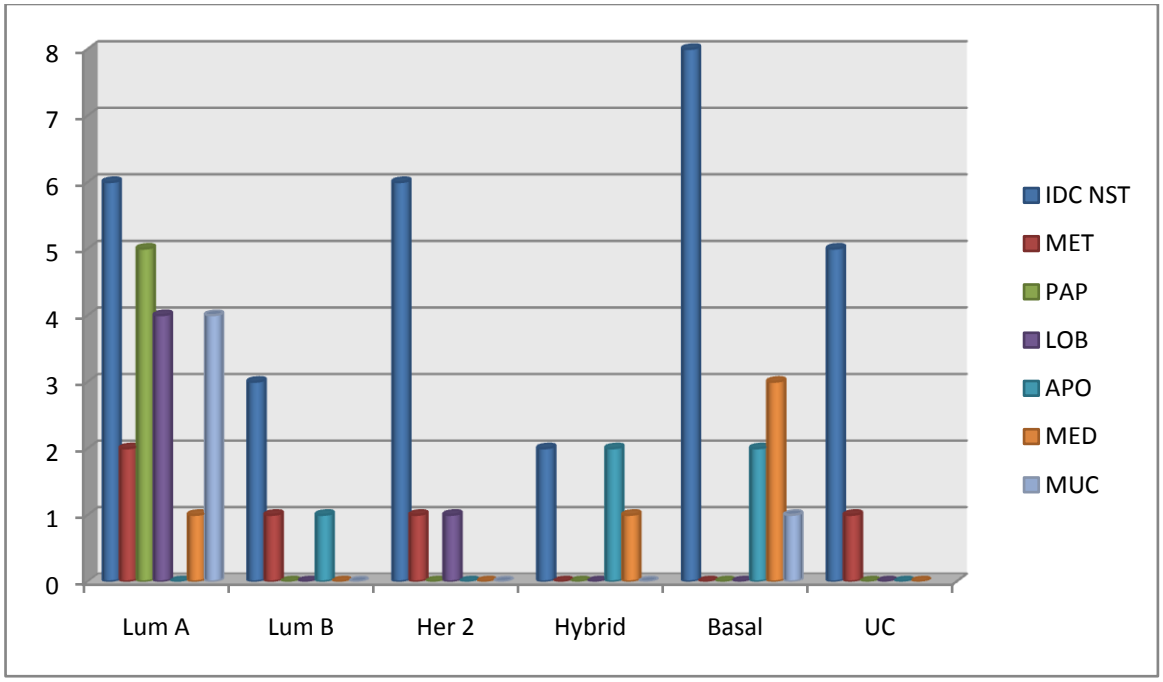


Chart 8: Association of histologic type with molecular classification.



Breast carcinomas are graded according to The Modified Scarff Bloom Richardson's grading system. This study included grade I, II and III with 10 cases in each grade which is shown in Table 13 and Chart 9.

Table 13: Association of histological grade with molecular classification.

	GRADE				
Molecular Classification	**Special variants (% of MC)**	**Grade I (% of MC)**	**Grade II (% of MC)**	**Grade III (% of MC)**	**Total**
Luminal A	16(73)	2(9)	4(18)	0	22
Luminal B	2(40)	1(20)	1(20)	1(20)	5
HER2	2(25)	1(13)	1(13)	4(50)	8
Hybrid	3(60)	0	2(40)	0	5
Basal	6(43)	2(14)	2(14)	4(29)	14
Unclassified	1(17)	4(68)	0	1(17)	6
Total	30(50)	10(17)	10(17)	10(17)	60
Pearsons chi square test	**0.013**				

4 out of 8 cases (50%) of HER 2 type, 4 out of 14 cases (28%) of basal types were grade III tumors. Among luminal A tumors, (9%) were grade I and 4(18%) were grade II and none belonged to grade III tumors. Luminal B tumors had equal distribution of cases among the 3 grades with 1 case in each grade. Among 5 hybrid cases, 2(40%) were grade II and other were special variants. Among the 6 unclassified cases 4(66%) of them were grade I tumors and only 1 case was grade III. This comparison had statistical significance with the p value of 0.01. (Table 13 and Chart 9)

Associated lesions in the adjacent breast tissue were studied for all the 60 cases. The most common lesion associated was the fibrocystic disease constituting 73.3%, followed by ductal carcinoma in situ with 18.3 %. The least common lesion associated was the sclerosing adenosis with 8.3%. As shown in Table 14 and Chart 10.

Table 14: Association of molecular classification with other associated lesions

	Associated lesions			
Molecular Classification	**Fibrocystic disease (% of MC)**	**DCIS (% of MC)**	**Sclerosing adenosis (% of MC)**	**Total**
Luminal A	16(73)	5(23)	1(4)	22
Luminal B	4(80)	0	1(20)	5
HER2	5(63)	1(13)	2(25)	8
Hybrid	4(80)	0	1(20)	5
Basal	11(79)	3(21)	0	14
Unclassified	4(67)	2(33)	0	6
Total	44(73)	11(18)	5(8)	60
Pearsons chi square test	**0.472**			

Fibrocystic disease was the most common associated lesion with all the classes of molecular classification comprising 73% of luminal A, 80% of luminal B and hybrid, 79% of basal, 67% of unclassified types, 63% of HER 2 type. 5 (45%) out of 11 cases of ductal carcinoma in situ belongs to luminal A class followed by 27% and 18% of basal and unclassified types respectively.

Chart9: Association of histological grade with molecular classification.

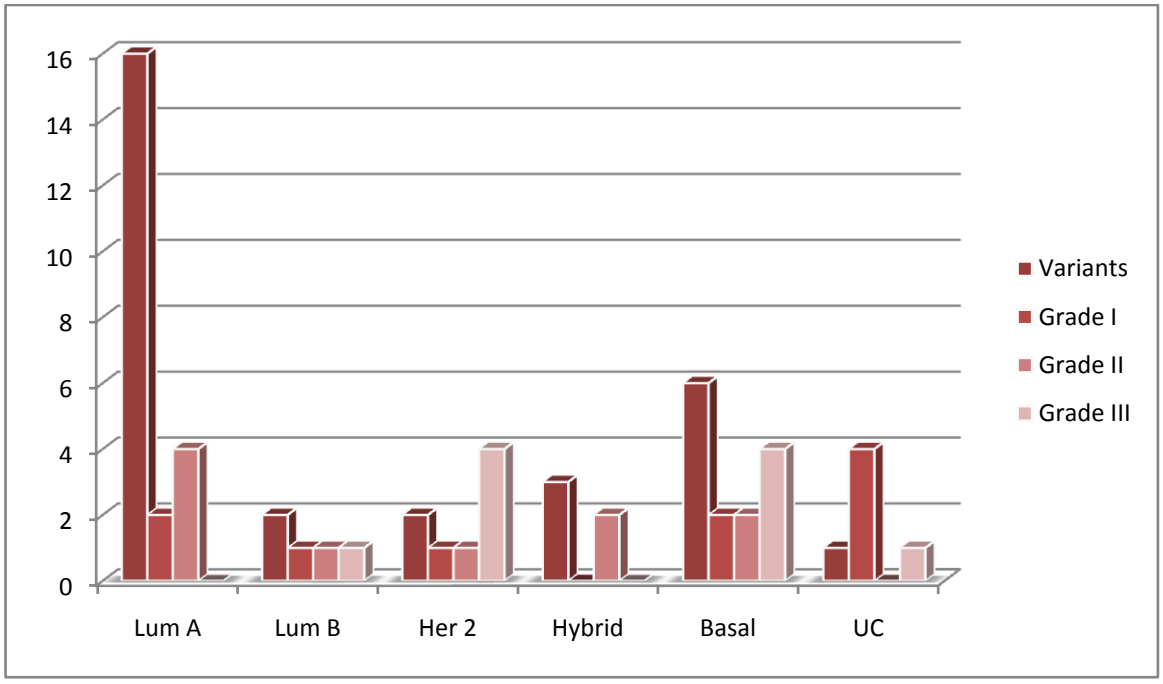
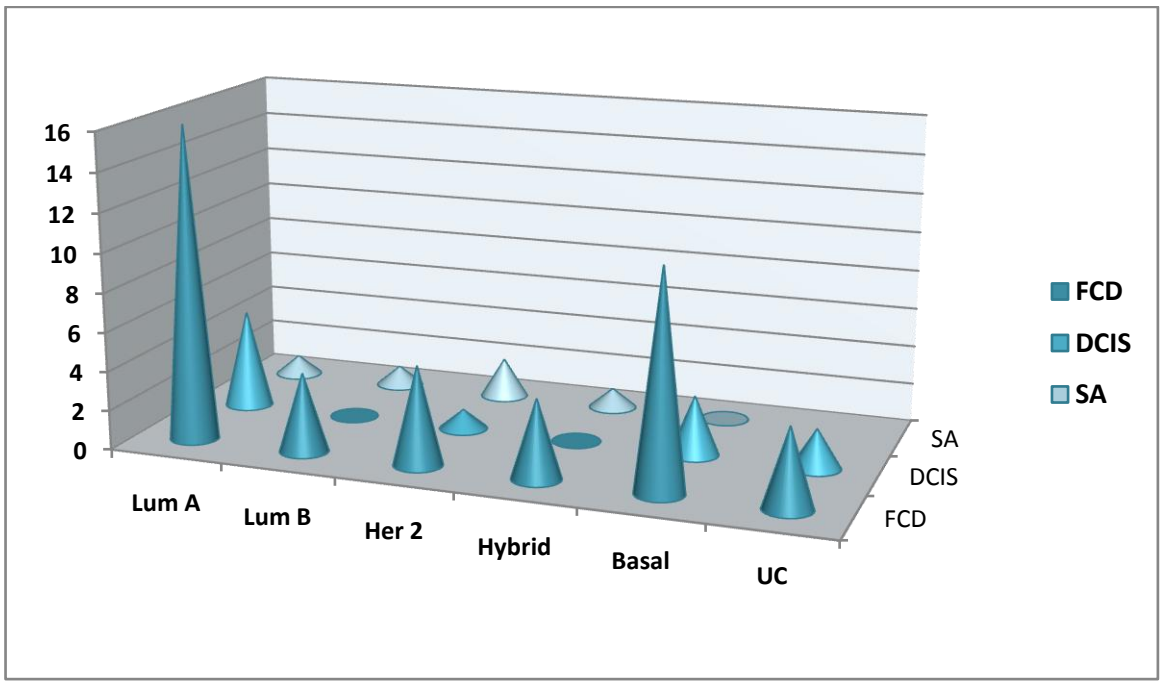


Chart10: Association of molecular classification with other associated lesions.



5 cases showed sclerosing adenosis in the adjacent breast of which 1 case from luminal A, luminal B and hybrid types and 2 cases from HER2 types. Basal and unclassified types did not show any association with sclerosing adenosis. This has no statistical significance.(Table 14 and Chart 10)

Lymphovascular invasion considered to be an adverse prognostic factor was assessed in all 60 cases. It was present in 66.7% of cases and absent in 33.3% of cases as shown in in Table 15 and Chart 11.

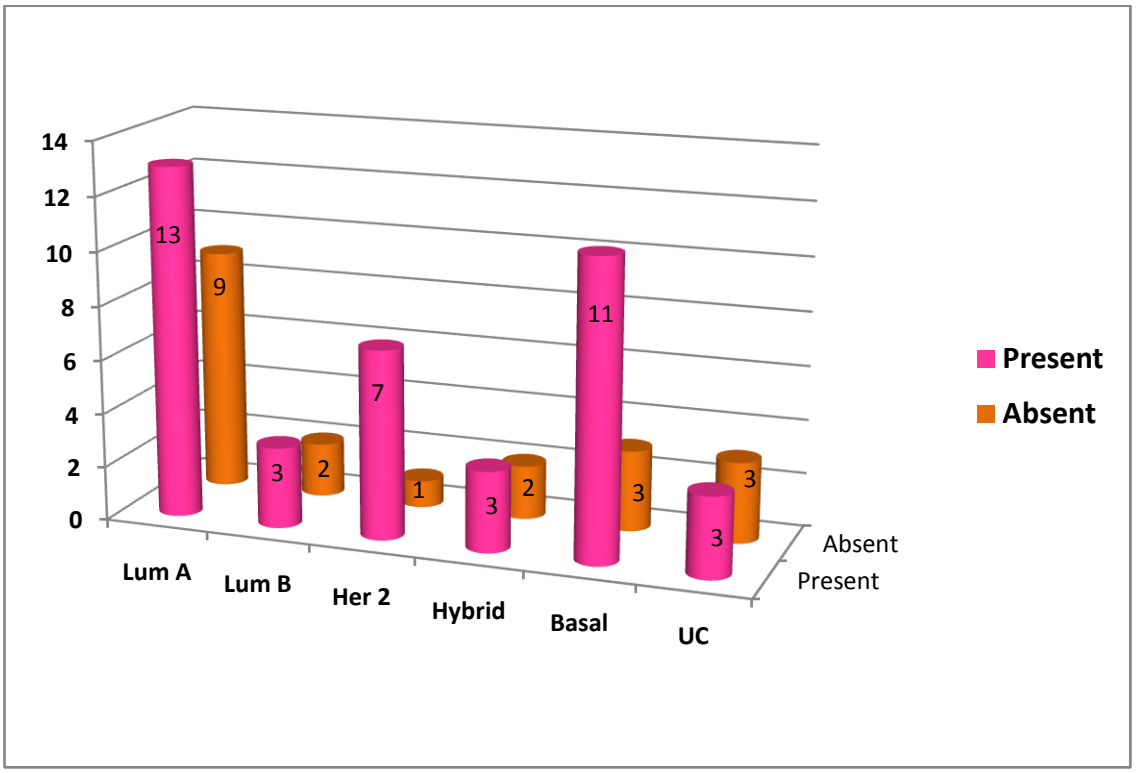
Table 15: Association of lymphovascular invasion with the molecular classification

	Lymphovascular invasion		
Molecular Classification	**Present (% of MC)**	**Absent (% of MC)**	**Total**
Luminal A	13(59)	9(41)	22
Luminal B	3(60)	2(40)	5
HER2	7(88)	1(12)	8
Hybrid	3(60)	2(40)	5
Basal	11(79)	3(21)	14
Unclassified	3(50)	3(50)	6
Total	40(67)	20(33)	60
Pearsons chi square test	**0.553**		

Among the different classes of molecular classification, all classes except unclassified type showed increased incidence of lymphovascular invasion. Especially the HER 2 and basal types in which 87% and 78% had respectively, whereas only 60% of luminal tumors and hybrid cases had lymphovascular invasion. Unclassified tumors had equal number of cases

with and without lymphovascular invasion. This parameter did not show any statistical significance. (Table 15 and Chart 11)

Chart11: Association of lymphovascular invasion with the molecular classification.



Lymphocytic infiltration when present they are considered to be associated with good prognosis. In this study 73% of tumors showed lymphocytic infiltration as shown in Table 16 and Chart 12.

All classes except HER 2 showed an increased incidence of lymphocytic infiltration, especially the luminal and hybrid tumors in which 80% of them showed presence, followed by basal type which showed 78% of lymphocytic infiltration. In HER 2 type only 50% of tumor had

lymphocytic infiltration. This parameter did not have any statistical significance. (Table 16 and Chart 12)

Table 16: Association of lymphocytic infiltration with the molecular classification

	Lymphocytic infiltration		
Molecular Classification	**Present (% of MC)**	**Absent (% of MC)**	**Total**
Luminal A	17(77)	5(23)	22
Luminal B	4(80)	1(20)	5
HER2	4(50)	4(50)	8
Hybrid	4(80)	1(20)	5
Basal	11(79)	3(21)	14
Unclassified	4(67)	2(33)	6
Total	44(73)	16(27)	60
Pearsons chi square test	**0.706**		

Increased amount of necrosis indicates a bad prognosis. 10 cases (16.7%) showed presence of necrosis. Majority of the cases (83.3%) showed no evidence of necrosis as shown in the Table 17 and Chart 13.

Luminal A tumors did not show evidence of necrosis. 38% of HER 2 type and 40% of luminal B showed presence of necrosis. 20% of hybrid and basal types and 17% of unclassified type had necrosis. There was no statistical significance with this comparison. (Table 17 and Chart 13)

Table 17: Association of necrosis with the molecular classification.

	NECROSIS		
Molecular Classification	**Present (% of MC)**	**Absent (% of MC)**	**Total**
Luminal A	0	22(100)	22
Luminal B	2(40)	3(60)	5
HER2	3(38)	5(62)	8
Hybrid	1(20)	4(80)	5
Basal	3(21)	11(79)	14
Unclassified	1(17)	5(83)	6
Total	10(17)	50(83)	60
Pearsons chi square test	**0.104**		

Involvement of skin is considered to be stage IV disease. In this study in 91.7% of cases, the skin was not involved. 8.3% of cases had involvement of skin as shown in Table 18 and Chart 14.

Table 18: Association of skin involvement with the molecular classification.

	Skin infiltration		
Molecular Classification	**Present (% of MC)**	**Absent (% of MC)**	**Total**
Luminal A	1(5)	21(95)	22
Luminal B	0	5(100)	5
HER2	0	8(100)	8
Hybrid	1(20)	4(80)	5
Basal	1(7)	13(93)	14
Unclassified	2(33)	4(67)	6
Total	5(8)	55(92)	60
Pearsons chi square test	**0.191**		

Chart 12: Association of lymphocytic infiltration with the molecular classification.

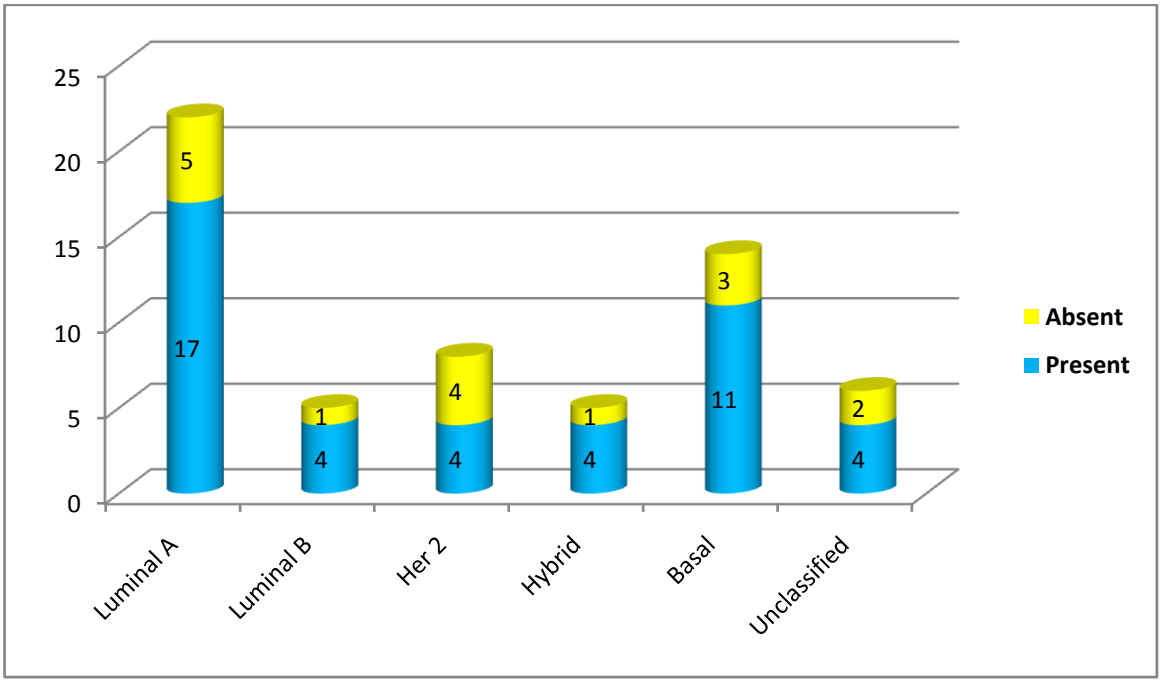
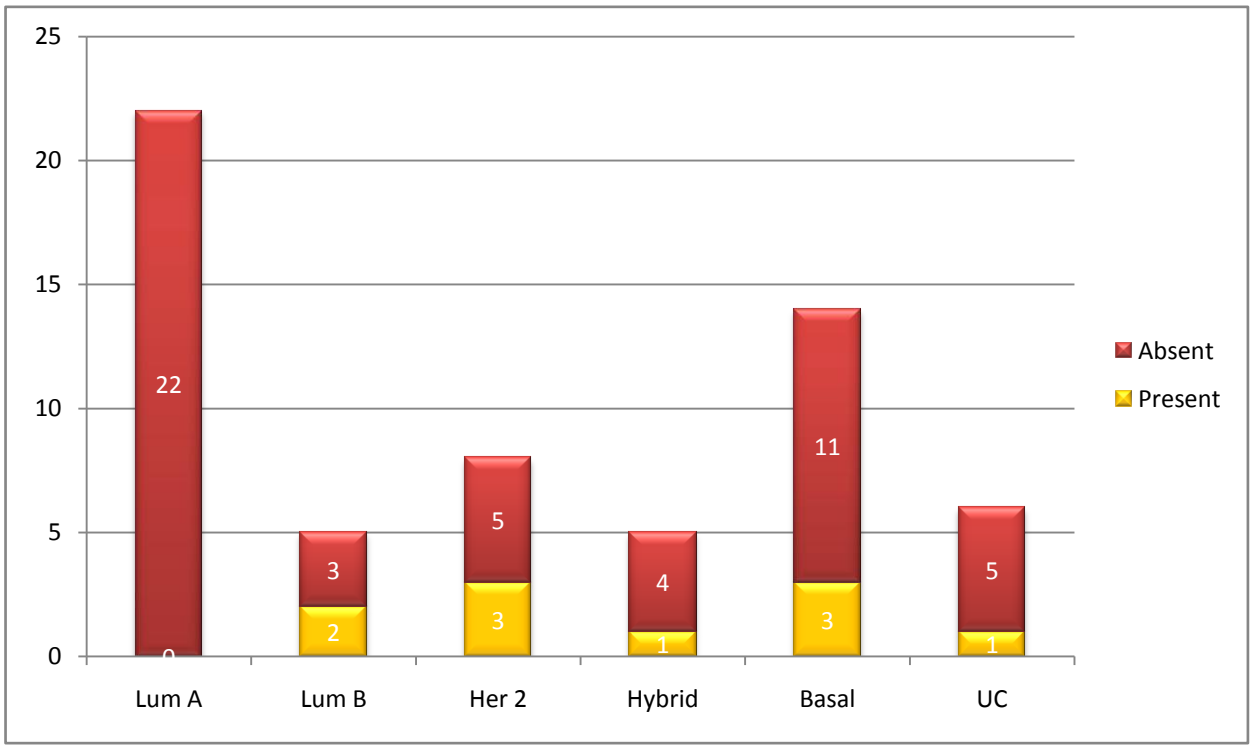


Chart 13: Association of necrosis with the molecular classification.



Out of 22 cases of luminal A only 1 case (4.5%) had skin involvement, none of the luminal B and HER 2 cases, 7% of basal and 20% of hybrid types showed involvement. Among the molecular subtypes the unclassified types had an increased incidence of skin involvement with 40%. This comparison did not have any statistical significance.(Table 18 and Chart 14)

5 cases (8.3%)did not show any lymphnode involvement. Majority of cases (35%) had lymph nodes positive between 4 and 9 nodes. 26% had 1 to 3 nodes positive and 30% had more than 10 nodes positive.(Table 19, Chart 19)

Table 19: Association of lymph node involvement with molecular classification.

	Number of Lymph nodes involved				
Molecular Classification	**Nil (% of MC)**	**1-3 (% of MC)**	**4-9 (% of MC)**	**>9 (% of MC)**	**Total**
Luminal A	3(17)	9(40)	6(27)	4(18)	22(36.7)
Luminal B	0	2(40)	2(40)	1(20)	5(8.30
HER2	0	2(25)	(25)2	4(50)	8(13.3)
Hybrid	0	1(20)	3(60)	1(20)	5(8.3)
Basal	0	2(14)	4(28)	8(56)	14(23.3)
Unclassified	2(33)	0	4(67)	0	6(10)
Total	5(8.3)	16(27)	21(35)	18(30)	60(100)
Pearsons chi square test	**0.045**				

Among the molecular classification only luminal A (5%) and unclassified types (3.3%) had cases without lymph node involvement. Among luminal A class only 18% had more than 10 nodes involved.

Luminal A, luminal B, hybrid and unclassified cases had more cases with 4-9 nodes involvement. HER2 (50%) and basal types (57%) had more number of cases with more than 10 nodes involvement. None of the unclassified cases had nodal involvement of more than 10 nodes. Lymph node involvement had a statistical significance. (Table19 and Chart 15)

Margin status is an important prognostic factor. In this study, 14 cases (23.3%) had involved margins, and 46 (76.7%) cases had margins free of tumor infiltration as shown in Table 20 and Chart 16.

Table 20: Association of margin status with molecularclassification.

	Margins		
Molecular Classification	**Present (% of MC)**	**Absent (% of MC)**	**Total**
Luminal A	3(14)	19(86)	22
Luminal B	0	5(100)	35
HER2	3(38)	5(63)	8
Hybrid	1(20)	4(80)	5
Basal	5(36)	9(64)	14
Unclassified	2(33)	4(67)	6
Total	14(23)	46(77)	60
Pearsons chi square test	**0.399**		

HER 2, basal and unclassified types showed increased incidence of involvement of margins, comprising of 38%, 36% and 33% respectively. Luminal B tumors did not show any margin involvement. Luminal A and hybrid tumors had lesser involvement of margins constituting 14 and 20% respectively.

Chart 14: Association of skin involvement with the molecular classification

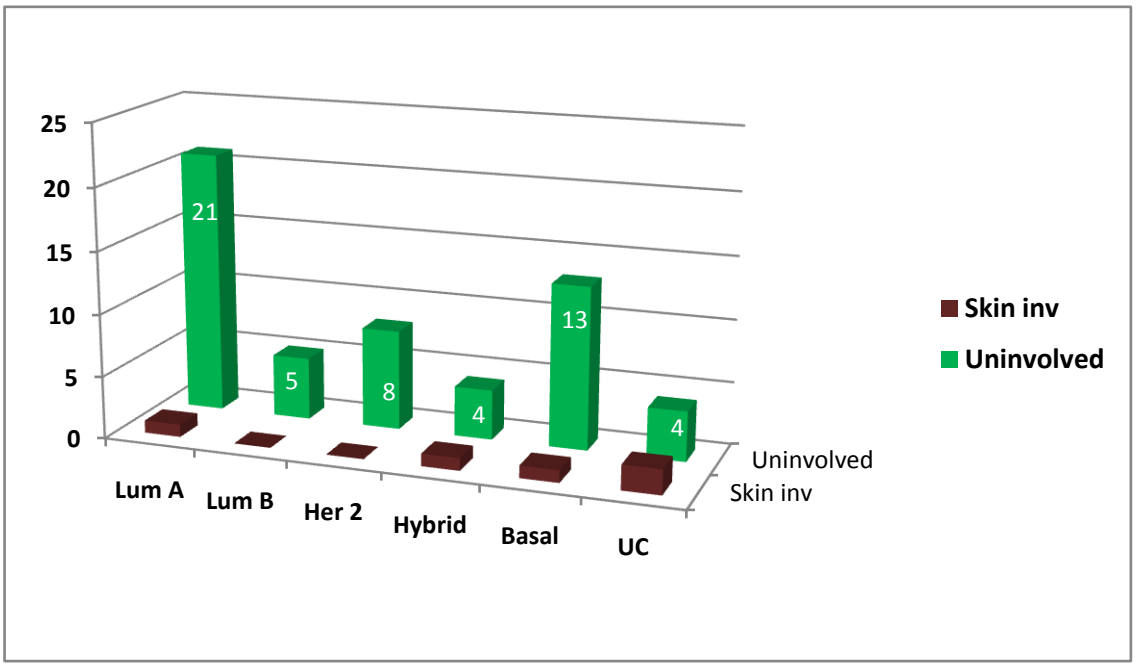
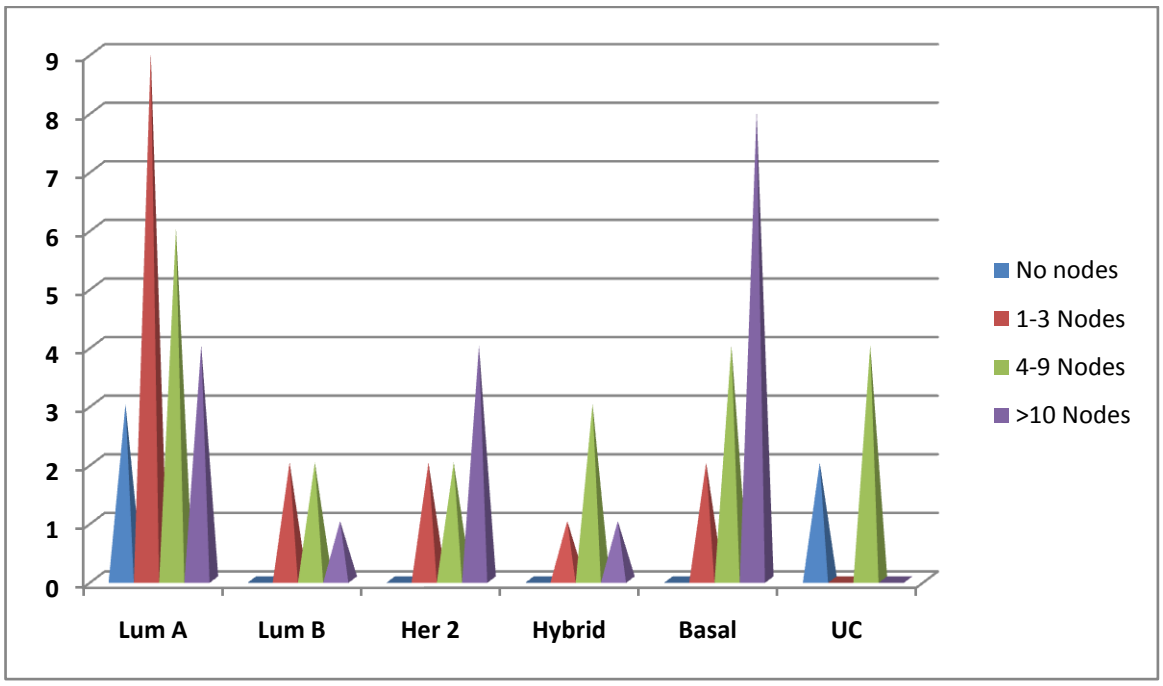


Chart 15: Association of lymph node involvement with molecular classification.



On analysing the stage of the tumor, majority of cases presented with the stage II tumors, among which were 20% of Stage II A and 42% of stage II B. 17% of cases landed up in stage III A. There were 2 cases (3.3%) with Stage I and 1 case (1.7%) with stage IV as shown in Table 21 and Chart 17.

Table 21: Association of molecular classification with stage of the tumor.

	STAGE							
Molecular Classification	**I (% of MC)**	**IIA (% of MC)**	**IIB (% of MC)**	**IIIA (% of MC)**	**IIIB (% of MC)**	**IIIC (% of MC)**	**IV (% of MC)**	**Total**
Luminal A	1(5)	6(28)	12(55)	2(9)	1(5)	0	0	22
Luminal B	0	1(20)	2(40)	2(40)	0	0	0	5
HER2	0	1	2(25)	5(63)	0	0	0	8
Hybrid	0	0	0	3(60)	1(20)	1(20)	0	5
Basal	0	3(21)	8(57)	2(14)	0	0	1(7)	14
Unclassified	1(17)	1(17)	1(17)	3(50)	0	0	0	6
Total	2(3)	12(20)	25(42)	17(28)	2(3)	1(2)	1(2)	60
Pearson chi square test	**0.083**							

Among the 22 cases of luminal A type tumors, 86% (18 cases with stage II and 1 case with stage I) of cases belonged to earlier stages (I&II) with only three cases (5%) in stage III. Luminal B and unclassified types had 40% and 50% respectively in stage IIIA tumors. HER 2type and unclassified types had tumors in stage IIIA but not beyond that. All the 5 cases of hybrid types and 21% of basal types were in stage III. The one case that presented in stage IV belongs to the basal type. This did not have any statistical significance.

Chart 16: Association of margin status with molecularclassification.

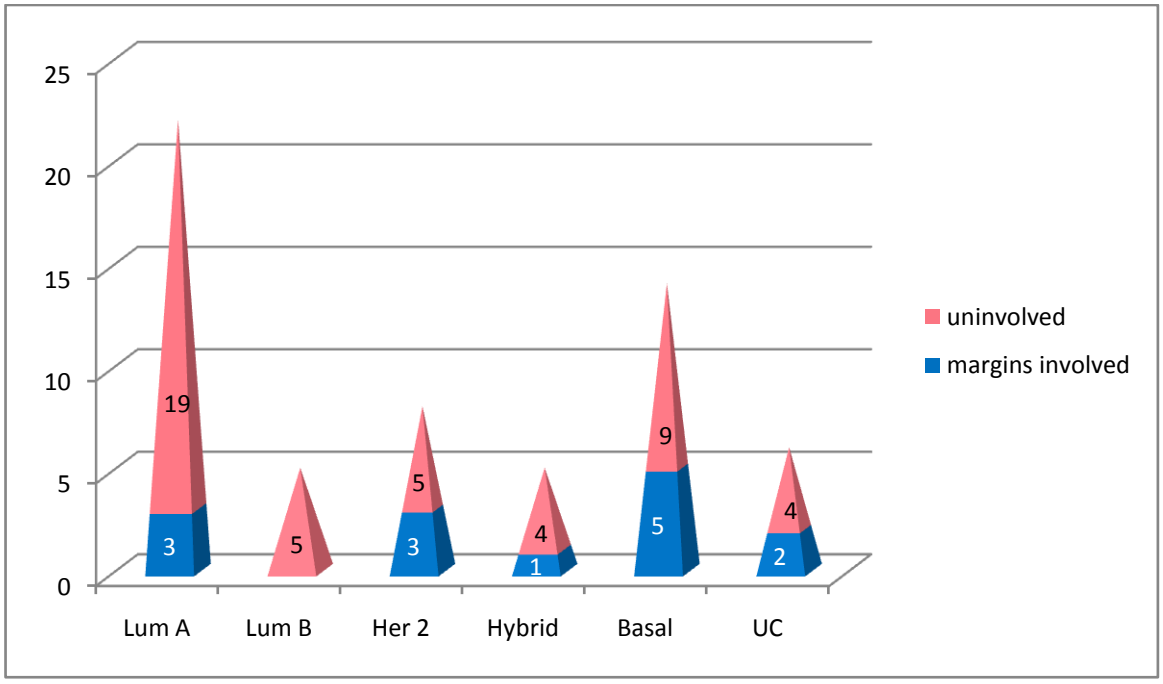
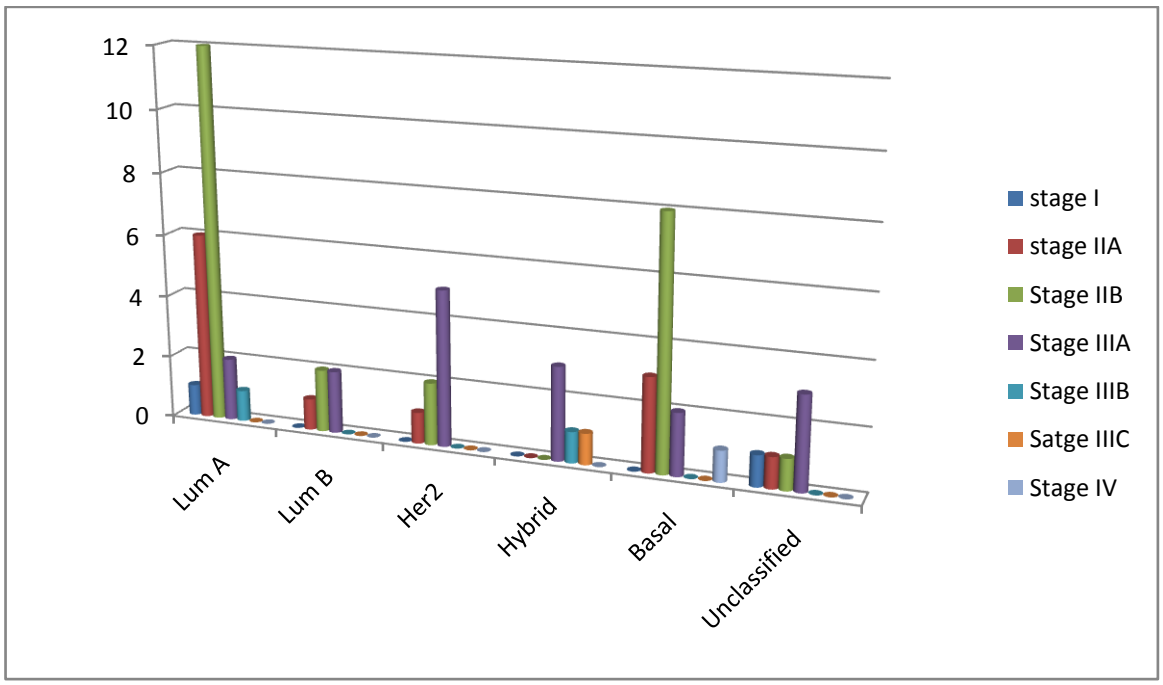


Chart 17: Association of molecular classification with stage of the tumor.



Hormone receptor analysis was done for all the 60 cases and their expression was analysed.

Table 22: ER expression among molecular subtypes.

	ER EXPRESSION				
Molecular Classification	**Negative (% of MC)**	**Weakly positive (% of MC)**	**Intermediate positive (% of MC)**	**Strongly positive (% of MC)**	**Total**
Luminal A	0	0	7(31)	15(69)	22
Luminal B	0	2(40)	3(60)	0	5
HER2	8(100)	0	0	0	8
Hybrid	0	0	5(100)	0	5
Basal	13(93)	1(7)	0	0	14
Unclassified	6(100)	0	0	0	6
Total	32(53)	3(5)	15(25)	15(20)	60
Pearsons chi square test	**0.003**				

Among the 22 luminal A cases, 69% showed strong positivity for estrogen receptor with a composite scoring of 7 and 8. (Annexure V). 31 % showed an intermediate positivity with a composite scoring of 4, 5, and 6. 40% of luminal B showed weakly positivity and 60% had intermediate scoring. All cases of the HER2 and unclassified types, 93% of basal types were negative for hormone receptors. All the 5 cases of hybrid tumors showed intermediate staining. (Table 22 and Chart 18). This had a statistically significant value.

Table 23: PR expression among molecular subtypes.

	PR EXPRESSION				
Molecular Classification	**Negative (% of MC)**	**Weakly positive (% of MC)**	**Intermediate positive (% of MC)**	**Strongly positive (% of MC)**	**Total**
Luminal A	3(14)	0	6(27)	13(59)	22
Luminal B	0	2(40)	2(40)	1(20)	5
HER2	8(100)	0	0	0	8
Hybrid	3(60)	0	2(40)	0	5
Basal	13(93)	1(7)	0	0	14
Unclassified	6(100)	0	0	0	6
Total	33(55)	5(8.3)	8(13)	14(23)	
Pearsons chi square test	**0.001**				

3 cases (14%) of luminal A was negative for PR expression, 27% with intermediate staining and 59% had strong nuclear expression. Among luminal B weak and intermediate staining was 40% each and 1 case (20%) had strong positivity. All the HER2 and unclassified types and 93% of basal types were negative. Among the 5 hybrid cases 3 were negative and 2 were intermediate. This comparison had statistical significance as shown in Table 23 and Chart 19.

Chart18: ER expression among molecular subtypes.

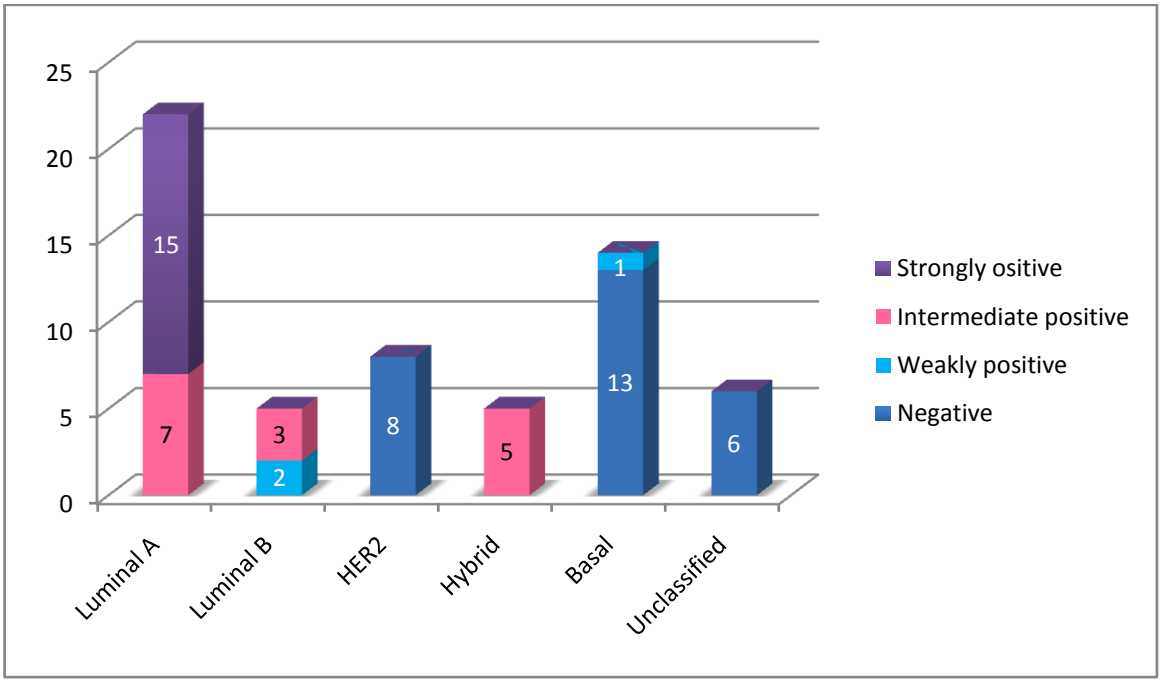


Chart19: PR expression among molecular subtypes.

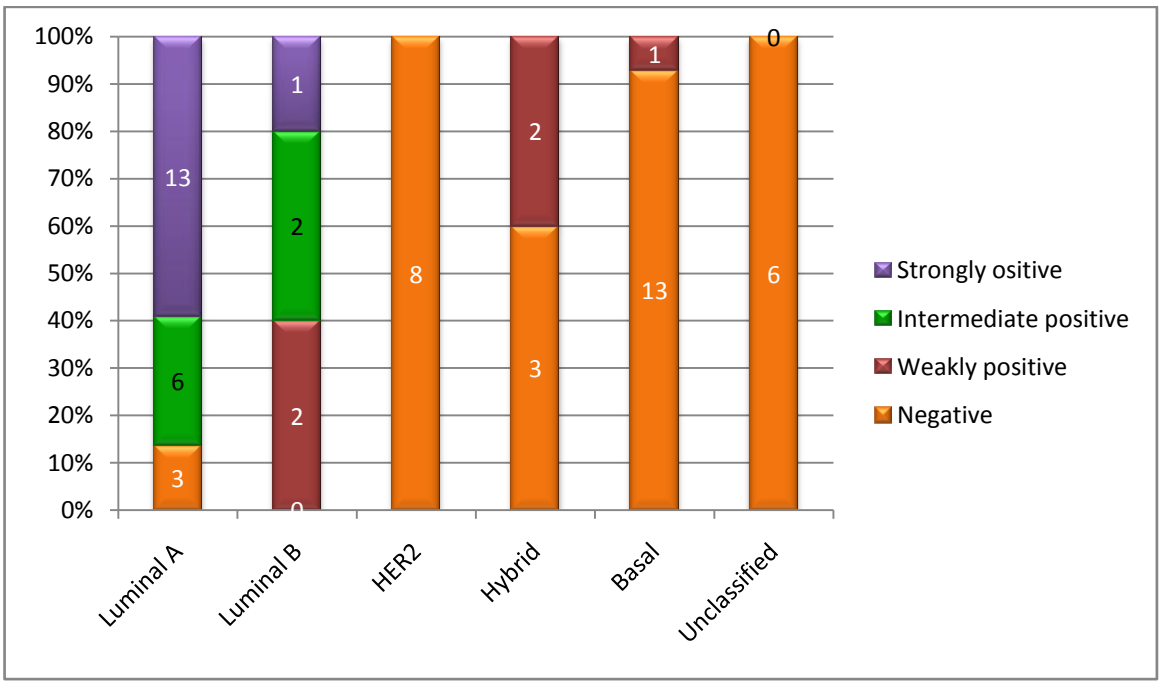


Table 24: HER2 neu expression among molecular subtypes.

	HER 2 neu EXPRESSION				
Molecular Classification	**0 (% of MC)**	**1+ (% of MC)**	**2+ (% of MC)**	**3+ (% of MC)**	**Total**
Luminal A	21(95)	0	1(5)	0	22
Luminal B	0	1(20)	3(60)	1(20)	5
HER2	0	0	1(12)	7(88)	8
Hybrid	0	0	0	5(100)	5
Basal	12(86)	1(7)	1(7)	0	14
Unclassified	5(83)	1(17)	0	0	6
Total	38(63)	3 (5)	4(7)	15(25)	60
Pearsons chi square test	**0.002**				

All the luminal A types except one was negative for HER 2 neu, and the 1 case had 2+ cytoplasmic membrane positivity. Luminal B types had 20 % of cases with 1+ and 2+ each, and 60% with strong 3+ positivity. 88% of HER2 had 3+ staining and rest 1 case (12%) had 2+ staining. All the hybrid cases were strongly positive (3+). 93% of basal were negative, and the rest 7% (1 case) showed 2+ staining. 100% of unclassified were negative. (0 and 1+). This is depicted in Table 24 and Chart 20.

Table 25: CK 5/6 expression among molecular subtypes.

	CK 5/6 EXPRESSION				
Molecular Classification	**1+ (% of MC)**	**2+ (% of MC)**	**3+ (% of MC)**	**4+ (% of MC)**	**Total**
Luminal A	18(82)	3(14)	1(4)	0	22
Luminal B	2(40)	2(40)	1(20)	0	5
HER2	5(63)	3(37)	0	0	8
Hybrid	2(40)	2(40)	1(20)	0	5
Basal	0	1(7)	5(36)	8(57)	14
Unclassified	4(67)	2(33)	0	0	6
Total	29(48)	11(18)	8(14)	12(20)	60
Pearsons chi square test	**0.001**				

On analysing the CK 5/6 expression, among luminal A type, 82% were negative, 14% had 2+ and 4% had 1+ staining. 40% of luminal B had negative and 2+ staining respectively and 1 case (20%) with 3+ staining. Among HER2 63% was negative and 37% had 2+ staining. 40% of hybrid cases had 1+ and 2+each, and 20% had 3+ staining. Among the basal cases 57% had 4+ strong positivity, 36% with 3+ and 7% with 2+ staining. 67% of unclassified were negative and 33% showed 2+ positivity. Only basal types had 4+ staining. This comparison had statistical significance.(Table 25 and Chart 21).

Chart 20: HER2 neu expression among molecular subtypes.

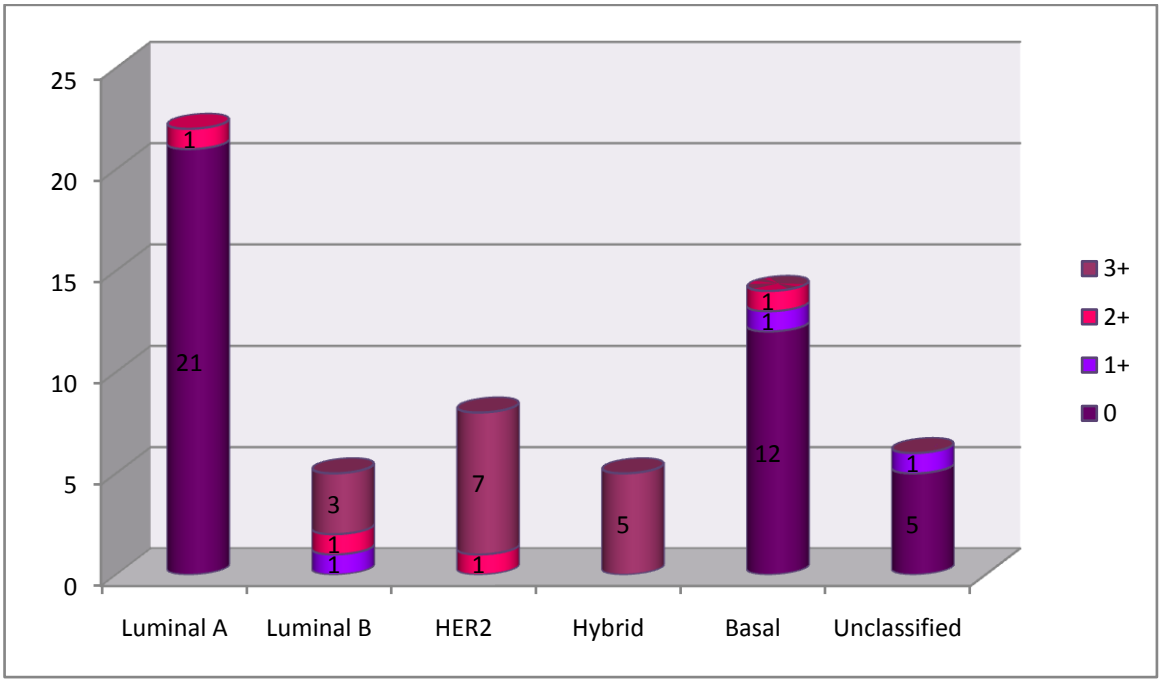
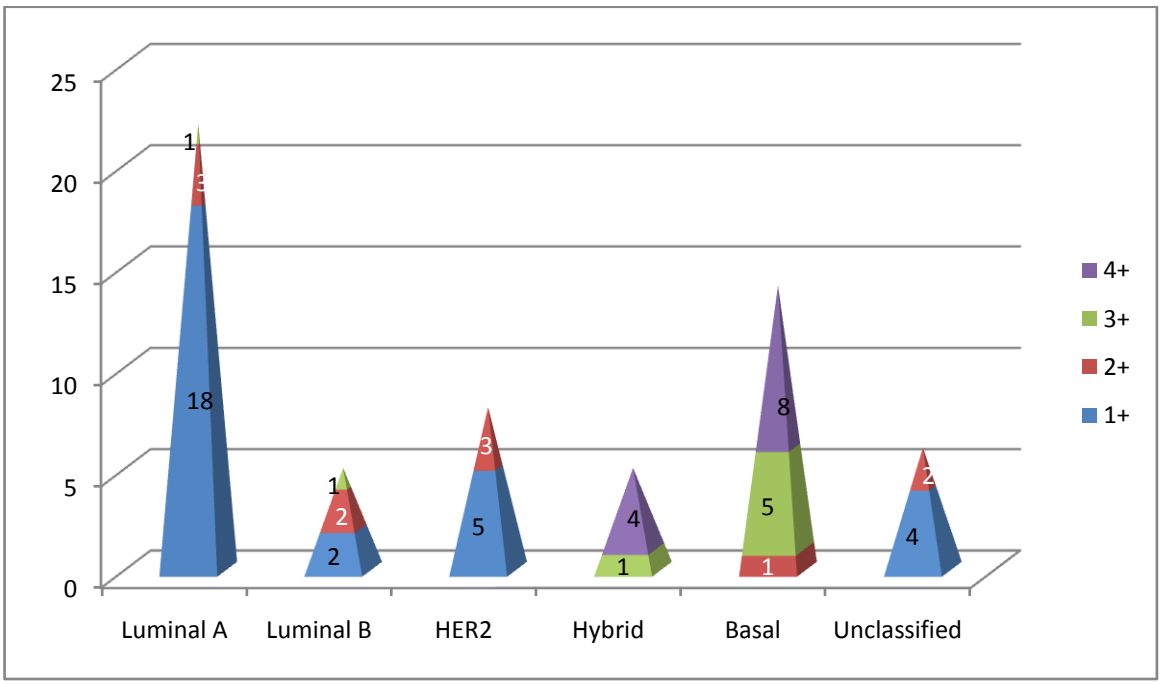


Chart 21: CK 5/6 expression among molecular subtypes.



On comparing the proliferation index within the molecular subtypes, it was found that luminal B, HER 2, hybrid and basal types had high proliferation index of 100%, 75%, 80% and 85% respectively. 86% of luminal A tumors had a low proliferation index. Unclassified tumors had an equal proportion (50%) of low and high proliferation indices. (Table 26& Chart 22)

Table 26: Comparison of proliferation index among molecular subtypes

	PROLIFERATION INDEX		
Molecular Classification	**HIGH (% of MC)**	**LOW (% of MC)**	**Total**
Luminal A	3(14)	19(86)	22
Luminal B	5(100)	0	5
HER2	6(75)	2(25)	8
Hybrid	4(80)	1(20)	5
Basal	12(86)	2(14)	14
Unclassified	3(50)	3(50)	6
Total	33(55)	27(45)	60
Pearsons chi square test	**0.01**		

The follow up was done for all cases for a minimum period of one year. Among the 60 cases, 47 cases (78%) were alive and healthy, 10 cases (16.7%) had recurrences and 3 cases (5%) were dead due to tumor complications. (Table 26 and Chart 23).

Table 27: Follow up of molecular subtypes of breast cancers.

	FOLLOW UP			
Molecular Classification	**Alive &Healthy (% of MC)**	**Alive & recurred (% of MC)**	**DEAD (% of MC)**	**Total**
Luminal A	21(95)	1(5)	0	22
Luminal B	5(100)	0	0	5
HER2	5(63)	2(25)	1(12)	8
Hybrid	3(60)	1(20)	1(20)	5
Basal	9(65)	4(28)	1(7)	14
Unclassified	4(67)	2(33)	0	6
Total	47(78)	10(17)	3(5)	60
Pearsons chi square test	**0.265**			

One case of luminal A (0.4%) and hybrid types (20%), 2 case of HER 2(25%) and unclassified types (30%) and 4 cases of basal type (28%) had recurrence. One case in each HER2, hybrid and basal type died during the follow up period. Rest of the cases were alive and healthy.

Chart 22: Comparison of proliferation index among molecular subtypes

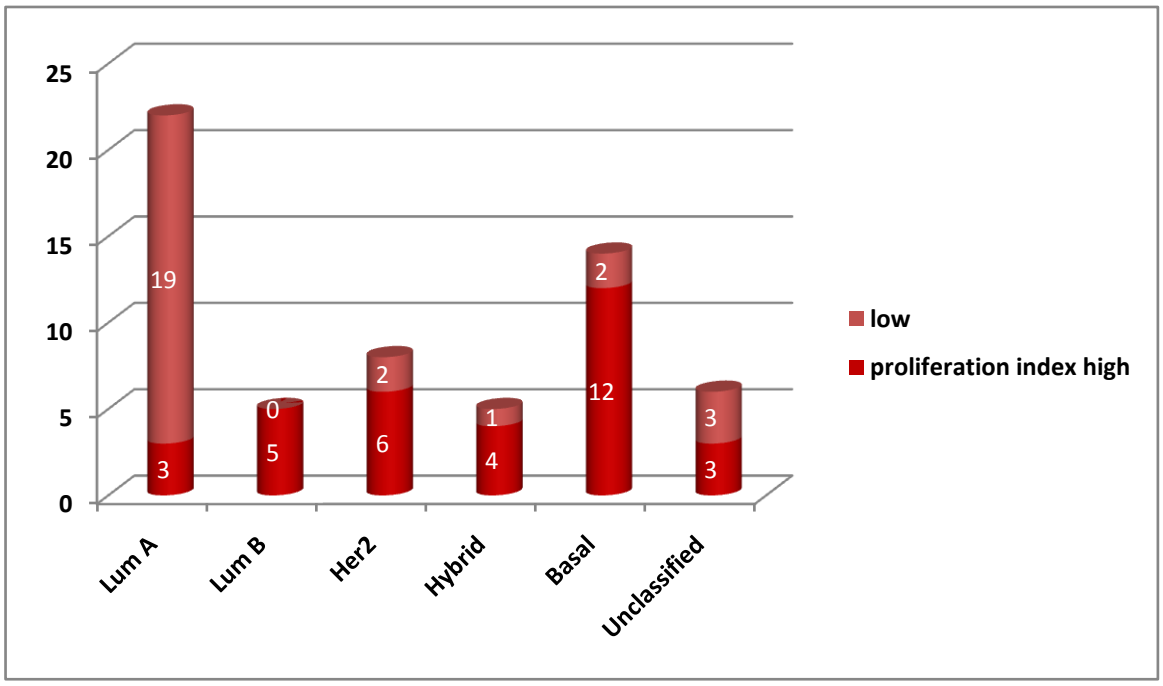
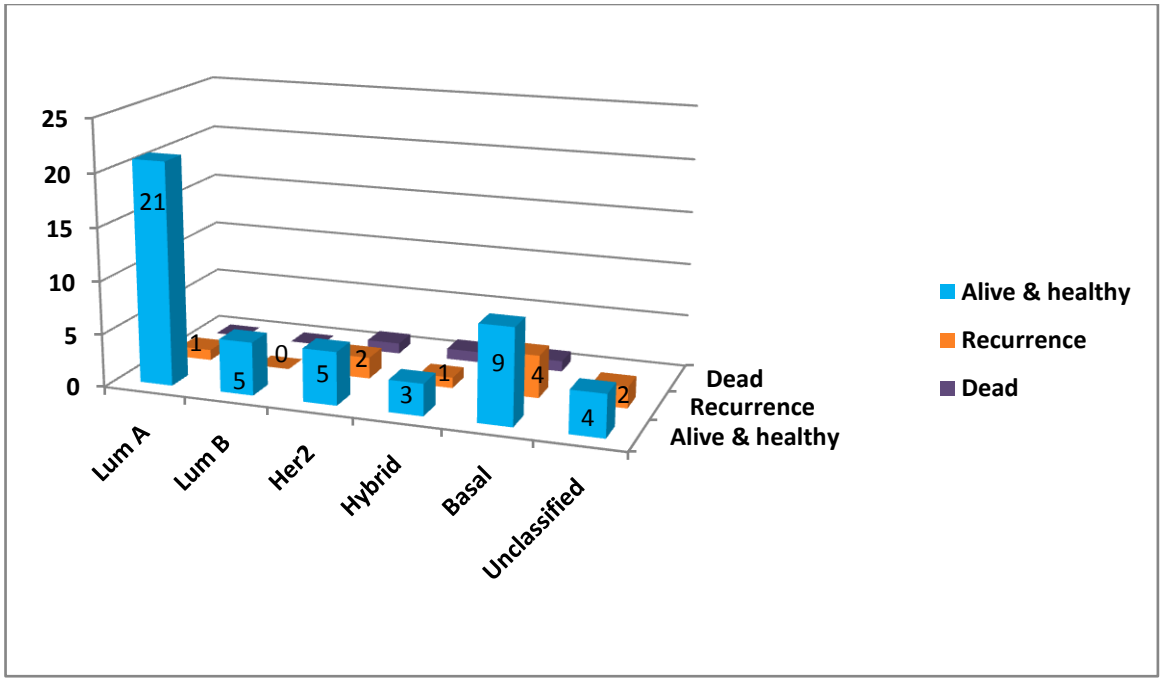


Chart 23: Follow up of molecular subtypes of breast cancers.



In this current study the same set of patients were given the histopathological classification and then subjected to immunohistochemical analysis and classified under molecular classification aiming targeted therapy to the patients.

Mc nemar's test and inter rater agreement:

The aim of this test is to evaluate the inter rater agreement between the histopathological and molecular classification systems and to quantitate the agreement with the KAPPA value by using the 2x2 classification tables.

Each subtype under the molecular classification is compared with histopathological classification which is divided as infiltrating ductal carcinoma no special types and special variants by using the 2x2 tables. P value and the inter rater agreement KAPPA value is derived and analysed. A negative KAPPA value indicates that the strength of agreement between these two classification systems is very poor.

Negative agreement between these two classification systems helps in substantiating the use of targeted therapy based on molecular classification.

Table 28: Comparison of luminal A type with histopathological classification by Mc nemar's test.

	MOLECULAR CLASSIFICATION		
HPE CLASSIFICATION	**LUMINAL A**	**Others**	**Total**
IDC NST	5	25	30 (50%)
VARIANTS	17	13	30 (50%)
Total	22 (36%)	38 (64%)	60 (100%)
95% CI	7.51% to 35.26%		
Significance level	**P=0.2115**		
Inter rater agreement- KAPPA value	**-0.367**		

When Luminal A subtype was compared with the histological subtypes it was found that the P value was 0.2 and the KAPPA value was -0.367, which indicates that strength of agreement between these two systems was very poor and the agreement between them is worse than chance. These values are depicted in Table 28.

Table 29: Comparison of luminal B type with histopathological classification by Mc nemar's test.

	MOLECULAR CLASSIFICATION		
HPE CLASSIFICATION	**LUMINAL B**	**Others**	**Total**
IDC NST	2	28	30
VARIANTS	3	27	30
Total	5	55	60
95% CI	25.05% to 49.56%		
Significance level	**P < 0.0001**		
Inter rater agreement- KAPPA value	**-0.0333**		

On comparing the Luminal B and histological classification, it had a significant P value of <0.0001 and the inter rater agreement KAPPA value was -0.033. This negative value proves the disagreement between these two systems. This is illustrated in the Table 29.

Table 30: Comparison of HER 2 type with histopathological classification by Mc nemar's test.

	MOLECULAR CLASSIFICATION		
HPE CLASSIFICATION	**HER 2**	**Others**	**Total**
IDC NST	6	24	30 (50%)
VARIANTS	2	28	30 (50%)
Total	8 (13%)	52 (86%)	60 (100%)
95% CI	28.6% to 40%		
Significance level	**P < 0.0001**		
Inter rater agreement- KAPPA value	**-0.200**		

On comparing the HER 2 subtype and histological classification, it was found that it had a significant P value of <0.0001 and the inter rater agreement KAPPA value was -0.200. This negative value proves the agreement between these two systems are worse than chance. This is illustrated in the Table30.

Table 31: Comparison of Hybrid type with histopathological classification by Mc nemar's test.

	MOLECULAR CLASSIFICATION		
HPE CLASSIFICATION	**Hybrid**	**Others**	**Total**
IDC NST	2	28	30 (50%)
VARIANTS	3	27	30 (50%)
Total	5 (8.3%)	55 (91.7%)	60 (100%)
95% CI	25.05% to 49.56%		
Significance level	**P < 0.0001**		
Inter rater agreement- KAPPA value	**-0.0333**		

On comparing the Hybrid subtype with the histopathological classification, it was found that it had a significant P value of <0.0001 and the inter rater agreement KAPPA value was -0.033. This negative value proves the disagreement between these two systems. This is illustrated in the Table31.

Table 32: Comparison of Basal type with histopathological classification by Mc nemar's test.

	MOLECULAR CLASSIFICATION		
HPE CLASSIFICATION	**BASAL**	**Others**	**Total**
IDC NST	9	21	30 (50%)
VARIANTS	5	25	30 (50%)
Total	14 (23.3%)	46 (76.7%)	60 (100%)
95% CI	4.79% to 36.69%		
Significance level	**P = 0.0140**		
Inter rater agreement- KAPPA value	**-0.0346**		

On comparing the Basal subtype with the histopathological classification, it was found that it had a significant P value of 0.01 and the inter rater agreement KAPPA value was -0.034. This negative value proves the disagreement between these two systems and agreement if any, is worse than chance. This is illustrated in the Table 32.

Table 33: Comparison of Unclassified type with histopathological classification by Mc nemar's test.

	MOLECULAR CLASSIFICATION		
HPE CLASSIFICATION	**UNCLASSIFIED**	**Others**	**Total**
IDC NST	5	25	30 (50%)
VARIANTS	1	29	30 (50%)
Total	6 (10%)	54 (90%)	60 (100%)
95% CI	23.14% to 44.18%		
Significance level	**P < 0.0001**		
Inter rater agreement- KAPPA value	**-0.100**		

On comparing the unclassified subtype with the histopathological classification, it was found that it had a significant P value of <0.0001 and the inter rater agreement KAPPA value was -0.100. This negative value proves the disagreement between these two systems. This is illustrated in the Table 33.

DUCTAL CARCINOMA

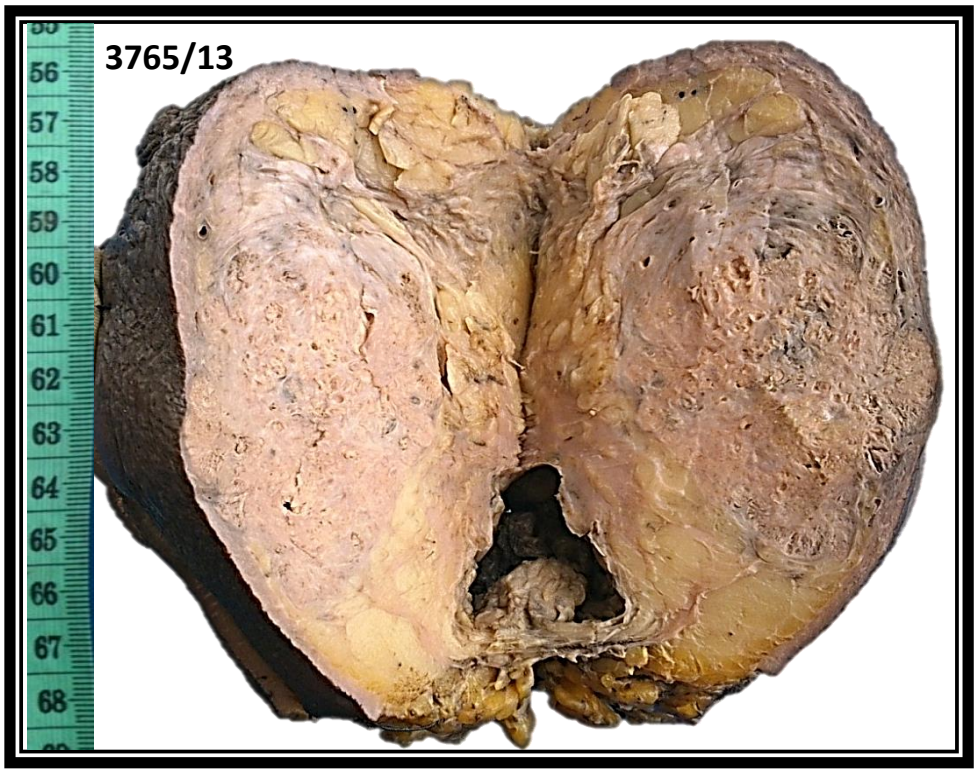


Figure 3: Grey white mass with infiltrating margins

APOCRINE CARCINOMA

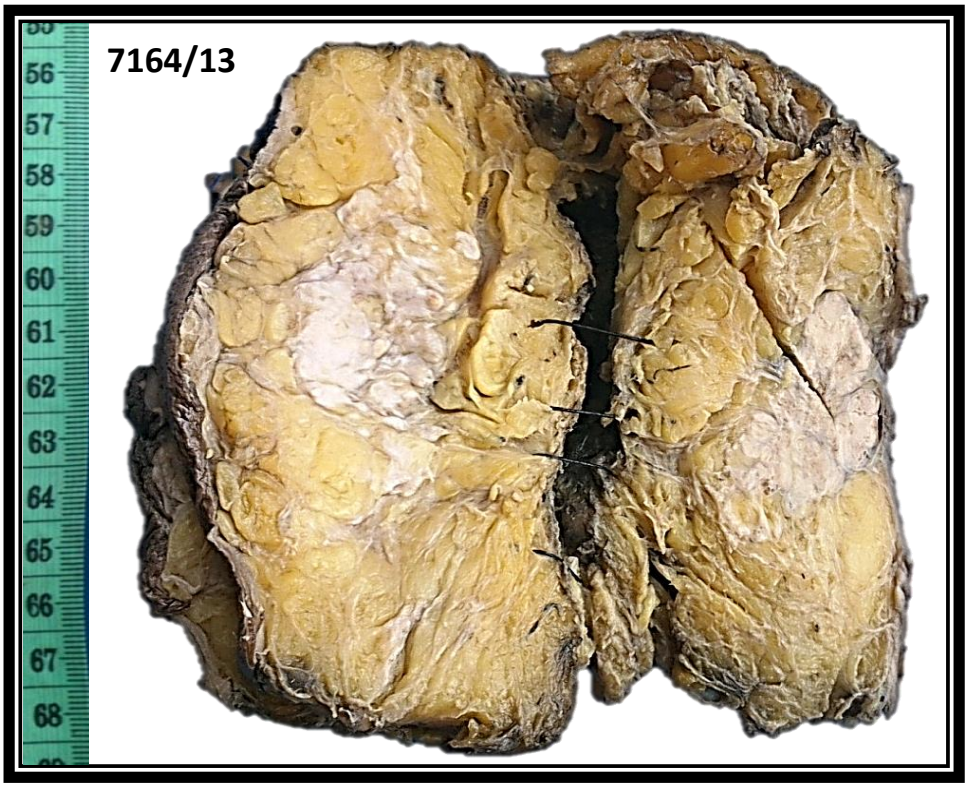


Figure 4: Well circumscribed, grey white nodule

LOBULAR CARCINOMA

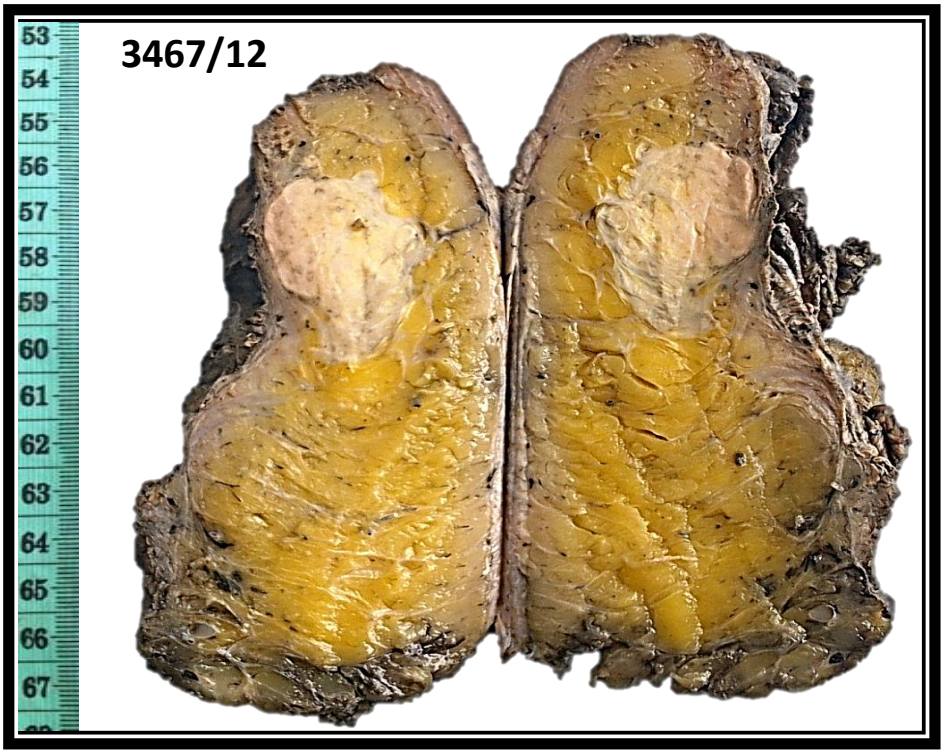


Figure5: Well circumscribed, grey white

METAPLASTIC CARCINOMA

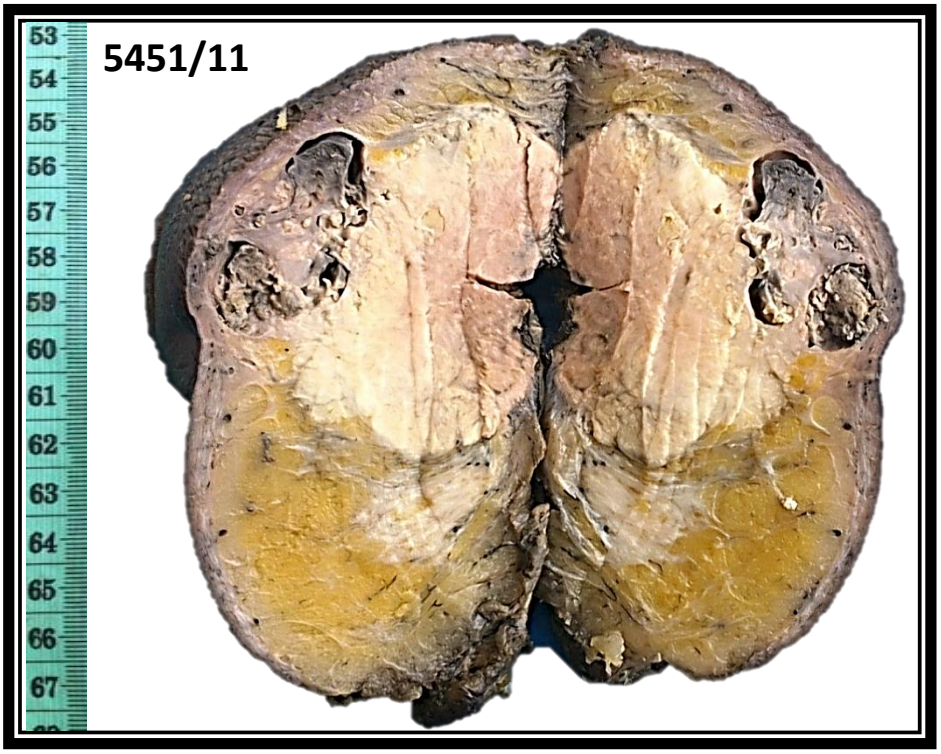


Figure 6: Well circumscribed, grey white growth with cystic degeneration

MUCINOUS CARCINOMA

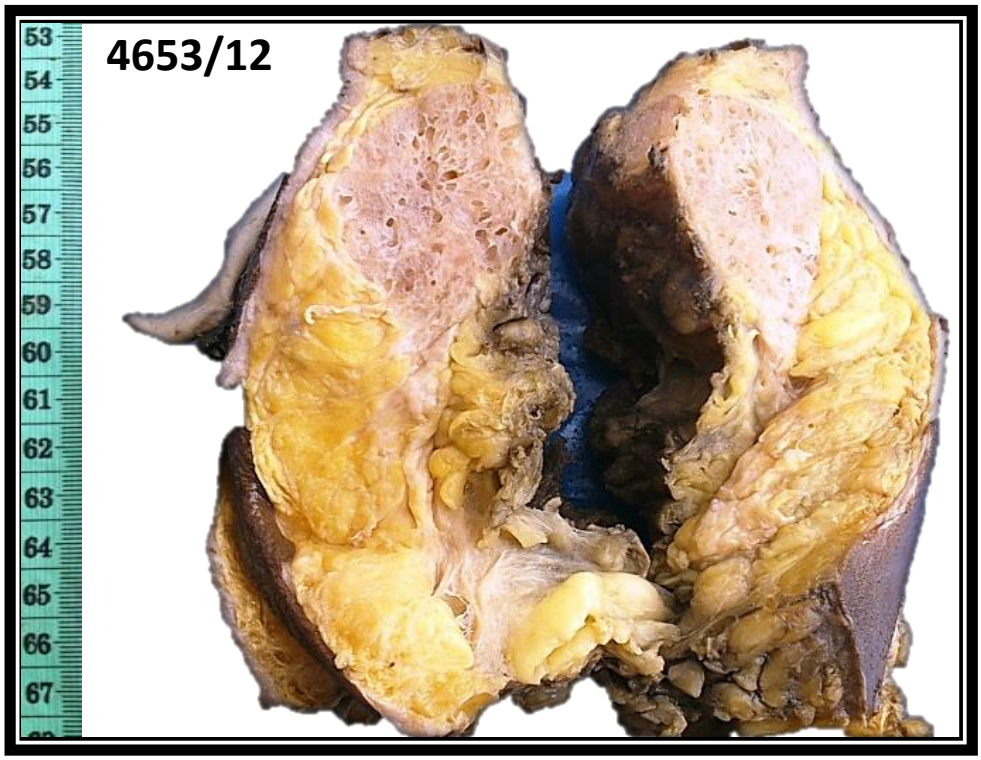


Figure7: Well circumscribed, multiple tiny cysts filled with gelatinous material

PAPILLARY CARCINOMA

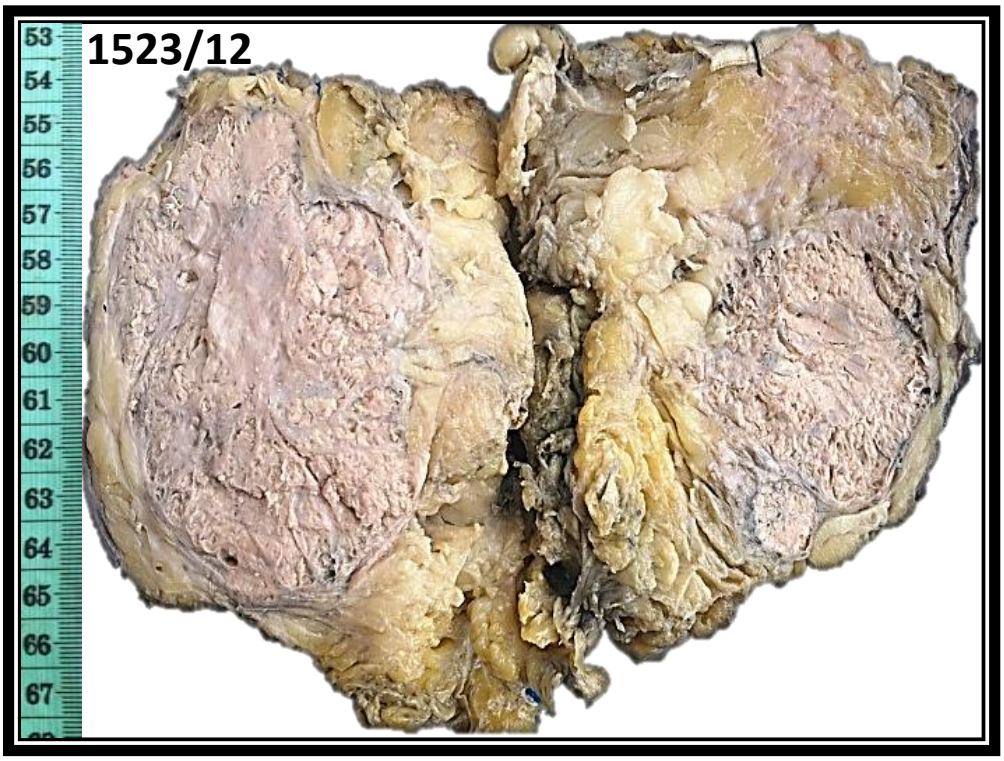


Figure8: Well circumscribed, grey tan with granular surface

MEDULLARY CARCINOMA

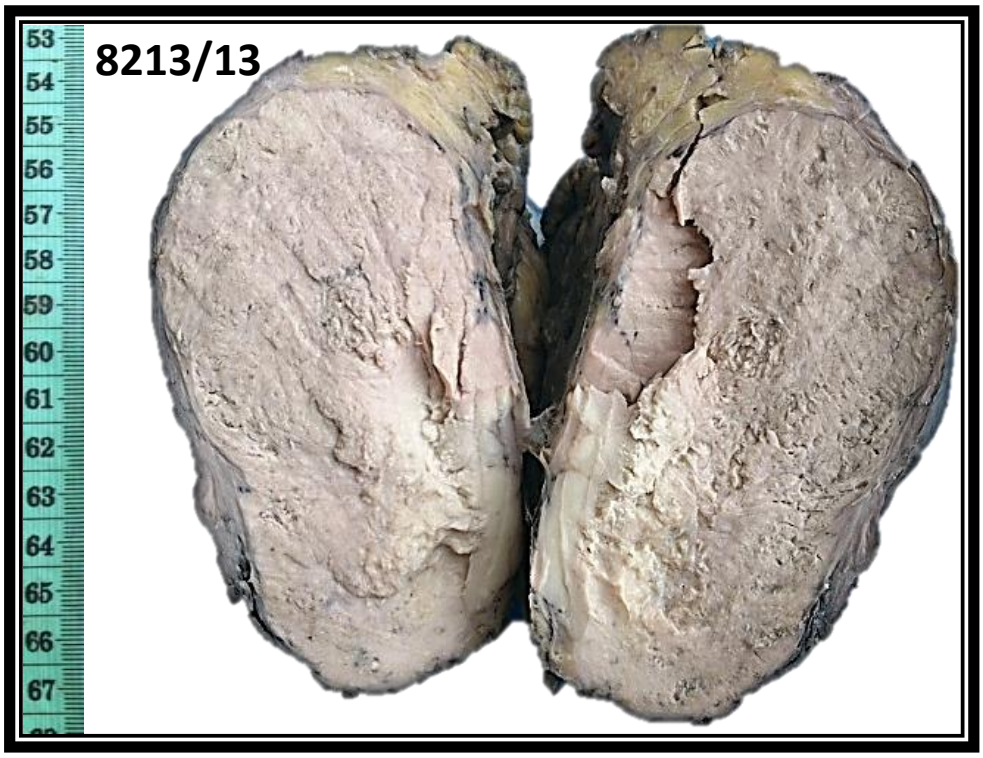


Figure9: Well circumscribed, fleshy mass.

INVASIVE DUCTAL CARCINOMA NOS - GRADE 1

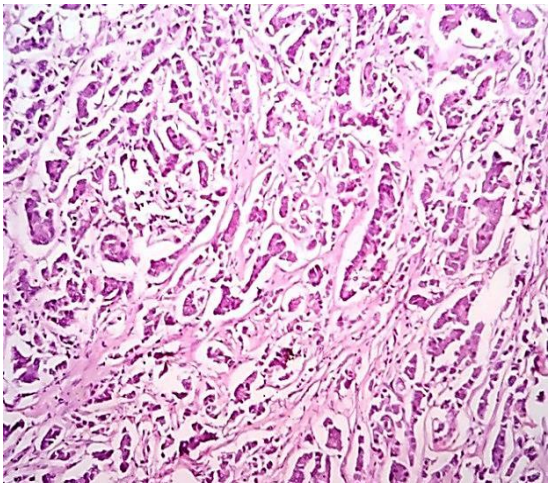


Figure 10: Invasive ductal carcinoma NST tubule formations in >75% tumor cells. HPE 3765/13, 40x

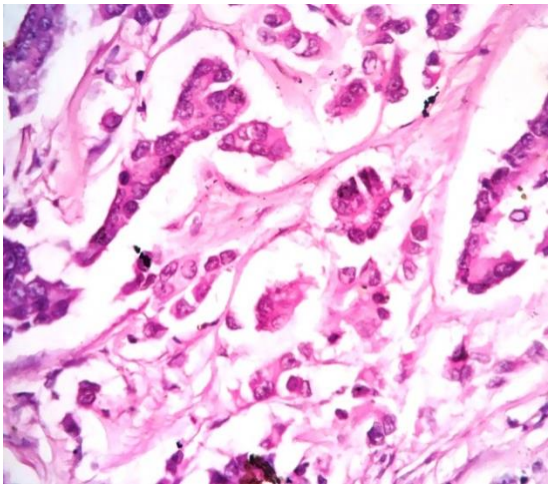


Figure11: Malignant ductal epithelial cells with mild nuclear pleomorphism & low mitosis. HPE 3765/13, 400x

INVASIVE DUCTAL CARCINOMA NOS GRADE 2

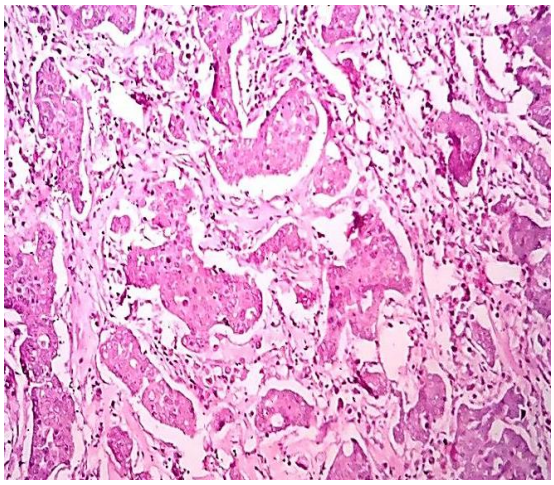


Figure 12: Sheets of malignant ductal epithelial cells, 30% tubule formation. HPE 3446/13, 100x

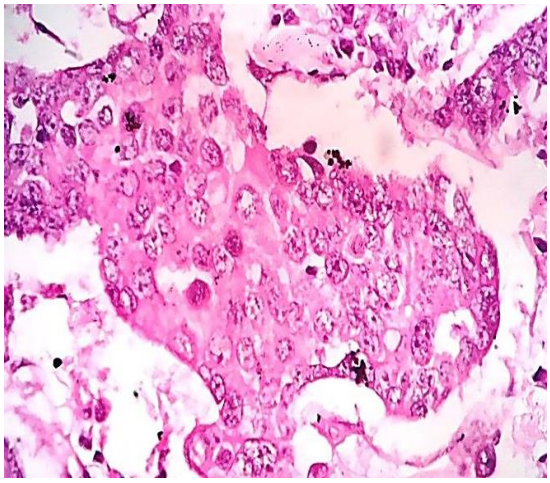


Figure 13: Malignant ductal epithelial cells in sheets, 30% tubules and mild nuclear pleomorphism. HPE 3446/13, 400x

INVASIVE DUCTAL CARCINOMA NOS - GRADE 3

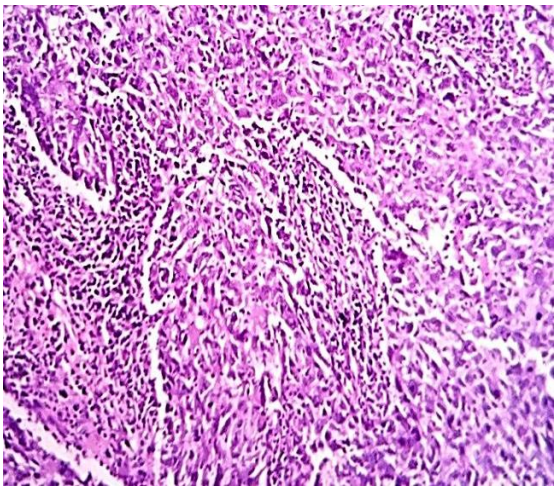


Figure 14: Malignant ductal epithelial cells in sheets. HPE 5379/13, 100x

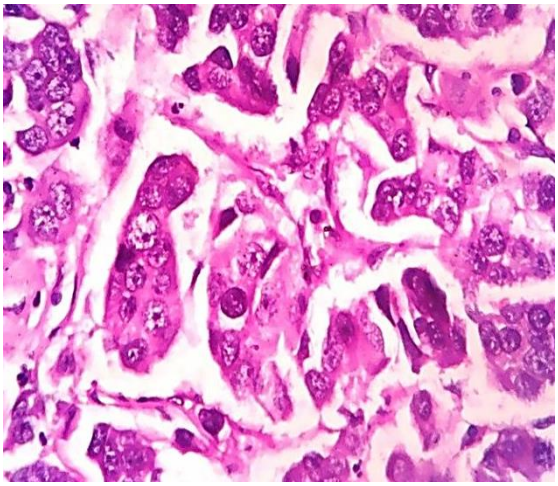


Figure 15: Malignant ductal epithelial cells with no tubules, marked nuclear, pleomorphism, increased mitosis . HPE 5379/13, 400x

MUCINOUS CARCINOMA

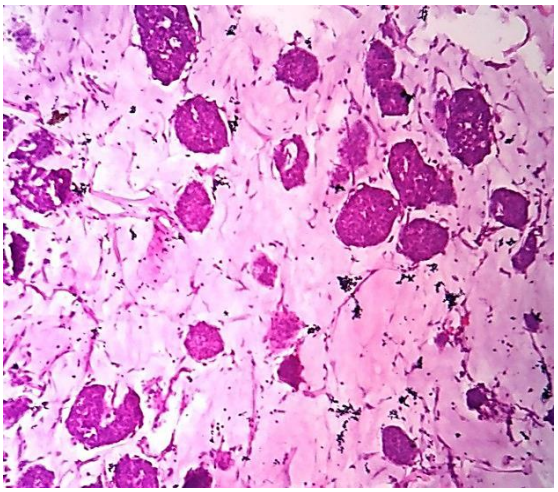


Figure 16: Tumor nests floating in mucin. HPE 4653/12, (100x)

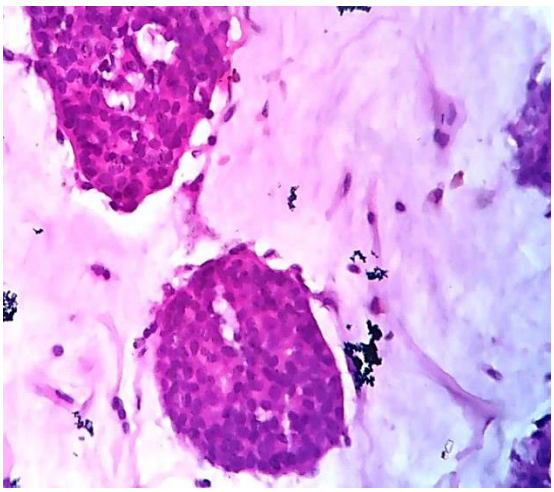


Figure 17: Malignant ductal epithelial cells with mild nuclear pleomorphism and no mitosis. HPE 4653/12, (400x)

LOBULAR CARCINOMA

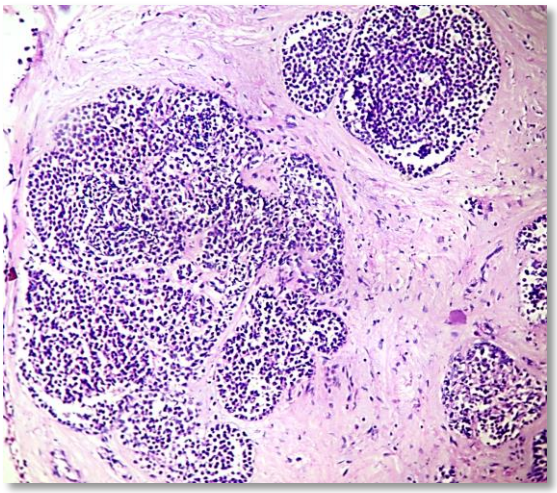


Figure 18: Tumor cells arranged in lobular pattern with pagetoid spread around ductal elements.
HPE 3467/12, (100x)

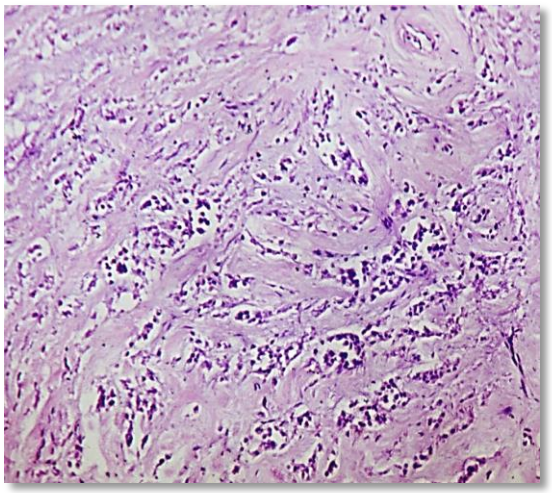


Figure 19: Tumor cells arranged in singles in Indian file pattern.
HPE 3467/12, (400X)

MEDULLARY CARCINOMA

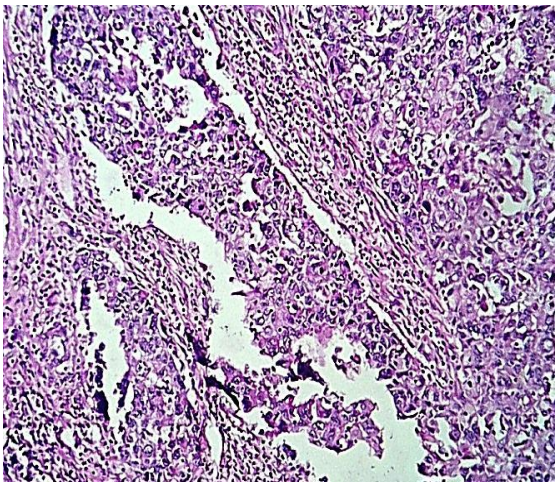


Figure 21: Nodular arrangement of tumor cells with lymphoplasmacytic infiltrate in periphery. HPE 8213/12 (100X)

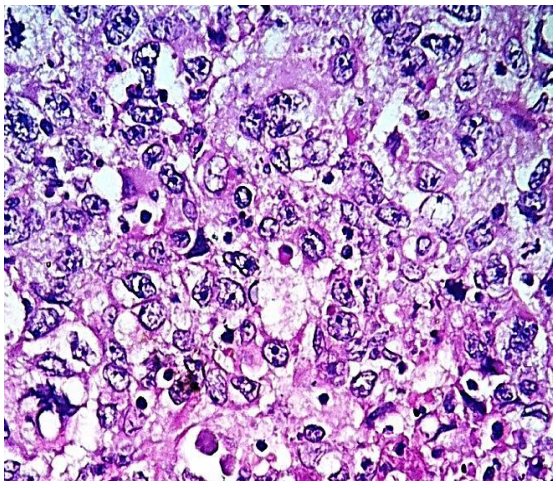


Figure 20: Tumor cells in syncytial pattern with marked nuclear pleomorphism and prominent nucleoli.
HPE 8213/12 (400X)

PAPILLARY CARCINOMA

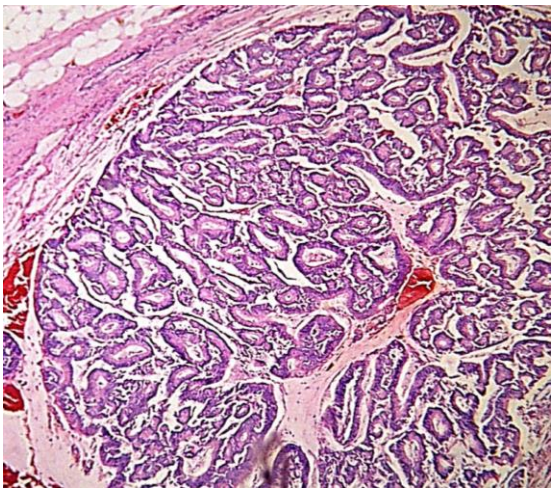


Figure 22: Tumor cells in papillary pattern. HPE 1523/12 (100x)

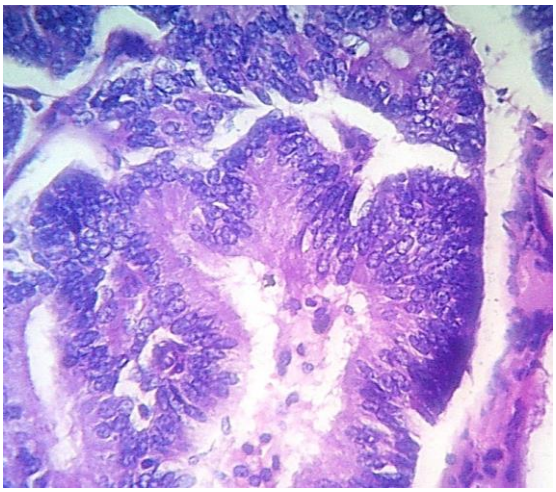


Figure 23: Tumor cells in delicate papillary pattern. HPE 1523/12 (400x)

APOCRINE CARCINOMA

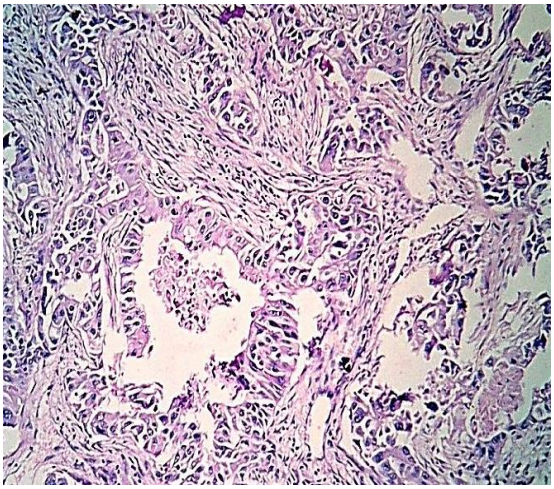


Figure 24 Apocrine cells in papillary pattern. HPE 6973/11 (100x)

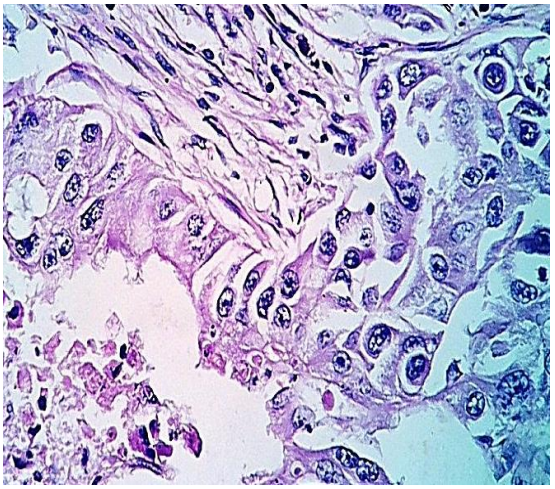


Figure 25: Apocrine cells with abundant granular eosinophilic cytoplasm. (400x) HPE 6973/11

METAPLASTIC CARCINOMA WITH SQUAMOUS DIFFERENTIATION

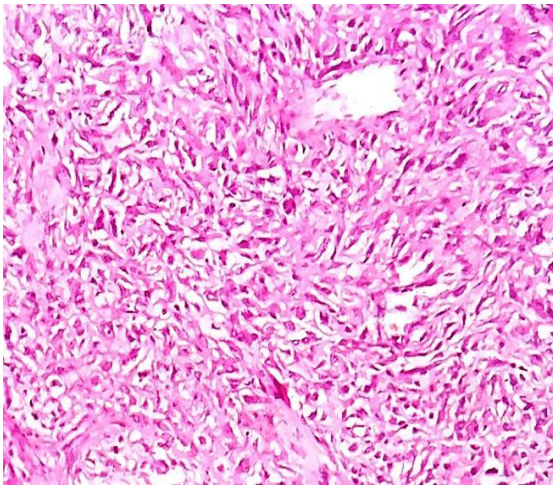


Figure 26: Nests of tumor cells with spindle cell differentiation. HPE 5451/11 (100x)

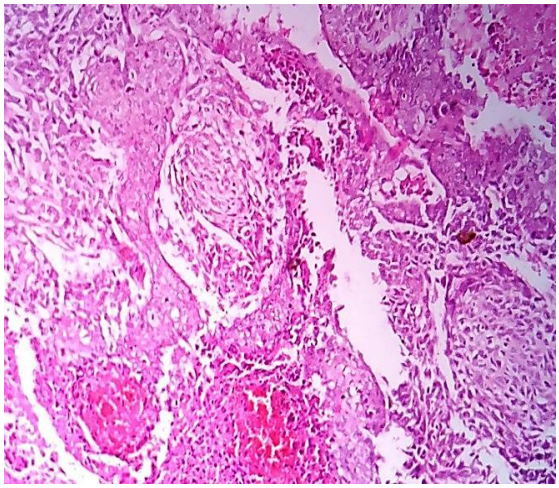


Figure 27: Squamous cell nests in between the tumour cells. HPE 5451/11 (400x)

OTHER PROGNOSTIC FACTORS

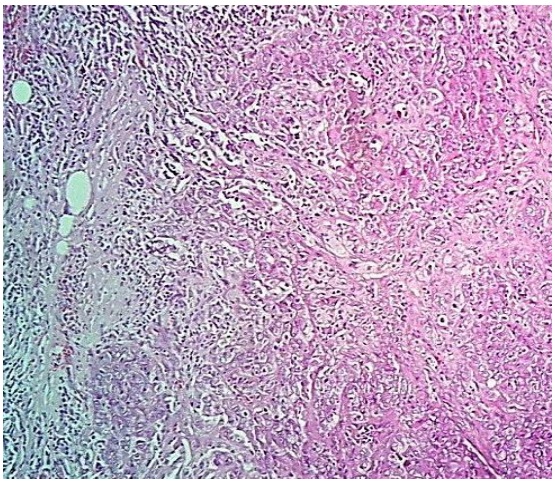


Figure 28: Metastatic deposit in node (100x). HPE 8469/13.

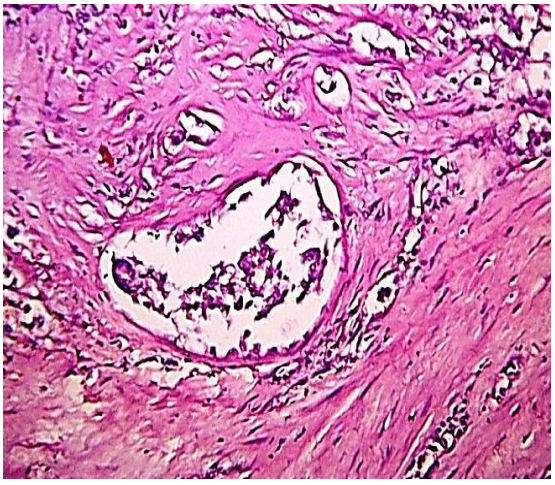


Figure 29: Lymphatic invasion. (100x). HPE 8407/13.

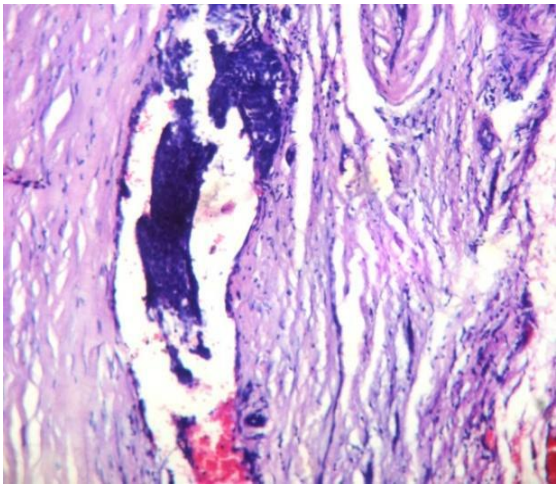


Figure 30: Vascular invasion (400x) HPE 8552/13

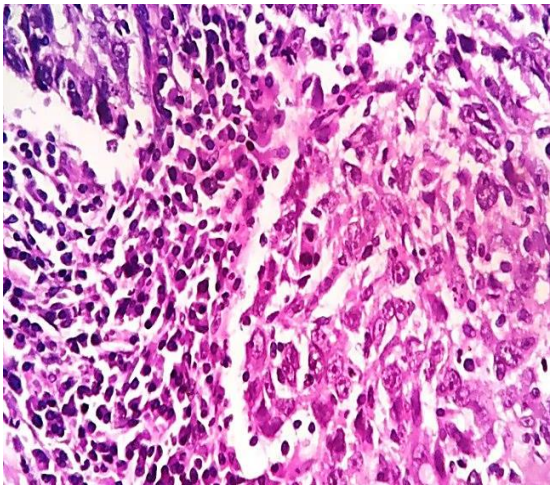


Figure 31: Lymphocytic infiltration. (400x) HPE 8213/12

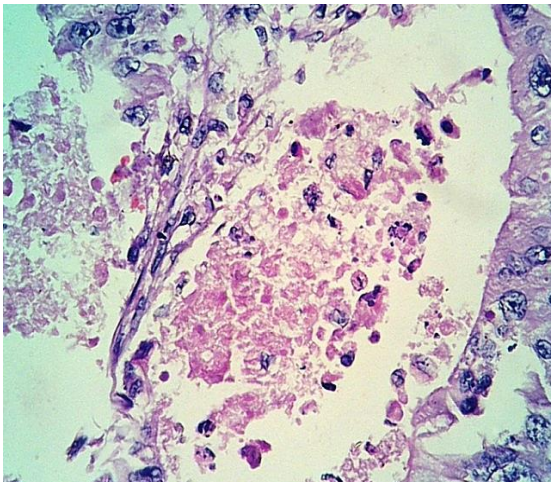


Figure 32: Necrosis. HPE 7164/13. (400x)

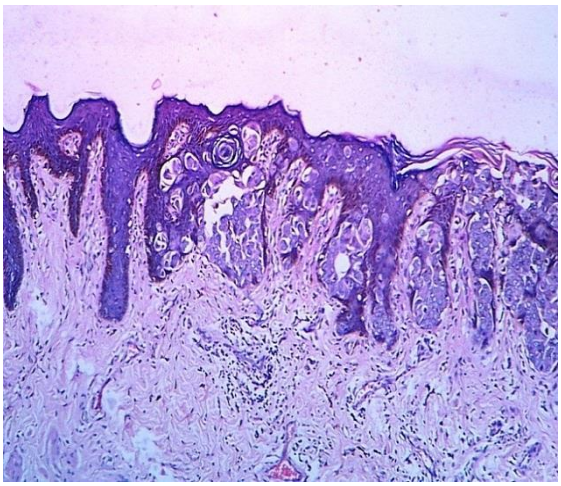


Figure 33: Skin infiltration HPE 8593/13. (100X).

ESTROGEN RECEPTOR EXPRESSION

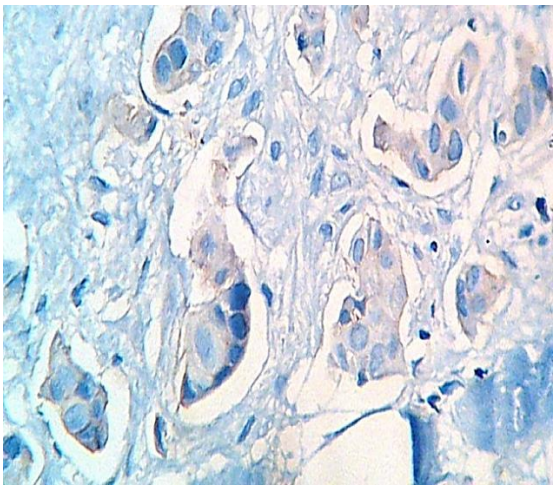


Figure 34: Invasive ductal carcinoma NST. Negative nuclear staining for estrogen receptor. HPE NO: 2351/13

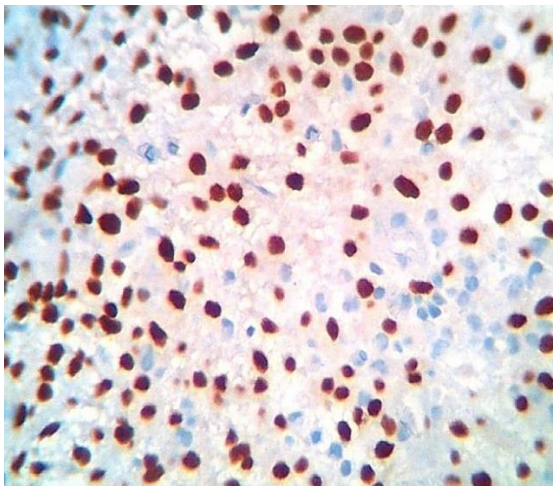


Figure 35: Invasie ductal carcinoma NST. Positie nuclear staining (5+3) for estrogen receptor. HPE NO: 7173/13

PROGESTERONE RECEPTOR EXPRESSION

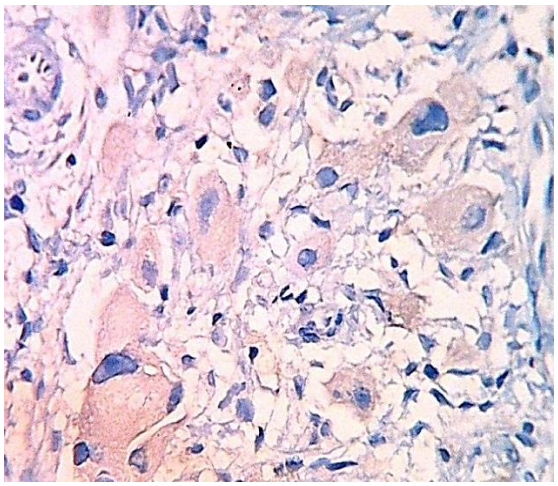


Figure 36: Invasive ductal carcinoma NST. Negative nuclear staining (1+1) for estrogen receptor.HPE NO: 7173/13

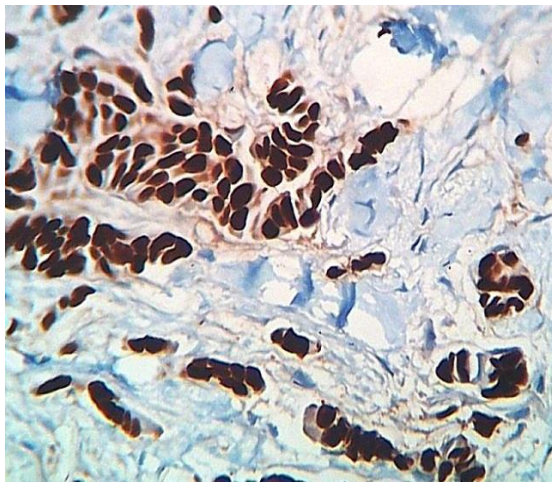


Figure 37: Invasie ductal carcinoma NST. Positive nuclear staining (5+3) for estrogen receptor.HPE NO: 7173/13

HER 2 neu EXPRESSION

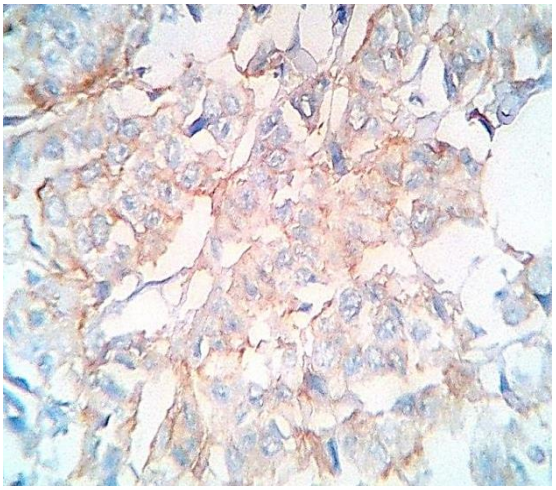


Figure 38: Invasive ductal carcinoma NOS
Negative cytoplasmic staining of HER 2 neu
HPE NO: 6959/13

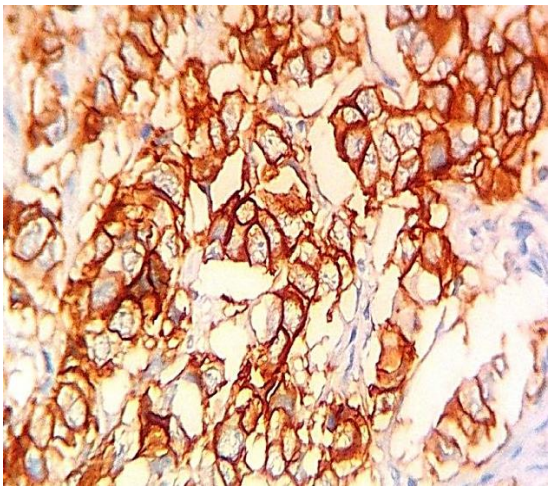


Figure 39: Invasive ductal carcinoma NOS
Positive (3+) cytoplasmic staining of
HER 2 neu, HPE NO: 3428/13

CK5/6 EXPRESSION

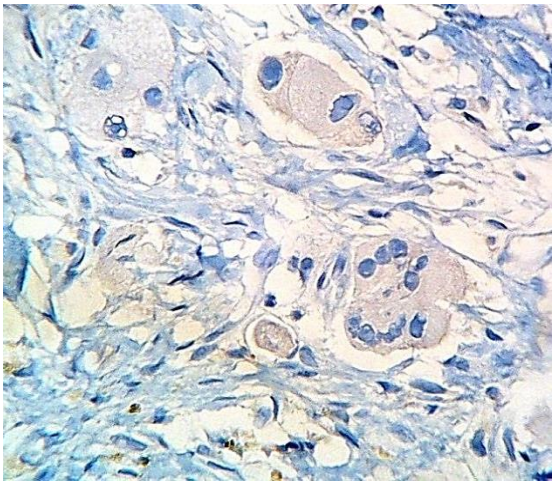


Figure 40: Invasive ductal carcinoma NST
Negative nuclear staining of
CK 5/6, HPE NO: 7891/11

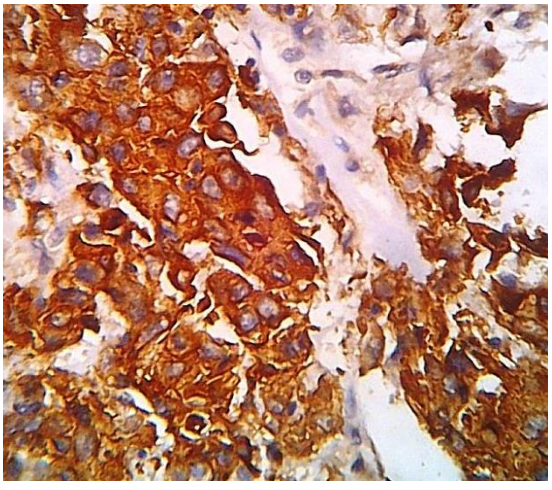


Figure 41: Invasive ductal carcinoma NST
Positive (4+) nuclear staining of
CK 5/6, HPE NO: 2778/13

Ki 67 PROLIFERATION INDEX

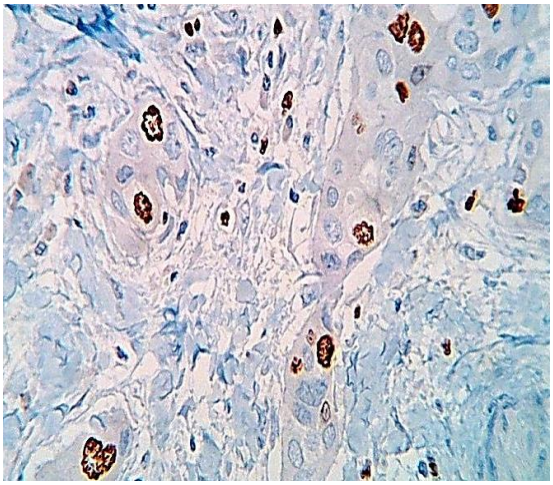


Figure 42: Invasive ductal carcinoma NST Low proliferation index (<14%); nuclear staining of Ki67. HPE NO: 2351/13

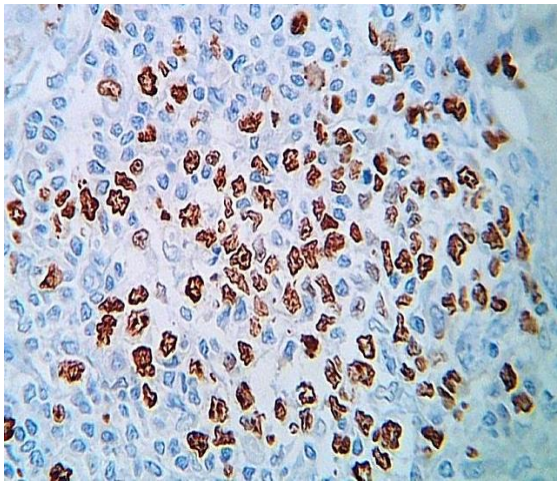


Figure 43: Invasive ductal carcinoma NST High proliferation index (>14%); nuclear staining of Ki67. HPE NO: 2778/13

LUMINAL A- HPE NO: 4161/13

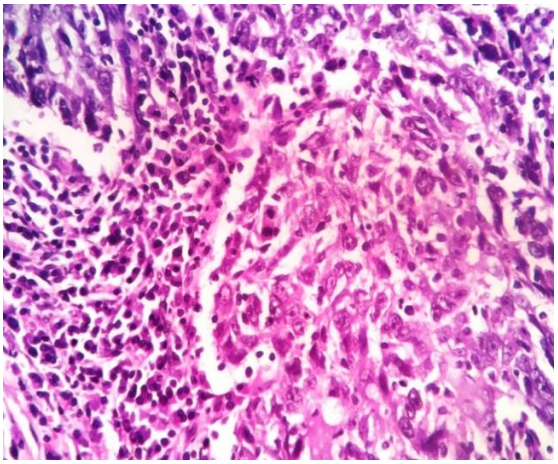


Figure 44: Invasive ductal carcinoma NST

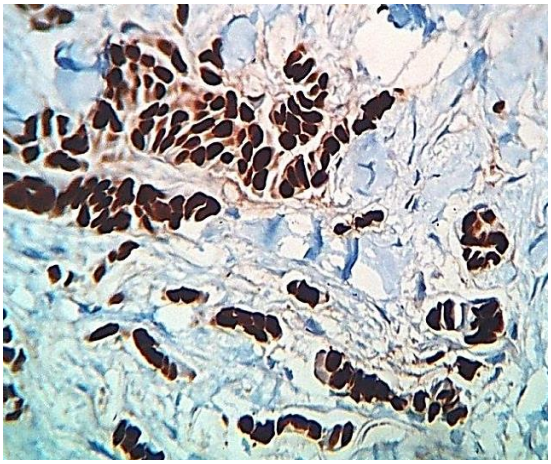


Figure 45: ER –Nuclear staining (5+3), 400x

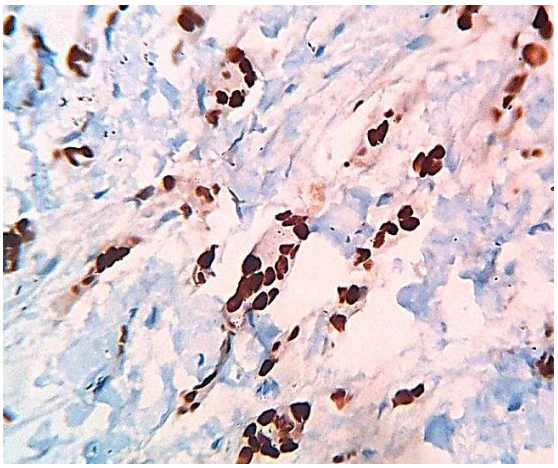


Figure 46: PR –Nuclear staining (2+3), 100x

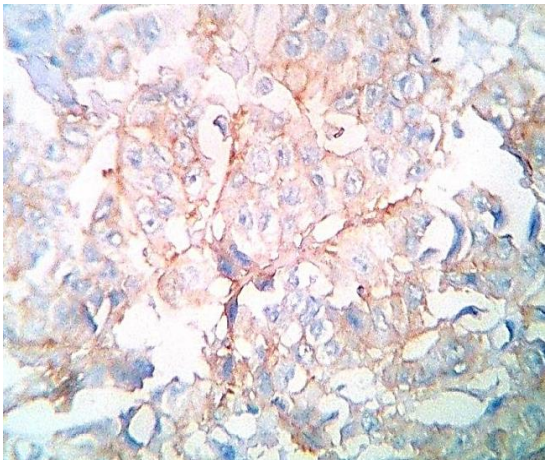


Figure 47: HER 2 Neu; cytoplasmic membrane positivity; 1+ Negative,

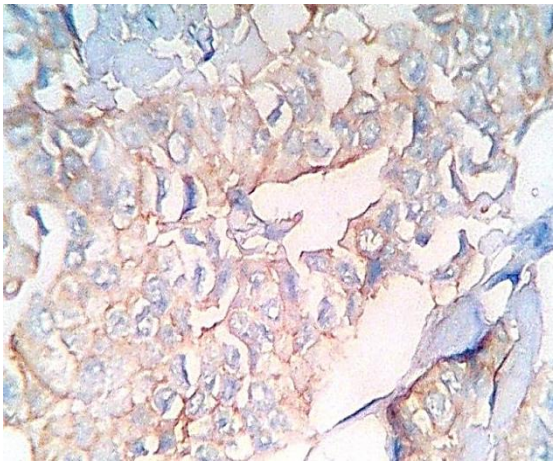


Figure 48: CK 5/6 – Negative, 400x.

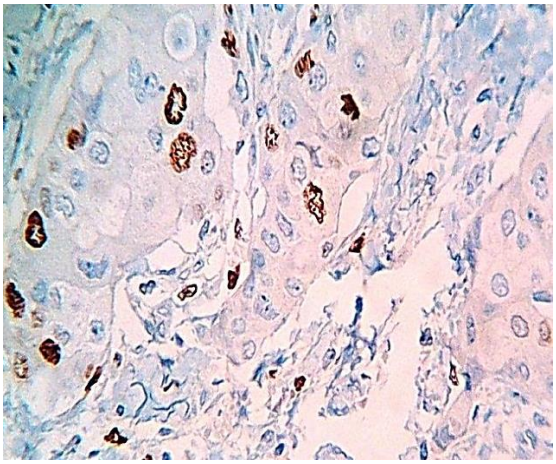


Figure 49: KI 67- Low proliferation index, 400x

LUMINAL B – HPE NO: 7478/13

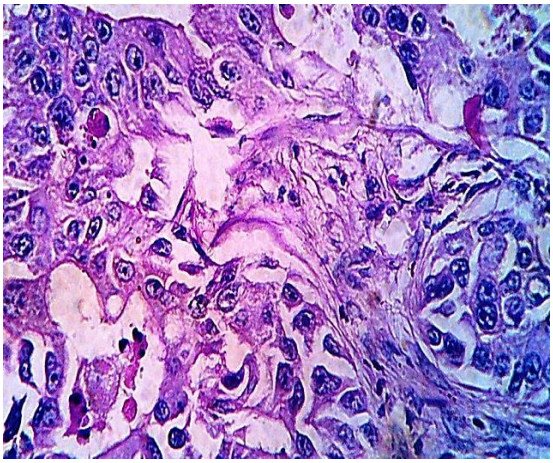


Figure 50: Apocrine carcinoma, H&E, 400x

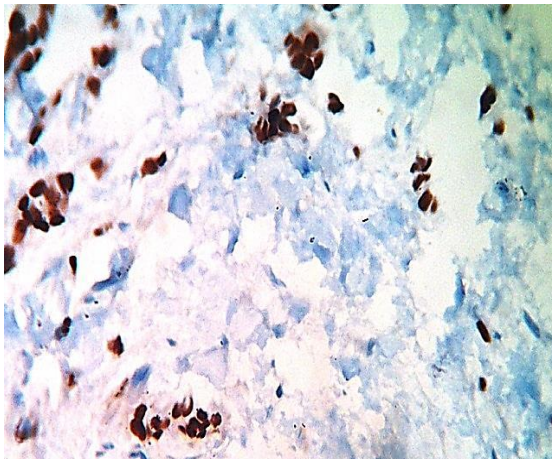


Figure 51: ER –Positive, 3+3, 100x

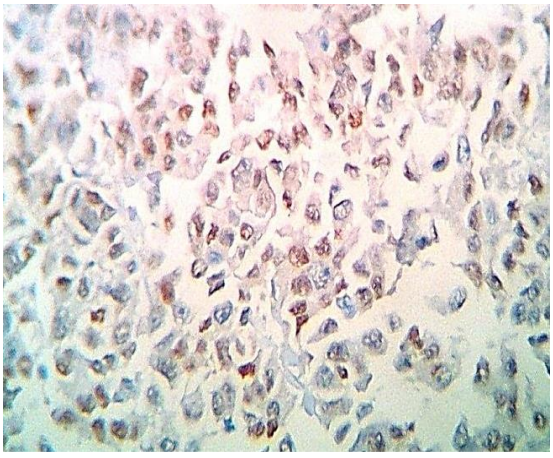


Figure 52: PR –Positive; 2+1,100x

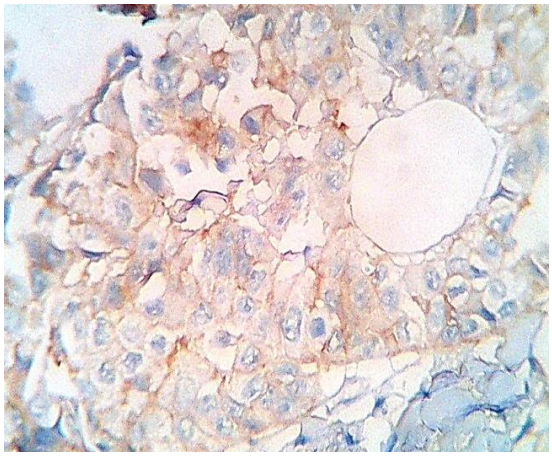


Figure 53: HER 2 NEU; cytoplasmic membrane positivity; 1+ Negative, 400x

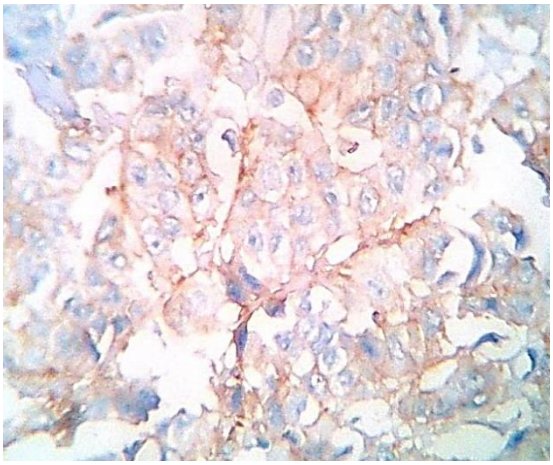


Figure 54: CK 5/6 –Negative, 400x.

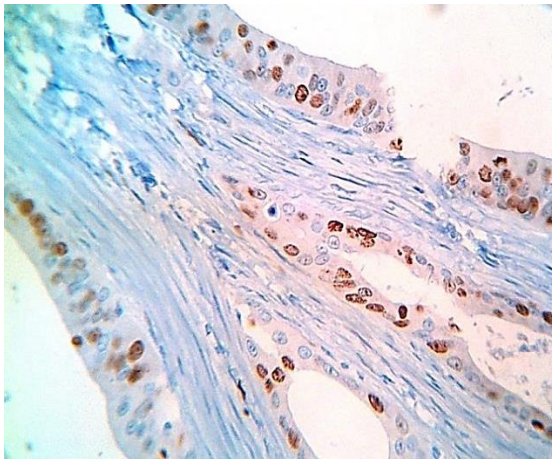


Figure 55: KI 67- High proliferation index. 100x

HER 2 TYPE- HPE NO: 5601/13

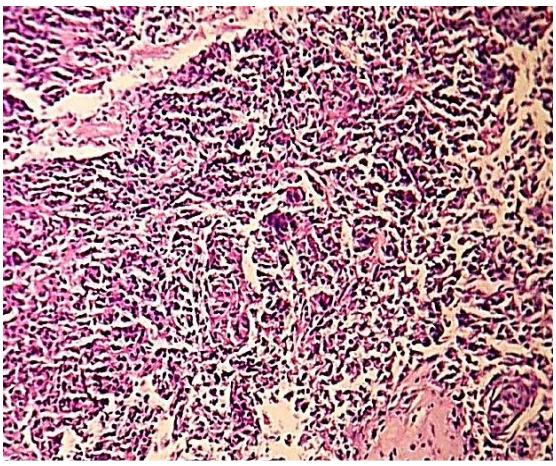


Figure 56: Invasive ductal carcinoma NST. H&E, 100x.

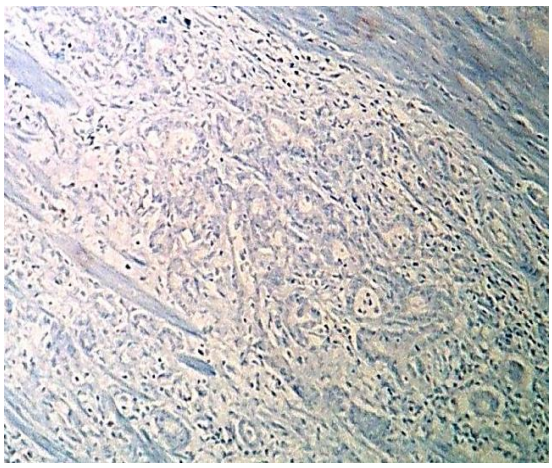


Figure 57: ER –Negative; 0+0, 100x

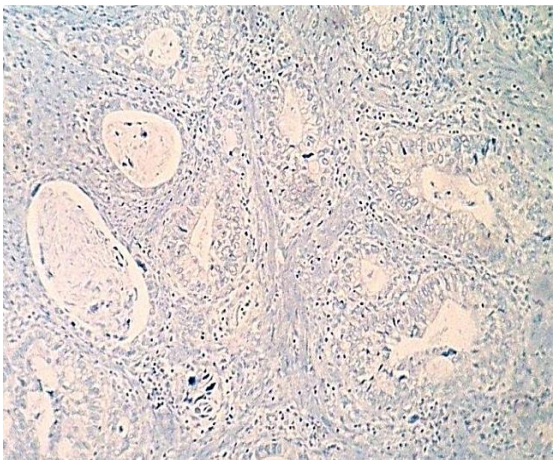


Figure 58: PR –Negative; 0+0, 100x

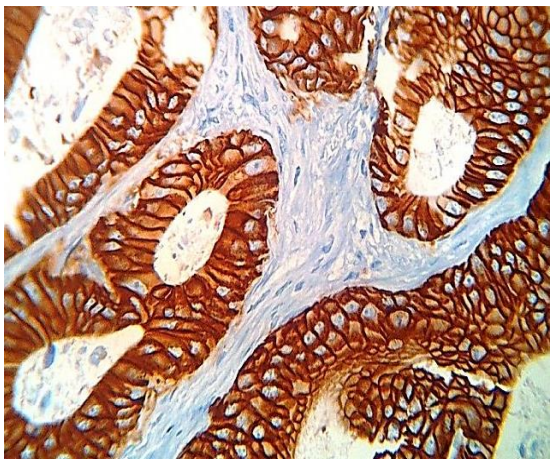


Figure 59: HER 2NEU–cytoplasmic membrane positivity; 3+, 400x

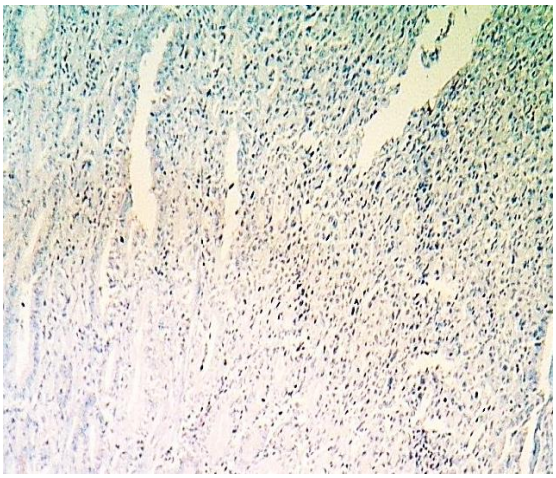


Figure 60: CK 5/6 – Negative, 100x

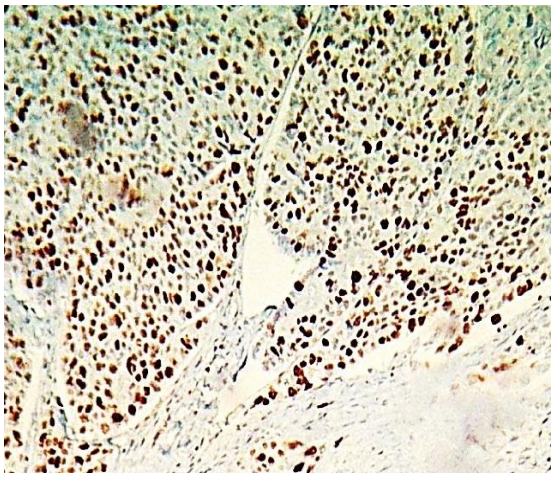


Figure 61: KI 67- High proliferation index, 100x

HYBRID TYPE- HPE NO: 3446/13

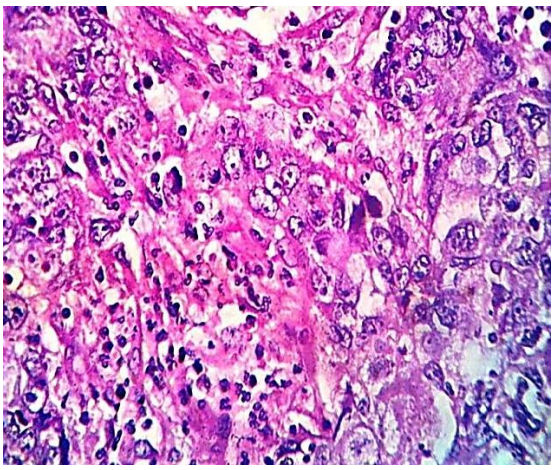


Figure 62: Invasive ductal carcinoma NST H&E, 400x

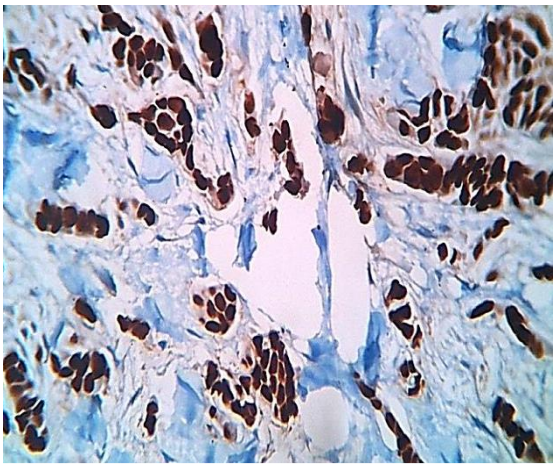


Figure 63: ER –Nuclear staining; 5+3, 100x

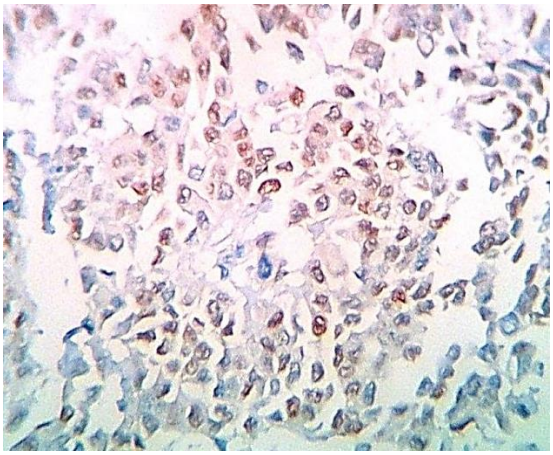


Figure 64: PR –Positive; 2+1,400x

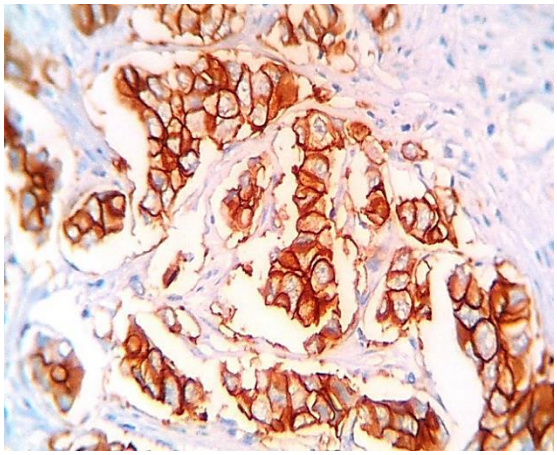


Figure 65: HER 2NEU–cytoplasmic membrane positivity; 3+, 400x

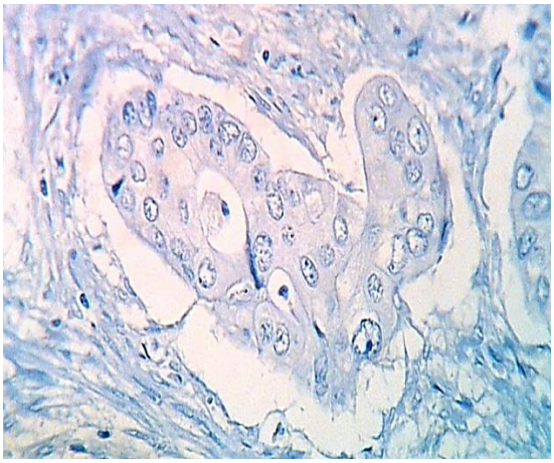


Figure 66: CK 5/6 – Negative, 400x.

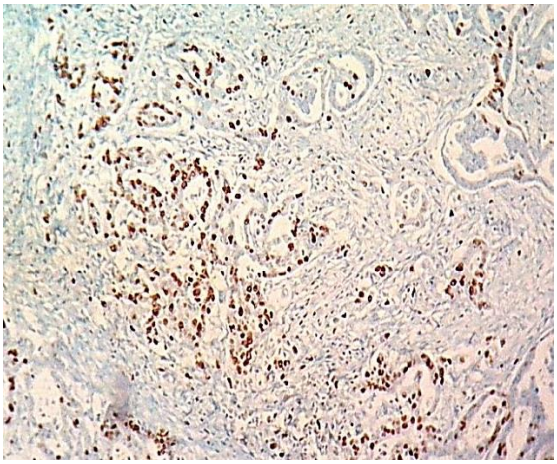


Figure 67: Ki 67- High proliferation index, 100x

BASAL TYPE- HPE NO: 1696/12

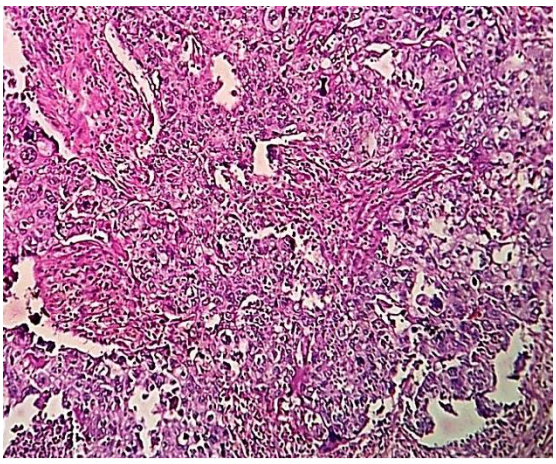


Figure 68: Medullary carcinoma, H&E, 100x

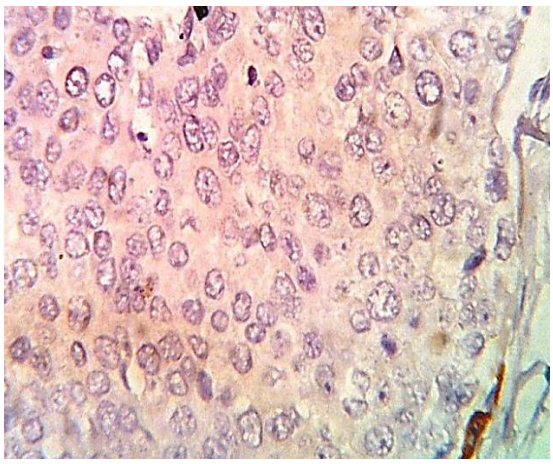


Figure 69: ER –Negative; 0+0, 400x

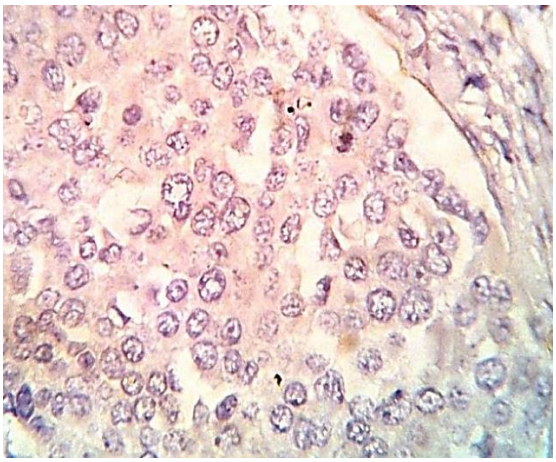


Figure 70: PR –Negative; 0+0,400x

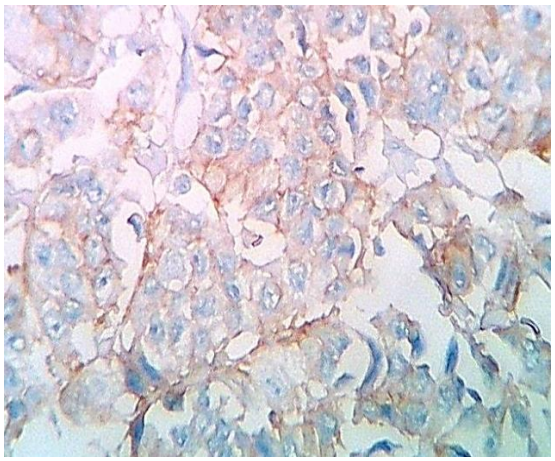


Figure 71: HER2 neu –Negative; 400x

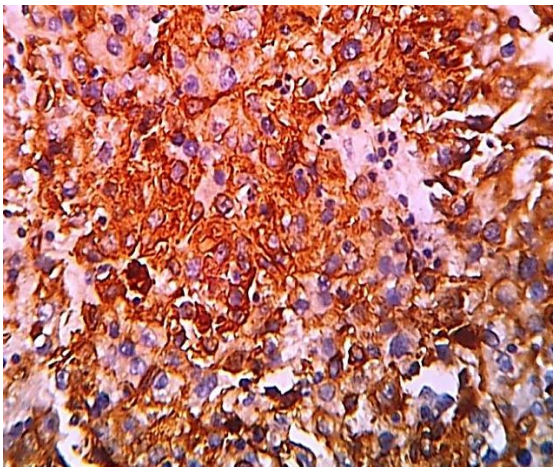


Figure 72: CK 5/6; 4+ positive, 400x.

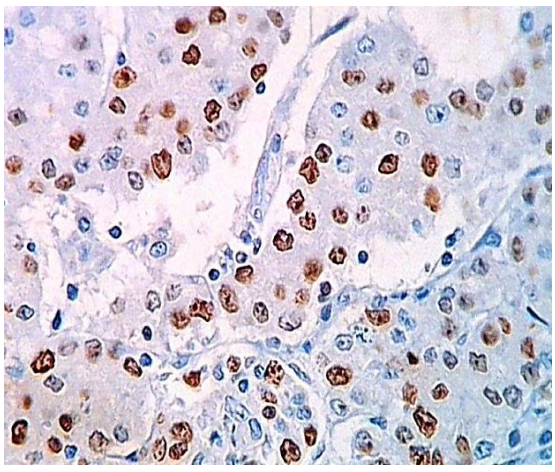


Figure 73: Ki67- High proliferation index, 400x.

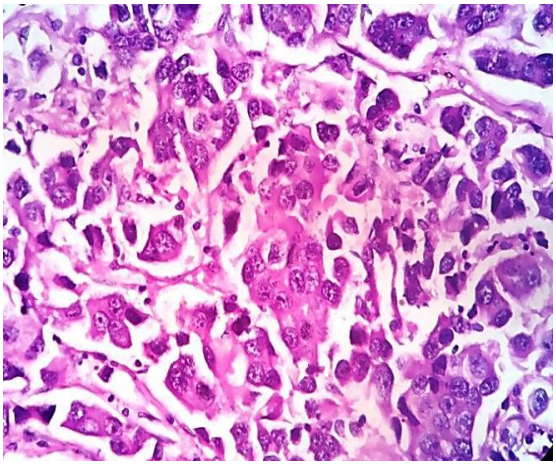


Figure 74: Invasive ductal carcinoma NST H&E, 400x

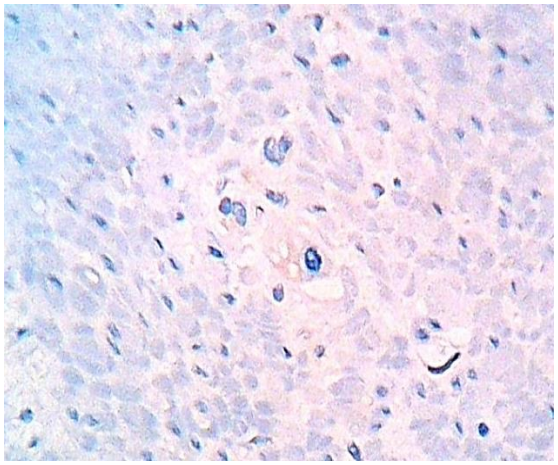


Figure 75: ER – Negative; 0+0, 400x

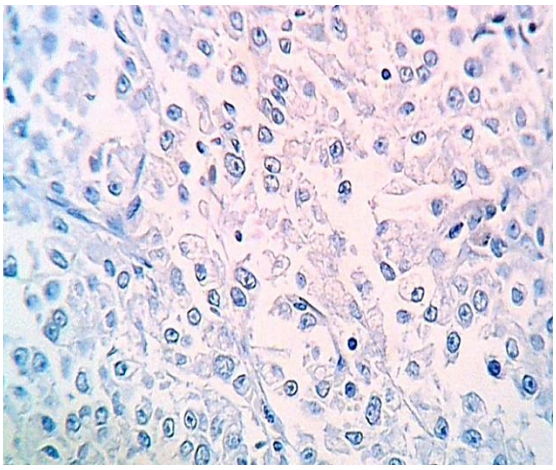


Figure 76: PR – Negative; 0+0, 400x

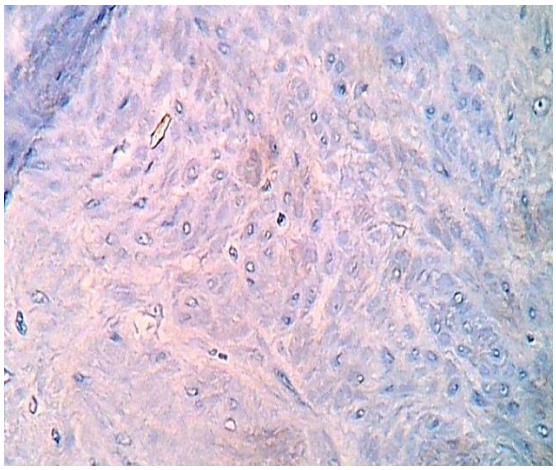


Figure77: HER2 neu–Negative; 400x

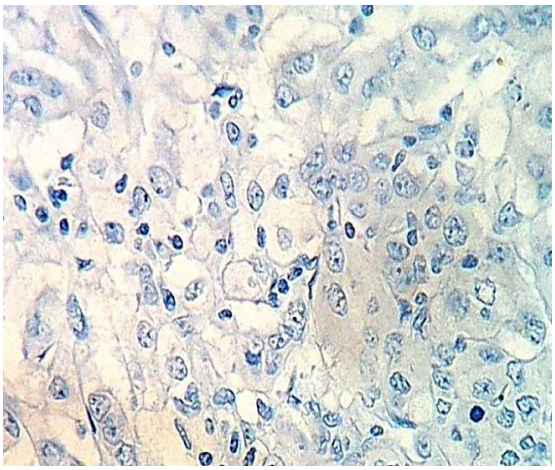


Figure 78: CK 5/6 – Negative, 400x.

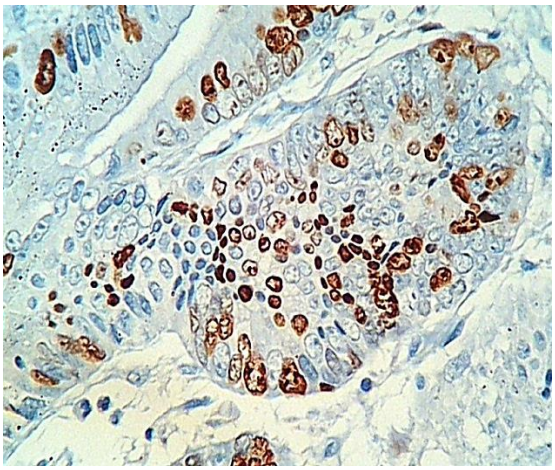


Figure 79: Ki67- High proliferation index, 400x

DISCUSSION

Breast carcinoma is one of the most commonly diagnosed cancers in females worldwide, comprising 16% of all female cancer cases. [1] Its incidence in India is 30-33% per 1,00,000 women and the relative risk is 0.033(1 in 30). [2] Early diagnosis and treatment will certainly reduce the mortality rates.

In this current study, immunohistochemical analysis was done for 60 cases of breast carcinomas, evaluated and scoring given as per ASCO –CAP guidelines. Based on the scoring those cases were classified as molecular subtypes. A comparative analysis of molecular classification with the clinical parameters, histological type, grade and prognostic factors were made.

Madras Medical College being a tertiary referral centre, in the study period, the relative frequency of breast cancers among the other surgical cases was 3.85%. Among the entire breast specimens received for histopathological examination, 46.11% of the cases were reported to be malignant.

60 cases of breast carcinomas were classified under molecular classification, based on the immunohistochemical markers. The most common was found to be the luminal A type comprising 37% and the least

common was the luminal B and hybrid types each comprising 8%. This was in concurrence with the study done by Perou and sorlie et al. [7]

The age of breast cancer patients ranged from 25 to 85 years with a mean age of 51.7 years. The highest incidence of breast cancer occurred in 50 to 59 year age group. This is in concurrence with the study done by Rajesh Singh Laishramet al.[127] Among the molecular classification the HER 2 and basal types had cases at an earlier age of presentation. This was in concurrence with the study done by Lajos Pusztai et al[124,125].

It was found that luminal A, luminal B, HER2 and basal types had more number of left sided tumors. Hybrid type had more right sided tumors. All the molecular subtypes showed an increase incidence of tumor located in the upper outer quadrant followed by central quadrant tumors.

Ahigherproportion of T2 sized tumors (68.3%) were seen (Table 25) similar to the study of Christine L. Carteret al (USA), [128]and Lakmini et al (India).[130]

Table 34: Comparison of size of tumors

Size	Christine L. Carter et al[128]	F S Al-Joudi et al[129]	Lakmini et al[130]	Current study
T1	33.6	3.14	14.5	6.7
T2	55.4	19.37	74	68.3
T3	11	77.49	11.5	25

Luminal B, HER 2 and unclassified types had tumors of T3 size of more than 5 cm. Majority of luminal A tumors were of T2 size. This was in concurrence with the study of bhumsukkaen et al.

The comparison of histological classification and the molecular classification shows that 72% of luminal A tumors belongs to histological variants of special types rather than infiltrating ductal carcinoma NST. Whereas 40%, 25%, 42% and 16% of luminal B, HER 2, basal and unclassified types respectively belongs to variants.

Among the 5 cases of mucinous tumors all except one were hormone receptor positive and all 5 were HER 2 negative. This was in concurrence with the study of Lacorixtriki et al. [132] (Table 35)

Table35: Immunohistochemical analysis of Mucinous carcinoma

IHC IN MUCINOUS CARCINOMAS	**LACORIX TRIKI ET AL[132]**	**CURRENT STUDY**
ER, PR positive	86%	80%
HER2 neu negative	96%	100%

Among the 5 cases of medullary tumors, 3 (60%) were triple negative, those were negative for both hormone receptors and HER 2 neu. This was in concurrence with the study of Jensen et al. [131](Table 36)

Table36: Immunohistochemical analysis of Medullary carcinoma

IHC IN MEULLARY CARCINOMAS	JENSEN ET AL	CURRENT STUDY
ER, PR positive	12%	20%
HER2 neu	22%	20%
Triple negative	76%	60%

All the 5 cases of papillary carcinomas belonged to luminal A and were positive for hormonal receptors and negative for HER 2 neu. Similar results were produced in the study of Chen et al[134] and Lotan et al[135] (Table 37)

Table37: Immunohistochemical analysis of papillary carcinoma

IHC in papillary carcinoma of breast	Chen et al[134]	Lotan et al[135]	Current study
ER,PR positive	84.8%	89.5%	100%
HER 2 neu positive	0%	15.8%	0%

Among the 5 cases of apocrine carcinomas none was luminal A. one case was luminal B and 2 cases were basal and hybrid tumors. This was in concurrence with the study done by Matsuo et al.[136] (Table 38)

Table38: Immunohistochemical analysis of apocrine carcinoma

IHC in apocrine carcinoma of breast	Matsuo et al[134]	Current study
ER,PR positive	17%	20%
HER 2 neu positive	33%	40%
Triple negative	46%	40%

Metaplastic carcinomas were described as triple negative tumors in the study done by GM Tse et al [138]. But surprisingly in the current study, among the 5 cases of metaplastic tumors none belonged to the basal types. 2 were luminal A, one was luminal B type, one was HER 2 and one was unclassified. (Table 39)

Table39: Immunohistochemical analysis of Metaplastic carcinoma

IHC in metaplastic carcinoma of breast	GM Tse et al [138]	Current study
ER, PR positive	8.8%	40%
HER 2 positive	0%	20%
Triple negative	93%	0%

Among the 5 cases of lobular carcinoma, 80% of them was luminal A tumors. This was in concurrence with the study done by Weidner et al [139] (Table 40)

Table40: Immunohistochemical analysis of Lobular carcinoma

IHC in lobular carcinoma	Weidner et al [139]	Current study
ER,PR positive	86%	80%
HER 2 positive	08%	20%
Triple negative	06%	0%

In the literature, studies have shown the most common of molecular subtypes was luminal A, followed by luminal B, least was HER 2 and basal type [140,141,142,143]. But to the contradiction in the current study, among the 30 cases of invasive ductal carcinoma, most common molecular subtype was basal, followed by luminal A and HER 2 type. (Table 41).

Table41: Immunohistochemical analysis of invasive ductal carcinoma.

IHC IN INVASIVE DUCTAL CARCINOMA	Constantinidou et al[140]	Correa Geyer et al[141]	Rebecca dent et al	Perou et al[143]	Current study
ER, PR positive	65%	72%	56%	62%	30%
HER2 positive	15%	22%	18%	!4%	20%
Tiple negative Ck5/6 positive	26%	28%	11.2%	15%	36%
Unclassified		14%		15%	14%

In the current study, 40% of grade I tumors were unclassified type. 50% of basal and 29% of HER 2 type tumors belonged to high grade (grade III). 18% of luminal A tumors were grade II tumors. None of the luminal A and hybrid types was grade III.

In the study of rakha et al they have concluded that among the ER positive Luminal A tumors, there was a considerable difference in their hazard ratio and ten year risk of relapse. It was found that hazard ratio has increased with an increase in the grade of the tumour. 10 year risk of relapse was 5% for grade 1 tumours, 24% for grade II tumours and 43% for grade III tumours, with a statistical significance.

In this current study, 27 cases of luminal tumours were graded as 11% of grade I, 22% of grade II and 5% of grade III tumours. After one year of follow up period these tumours there was an increased incidence of recurrence rate reported in grade III tumors. Also this study shows that the HER2 and basal types had more number of grade III tumors. This was in concurrence with the study done by Rakha et al. (Table 42)

Table 42: Comparison of grade with the recurrence.

	Rakha et al (% of risk of relapse)	**Current study (% of recurrence and dead)**
Grade I	5%	3.3%
Grade II	24%	6.6%
Grade III	43%	16.7%

Fibrocystic disease was the most common associated lesion with all the molecular subtypes. Ductal carcinoma in situ was more frequently associated with unclassified types. All classes of molecular classification showed an increased incidence of lymphovascular invasion. HER2 and basal types showed increase percentage of lymphovascular invasion. Luminal tumors had lesser percentage. This gives a better prognostic significance of luminal tumors and adverse for HER 2and basal types. This was in concurrence with the study done by Cheang Maggie et al[148].

Table 43: Comparison of lymphovascular invasion with molecular classification.

	Cheang Maggie et al[148] (% of cases with lymphovascular invasion)	Current study (% of cases with lymphovascular invasion)
Luminal A	39%	59%
Luminal B	50%	60%
HER 2 type	60%	88%
Basal	64%	79%

In their study they have also shown the percentage of lymph nodal involvement which was in concurrence with the current study. HER2 and basal types showed increased incidence of tumors with N3 nodal stage.(Table 44)

Table 44: Comparison of lymph node involvement with the molecular classification

	Cheang Maggie et al[148] (no. of nodes involved)			Current study (no. of nodes involved)		
	Nil	1-3	>4	Nil	1-3	>4
Luminal A	55%	28%	11%	17%	40%	45%
Luminal B	28%	28%	28%	0	40%	60%
HER 2 type	11%	16%	24%	0	25%	75%
Basal	10%	22%	28%	0	14%	86%

Most of the tumors had lymphocytic infiltration, especially the luminal tumors showed an increased incidence. The other prognostic factors like necrosis, skin infiltration and involvement of margins were predominantly associated with luminal B, HER2 type and the basal types.

Table 45: Comparison of distribution of AJCC staging in breast cancers

	Rajesh singhlaishram[127] (%)	Carey et al[146] (%)	Current study (%)
Stage I	3.3	39	3
Stage II	12.3	51	62
Stage III	76.4	8	34
Stage IV	7.8	3	2

Most of the cases presented in stage II followed by stage III which was similar to the study of Carey et al. Rajesh Singh Laishram et al[127] who studied 142 breast cancers in Manipur and reported the increased incidence

ofhigherstage tumors in their population when compared to the western studies done by Carey et al[146](Table 46). In this study it was found that HER2 and basal types had tumors withhigherstage.

Table 46: Comparison of KI67 index in hormone receptor positive tumors and its relapse free survival.

Ki 67 index in hormone receptor positive tumors	**Cheang et al.[147] (10 year relapse free survival)**	**Current study. (1 year relapse free survival)**
High	42%	61%
Low	69%	100%

In the current study those hormone receptor positive tumors with high proliferation were considered as luminal B category. This was similar to the study of Bentran and Philippe bedard et al in which they have concluded that although both luminal A and luminal B tumours express estrogen receptor positivity, the luminal B subtype end up with early relapse when treated with endocrine therapy compared to the luminal A subtypes. Early distant metastases were identified in luminal B subtypes with a hazard ratio of 2.86 when compared to 1 of luminal A tumours. This has led to the identification and segregation of aggressive luminal B tumours from the indolent luminal A tumours, as those tumours with reduced hormonal receptor expression, variable HER 2 expression and high proliferation index.

According to Maggie cheang et al[148] in their study of breast cancers found that among the ER positive cancers that expressing high proliferation index with ki67, a nuclear marker for cell proliferation gained minimally with adjuvant chemotherapy and were associated with worst outcomes irrespective of their histological and molecular subtypes. Ki67 index was given a visually assessable cut-off point of 14 % and its prognostic significance was assessed [148].

Beyond the three biomarkers it is essential to have Ki 67 proliferation index to categorise luminal B tumours which are generally tamoxifen resistant.This is an effort to improve survival in these patients and development of novel therapeutic agents that will alter the natural course of the illness. (Table 47)

Table 47: Comparison of number of basal tumors among the triple negative tumors

	Cheang et al[147] (%)	**SeemaSethi et al (%)**	**Current Study (%)**
Triple negative tumors	17%	24%	33%
CK 5/6 positive tumors	9%	24%	23%

According to cheang et al [147], they have concluded that the expanded immunopanel of five markers which composed of ER, PR, HER2neu,

EGFR, and CK 5/6 than the usual triple biomarkers of ER, PR and HER2neu provides a better definition of basal like tumours and its disease free survival more specifically. They have found that not all the triple negative tumors express the basal markers. Only 9% out of 17% of triple negative tumors expressed basal markers. This similar finding was found in the current study, in which among 33% of triple negative tumors only 23% showed positivity for basal markers. Another study of seemaseethi showed that all cases of triple negative tumors expressed basal markers.

Triple negative tumours which are considered to have poor outcome where conferred as basal type but those cohort of triple negative tumours with positive basal markers are found to have almost entirely and significantly bad outcome. The significance of demonstrating the basal type tumours is that they may benefit from EGFR targeted therapy and specified chemotherapy.

Table 48: Comparison of CK5/6 expression among molecular classification.

	Seemasethi et al[149] (EGFR, CK 5/6 expression)	**Current study (CK 5/6 expression)**
Luminal A	0	5%
Luminal B	2%	20%
HER 2 type	3%	0
Basal	100%	92.8%

In the current study it was found that the basal type tumors had the highest expression of CK5/6 expression. This was concurrent with the study done by Seemasethi et al[149] who has found that epithelial to mesenchymal transition occurs during the development of carcinogenesis, resulting in increased metastatic potential of the tumour cells and resistance to the therapy. Expression of vimentin, EGFR, CK5/6 are involved in this trasnsition. In this study they have concluded that these markers were statistically significantly expressed in triple negative tumours when compared to luminal A and luminal B and consistently reflected the aggressiveness of the tumour.

Under the umbrella of triple negative tumours which has an overall poor survival and early recurrence, there are tumours with good prognosis as adenoid cystic carcinoma and secretory carcinoma. So it is essential to subtype triple negative tumours with basal markers. This is concluded in this study by constantindou et al [140].

Table 49: Cancer specific survival among molecular classification

	Maggie cheang et al.[147](cancer specific survival)	**Current study.(cancer specific survival)**
Luminal A	79%	93%
Luminal B	64%	100%
Hybrid	57%	60%
Basal		62%

Luminal B and hybrid types had an increased incidence of recurrence in the study of Maggie cheang et al [147]. In their study of immunohistochemical analysis of breast cancers have concluded that all the luminal B and luminal -HER 2 hybrid tumours were associated with poor recurrence free survival and disease survival, those who were treated with adjuvant systemic therapy. But in the current study, after the one year follow up period it was found that all he luminal B tumors were alive and healthy, whereas the luminal-HER2 hybrid and basal types had only 60% of cases with disease free survival.Therefore it is essential to identify the basal tumours and hybrid categories so that they are provided with additional therapies.

Treatment for breast cancers, given based on the histopathological classification is broad based and includes endocrine therapy, systemic chemotherapy and Herceptintherapy. Whereas when breast cancers were classified under molecular classification a better targeted therapy is provided, avoiding unnecessary drugs to patients who do not need it, thereby preventing unnecessary drug related toxicities and reducing the costs of the treatment.

There exist a difference in the treatment options between the histopathological classification and molecular classification. Therefore it is

necessary to prove the disagreement between these two classification systems.

In this current study, each molecular subtype was compared with the histopathological classification and it was found that all the subtypes had a disagreement with the histopathological classification that was proved by the negative inter rater agreement KAPPA value. Disagreement between the two systems substantiates the value of molecular classification in the field of targeted therapy.

In the current study the treatment was given for some cases based on the histopathological classification and for some based on the immunohistochemical analysis of triple markers. In other studies of Hess KR *et al*[150], *Ayers* M *et al*[151], Gianni L *et al*[152],they have found a significant reduction in the incidence of relapse, when treatment was targeted therapy based on molecular classification.

LIMITATIONS OF THE STUDY

- The cases were selected on the basis of histopathological classification in the tertiary care centre and not a population base study, which will not reflect the true prevalence of the general population
- Her 2 neu expression has an intermediate stain scoring of 2+ which requires FISH for grading it as negative or positive.

- Gene expression profiling will give more accurate molecular subtypes than immunohistochemistry, but being expensive it cannot be applied to all patients.
- Being a retrospective study the targeted therapy according to molecular classification was not given and hence the prognostic inference could not be ascertained.

SUMMARY

This study is a prospective and retrospective descriptive study of invasive breast cancers conducted in the Institute of Pathology, Madras Medical College, Chennai during the period between Jan 2011 to Jun 2013. A total of 26,536 specimens were received. Total numbers of breast specimens received were 1412 cases, of these breast tumours accounted for 1023 cases with a percentage of 3.85% of all cases (including both incisional and excisional biopsies). The total number of non-neoplastic, benign and malignant cases was 289, 472 and 651 respectively. Thus the distribution of non-neoplastic breast lesions was 20.46%, benign tumours were 33.42% and of malignant tumours were 46.11%. Of these, a total of 369 mastectomy specimens (simple, modified radical or radical mastectomy) were received. All invasive breast carcinomas no special type (ductal and lobular), medullary, mucinous, papillary, apocrine and metaplastic carcinomas irrespective of the age and sex were included for the study

Detailed history of the cases regarding age, sex, side of the breast, type of procedure, history of neo adjuvant therapy, details of gross characteristics such as tumour size, nodal status details were obtained for those 60 cases included in the study. 10 cases of each grade from Invasive ductal carcinoma NOS subtype and 5 cases from special type as medullary,

metaplastic, mucinous, apocrine, papillary and invasive lobular were randomly selected from the total cases. Samples were subjected to immunohistochemical analysis of 5 markers which includes ER,PR,H2N,CK5/6 and Ki67. Slides were evaluated and scoring was given. Based on which they were classified into luminal A, luminal B, HER 2 , basal and normal like/ unclassified as per molecular classification.

LUMINAL A TYPE

- Most common age group is 50 -59 years (40%).
- Most common on the left side.
- The tumor was commonly located in the UOQ (50%).
- The tumor size commonly ranged between 2cm and 5 cm. (73%)
- Most of the tumors in Luminal A group were the histological special variants (73%). Among which, papillary constituted 23%.
- Among the IDC NST tumors, grade II (18%) was the most common grade.
- The most common associated lesion was fibrocystic disease (73%).
- 59% of the tumors showed lymphovascular invasion.
- 77% of the tumors showed lymphocytic infiltration.
- None of the cases had necrosis.
- Only 5% of the tumors showed skin involvement.

- 40% of the tumors had N2 stage of nodal involvement. 17% of the cases did not have lymph node involvement.
- 14% of the cases showed involvement of margins.
- Most of the tumors belonged to Stage II (83%).
- 69% and 59% of the tumors showed strong ER and PR expression respectively.
- Only 5% expressed HER2 neu positivity. (2+)
- 82% of the tumors did not express CK5/6.
- 86% of the tumors had low proliferation index with Ki-67.
- On follow up, 95% of cases were alive and healthy and one case had recurrence.
- By McNemar's test, the comparison of Luminal A type with the histopathological classification showed an inter rater agreement Kappa value of – 0.367 which indicates disagreement.

LUMINAL B TYPE

- Most common age group is 50 -59 years (40%).
- Most common on the left side.
- The tumor was commonly located in the UOQ (80%).
- The tumor size commonly ranged between 2cm and 5 cm (60%).

- Most of the tumors in Luminal B group were infiltrating ductal carcinoma NST (60%).
- Among the variants, one case of metaplastic carcinoma and one case of apocrine carcinoma belonged to Luminal B group.
- Among the IDC NST tumors, all the grades were of equal proportion.
- The most common associated lesion was fibrocystic disease (80%).
- 60% of the tumors showed lymphovascular invasion.
- 80% of the tumors showed lymphocytic infiltration.
- 40% of the cases had necrosis.
- None of them showed skin involvement.
- 40% of the tumors exhibited N1 and N2 stage of nodal involvement. All the cases showed lymph node involvement.
- Margins were free in all the cases.
- 40% of the tumors were in Stage IIB and IIIA each.
- 60% of the tumors showed intermediate ER expression.
- 40% of the tumors showed weak and intermediate PR positivity each.
- 60% expressed HER2 neu positivity. (3+)
- 40% of the tumors had negative and 2+ expression of CK5/6 each.
- All the cases had high proliferation index with Ki67.
- On follow up, all the cases were alive and healthy.

- By McNemar's test, the comparison of Luminal B type with the histopathological classification showed an inter rater agreement Kappa value of – 0.033 which indicates disagreement.

HER2 TYPE

- Most common age group is 40 - 49 years (38%).
- Most common on the left side (82%).
- The tumor was commonly located in the UOQ (63%).
- The tumor size commonly ranged between 2cm and 5 cm (63%).
- Most of the tumors in HER2 group were infiltrating ductal carcinoma NST (75%).
- Among the variants, metaplastic carcinoma (one case) and lobular carcinoma (one case) belonged to HER2 group.
- Among the IDC NST tumors, Grade III was the most common grade (50%).
- The most common associated lesion was fibrocystic disease (63%).
- 88% of the tumors showed lymphovascular invasion.
- 38% of the cases had necrosis.
- None of them showed skin involvement.
- 50% of the tumors belonged to N3 stage of nodal involvement. All the cases had lymph node involvement.
- 38% of the cases had involvement of margins.

- 63% of the tumors were in Stage IIIA.
- All the cases were negative for ER and PR expression.
- 88% expressed 3+ HER2 neu positivity.
- 63% of the tumors were negative for CK5/6.
- 75% of the cases had high proliferation index with Ki -67.
- On follow up, 63% of the cases were alive and healthy. One case had recurrence and one death was reported.
- By McNemar's test, the comparison of HER2 type with the histopathological classification showed an inter rater agreement Kappa value of – 0.2 which indicates disagreement.

HYBRID TYPE (Luminal + HER2)

- Both 40 -49 and 50-59 years had equal distribution of cases (40%).
- Most common on the right side (60%).
- The tumor was commonly located in the UOQ (80%).
- The tumor size commonly ranged between 2cm and 5 cm (80%).
- The histological special variants constituted the most common type. Among which two cases were apocrine carcinoma and one was medullary carcinoma.
- Among the IDC NST tumors, most common grade was grade II (40%).

- The most common associated lesion was fibrocystic disease (80%).
- 60% of the tumors showed lymphovascular invasion.
- 80% of the tumors showed lymphocytic infiltration.
- 20% of the cases had necrosis.
- One case had skin involvement.
- 60% of the tumors exhibited N2 stage of nodal involvement. All the cases showed lymph node involvement.
- Margins were involved in 20% of the cases.
- All the cases belonged to Stage III.
- All the cases showed intermediate ER expression.
- 40% of the tumors showed intermediate PR positivity.
- All the cases showed 3+ HER2 neu positivity.
- 40% of the tumors had negative and 2+ expression of CK5/6 each.
- 80% of the cases had high proliferation index with Ki67.
- On follow up, 60% of the cases were alive and healthy. One case of recurrence and one death was reported.
- By McNemar's test, the comparison of Hybrid type with the histopathological classification showed an inter rateragreement Kappa value of – 0.033 which indicates disagreement.

BASAL TYPE

- Most common age group is 40 - 49 years (29%).
- Most common on the left side (57%).
- The tumor was commonly located in the UOQ (64%).
- The tumor size commonly ranged between 2cm and 5 cm (72%).
- Most of the tumors in basal type were infiltrating ductal carcinoma NST (57%).
- Among the variants, two cases of apocrine carcinoma, three medullary carcinomas and one case of mucinous carcinoma belonged to Basal group.
- Among the IDC NST tumors, Grade III was the most common grade (29%).
- The most common associated lesion was fibrocystic disease (79%).
- 79% of the tumors showed lymphovascular invasion and lymphocytic infiltration.
- 21% of the cases had necrosis.
- One case had skin involvement.
- 56% of the tumors belonged to N3 stage of nodal involvement. All the cases had lymph node involvement.
- 36% of the cases had involvement of margins.

- 57% of the tumors were in Stage IIB. The only case in Stage IV disease belonged to this group.
- 93% of the tumors were negative for ER and PR expression.
- 86% were negative for HER2 neu.
- 57% of the tumors had 4+ expression of CK5/6.
- 86% of the cases had high proliferation index with Ki -67.
- On follow up, 65% of the cases were alive and healthy. Four cases had recurrence and one death was reported.
- By McNemar's test, the comparison of Basal type with the histopathological classification showed an inter rater agreement Kappa value of – 0.133 which indicates disagreement.

UNCLASSIFIED TYPE

- Both 40 -49 and 50-59 years had equal distribution of cases (33%).
- Both right and left sides had equal proportion of cases.
- The tumor was commonly located in the UOQ (50%).
- The tumor size commonly ranged between 2cm and 5 cm (50%).
- Most of the tumors in unclassified group were infiltrating ductal carcinoma NST (83%).
- Among the variants, metaplastic carcinoma (one case) belonged to unclassified group.

- Among the IDC NST tumors, Grade I was the most common grade (68%).
- The most common associated lesion was fibrocystic disease (68%).
- 67% of the cases showed lymphocytic infiltration.
- 17% of the cases had necrosis.
- 33% of the cases had skin involvement.
- 67% of the tumors belonged to N2 stage of nodal involvement. Two cases did not have lymph node involvement.
- 33% of the cases had involvement of margins.
- 50% of the tumors were in Stage IIIA.
- All the cases were negative for ER, PR and HER2 neu expression.
- 67% of the tumors were negative for CK5/6.
- On follow up, 67% of the cases were alive and healthy. Two cases had recurrence.
- By McNemar's test, the comparison of HER2 type with the histopathological classification showed an inter rater agreement Kappa value of – 0.100 which indicates disagreement.

COMPARISON AMONG MOLECULAR CLASSIFICATION

- Luminal A and Luminal B showedhigherincidence of breast cancer in 50-59 age group with 40% incidence.HER 2(23%) and

basal types (20%) had ahigherincidence at an earlier age group of 40-49 years.

- Left side (58%) tumors were more common in all subtypes except hybrid type.
- Upper outer quadrant (60%) was the most common site in all types.
- Most of the tumors were between 2cm and 5cm (68%). Luminal B, HER2 and Unclassified types had a relatively increased incidence of tumors with more than 5cm size.
- 72% of luminal A tumors belongs to histological variants of special types rather than infiltrating ductal carcinoma NST. Whereas 40%, 25%, 42% and 16% of luminal B, HER 2, basal and unclassified types respectively belongs to variants.
- The most common grade for HER2 was Grade III (50%). The association of histological grade with the molecular classification was statistically significant with the p value of 0.01.
- The most common associated lesion was fibrocystic disease (73%) in all subtypes. DCIS was commonly associated with Basal (21%) and Unclassified types (33%).
- 66.7% of the tumors had lymphovascular invasion. Among which HER2 group had the highest incidence (88%).

- 73% of the tumors had lymphocytic infiltration. Among which Luminal A had the highest incidence (83%).
- Only 17% of the tumors had necrosis. Luminal B type (40%) tumors had the highest incidence.
- 8% of the tumors had skin involvement. Unclassified type (33%) tumors had the highest incidence.
- Most of the tumors had N2 stage of nodal involvement. Basal type (56%) had the highest incidence of N3 stage.
- 23% of the tumors showed involvement of the margins. HER2 type (38%) had the highest incidence.
- Most of the tumors belonged to Stage II (62%). One case of Stage IV was Basal type.
- ER, PR, HER2 neu, CK5/6 expression and proliferation index with Ki67 had a statistically significant association with the molecular classification.
- High proliferation index (>14%) with Ki67 was noted in Luminal B, Basal and Hybrid types.
- 78% of the total 60 cases were alive and healthy. Unclassified type (33%) had the highest incidence of recurrence. One death was reported in HER2, Hybrid and Basal types.

INTER RATER AGREEMENT KAPPA

- On evaluating the inter rater agreement between the histopathological and molecular classification which is quantified by the kappa statistic by using Mc Nemar's test, it was found that all the subtypes showed negative kappa value.
- This indicates that the agreement is worse than chance and hence the importance of molecular classification is substantiated for the targeted therapy.

CONCLUSION

Breast carcinoma is one of the most commonly diagnosed cancers in females worldwide comprising 16% of all female cancer cases. Study of tumor molecular characteristics has enhanced our understanding of both the tumor behaviour and the response to therapy. In this study of 60 cases which included invasive ductal carcinoma NST and its variants, an attempt has been made to evaluate the hormonal status and proliferation index by immunohistochemistry.

Luminal A and Luminal B showedhigherincidence of breast cancer in 50-59 age group.HER 2 and Basal types had ahigherincidence at an earlier age group of 40-49 years. Most of the tumors were left sided and situated in upper outer quadrant. Luminal B, HER2 and Unclassified types had a relatively increased incidence of tumors with more than 5cm size. 72% of luminal A tumors belongs to histological variants of special types rather than infiltrating ductal carcinoma NST. Whereas 40%, 25%, 42% and 16% of luminal B, HER 2, basal and unclassified types respectively belongs to variants. The association of histological grade with the molecular classification was statistically significant with the p value of 0.01.The most common associated lesion was fibrocystic disease in all the subtypes. DCIS was commonly associated with Basal and Unclassified types. Unclassified typeof tumors had the highest incidence of skin involvement.Most of the

tumors had N2 stage of nodal involvement. Basal type (56%) had the highest incidence of N3 stage.Most of the tumors belonged to Stage II. One case of Stage IV was Basal type. ER, PR, HER2 neu, CK5/6 expression and proliferation index with Ki67 had a statistically significant association with the molecular classification.High proliferation index (>14%) with Ki67 was noted in Luminal B, Basal and Hybrid types.78% of the total 60 cases were alive and healthy. Unclassified type had the highest incidence of recurrence. One death was reported in HER2, Hybrid and Basal types.

Not all triple negative tumors are basal types, only those tumors which express basal markers are categorised as basal type tumors and the rest which do not express are unclassified which has got a better prognosis.Ki67 plays an important role in categorising luminal tumors.

On evaluating the inter rater agreement between the histopathological and molecular classification which is quantified by the kappa statistic by using Mc Nemar's test, it was found that all the subtypes showed negative kappa value.This indicates that the agreement is worse than chance and hence the importance of molecular classification is substantiated for the targeted therapy.

To conclude, breast cancers are heterogenous and having diverse clinical outcomes, these researches on molecular subgroups would pave way towards the "personalisation" of treatment for breast cancers with the more feasible and economic tool of immunohistochemistry.

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ANNEXURE – I

PROFORMA

Case number : Name :

HPE numbe : Age :

IP number : Sex :

Clinical diagnosis :

Menstrual status :

Risk factors if any :

Side of breast : Right/Left

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

GROSS

Specimen size :

Nipple areola and Skin :

Tumor size : Tumor margin :

Appearance :

Resected margins : Superior : Inferior :

Medial : Lateral :

Posterior :

Associated findings :

Total number of nodes dissected :

Largest node size :

MICROSCOPY

Histological subtype :

Histological score : Nuclear score: Mitotic score:

Modified Scarf Bloom Richardson Grade: I / II / III

Skin : Free / Involved

Nipple & Areola : Free / Involved

Margins : Superior : Free / Involved Inferior : Free / Involved

Medial : Free / Involved Lateral : Free / Involved

Posterior : Free / Involved

Lymphatic invasion : Present / Absent

Vascular invasion : Present / Absent

Lymphocytic infiltration : P resent / Absent

Necrosis : P resent / Absent

Associated breast lesions :

Total number of nodes dissected :

Number of nodes involved :

ANNEXURE II

WHO HISTOLOGICAL CLASSIFICATION OF EPITHELIAL BREAST TUMORS

INVASIVE BREAST CANCERS

Invasive ductal carcinoma not otherwise specified
Mixed type carcinoma
Pleomorphic carcinoma
Carcinoma with osteoclastic type of giant cells
Carcinoma with choriocarcinomatous features
Carcinoma with melanotic features
Invasive lobular carcinoma
Tubular carcinoma
Invasive cribriform carcinoma
Medullary carcinoma
Mucinous carcinoma
Cystadenocarcinoma
Signet ring carcinoma
Neuroendocrine tumors
Solid neuroendocrine carcinoma
Atypical carcinoid tumor
Small cell/oat cell carcinoma
Large cell neuroendocrine carcinoma
Invasive papillary carcinoma
Invasive micropapillary carcinoma
Metaplastic carcinoma
Apocrine carcinoma
Pure epithelial metaplastic carcinoma
Squamous cell carcinoma
Adenocarcinoma with spindle cell metaplasia
Adenosquamous carcinoma
Mucoepidermoid carcinoma
Mixed epithelial/mesenchymal metaplastic carcinoma
Lipid rich carcinoma
Secretory carcinoma
Oncocytic carcinoma
Adenoid cystic carcinoma
Acinic cell carcinoma
Glycogen rich carcinoma
Sebaceous carcinoma
Inflammatory carcinoma
Intraductal papillary carcinoma
Intracystic papillary carcinoma
Microinvasive carcinoma

NON INVASIVE BREAST CANCERS

Ductal carcinoma in situ
Lobular carcinoma in situ
Atypical papilloma

BENIGN EPITHELIAL TUMORS

Tubular adenoma
Lactating adenoma
Apocrine adenoma
Pleomorphic adenoma
Ductal adenoma
Papilloma

FIBROEPITHELIAL TUMORS

Fibroadenoma
Phyllodes tumor
Benign
Borderline
Malignant
Periductal stromal sarcoma
Mammaryhamartoma

INTRADUCTAL PROLIFERATIVE LESIONS

Atypical ductal hyperplasia
Flat epithelial atypia
Usual epithelial hyperplasia

METASTATIC TUMORS

ANNEXURE III

NOTTINGHAM MODIFICATION OF SCARF BLOOM

RICHARDSON GRADING SYSTEM

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

NUCLEAR PLEOMORPHISM	**SCORE**
Minimal variation in size and shape of nuclei	1
Moderate variation in size and shape of nuclei	2
Marked variation in size and shape of the nuclei	3

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

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

ANNEXURE IV

IMMUNOHISTOCHEMISTRY PROCEDURE

1. 4µ thick sections were cut from formalin fixed paraffin embedded tissue samples and transferred to gelatin-chrome alum coated slides.
2. The slides were incubated at 58ºC for overnight.
3. The sections were deparaffinized in xylene for 15 minutes x 2 changes.
4. The sections were dehydrated with absolute alcohol for 5 minutes x 2 changes.
5. The sections were washed in tap water for 10 minutes.
6. The slides were then immersed in distilled water for 5 minutes.
7. Heat induced antigen retrieval was done with microwave oven in appropriate temperature with appropriate buffer for 20 to 25 minutes.
8. The slides were then cooled to room temperature and washed in running tap water for 5 minutes.
9. The slides were then rinsed in distilled water for 5 minutes.
10. Wash with appropriate wash buffer (phosphate buffer) for 5 minutes x 2 changes.
11. Apply peroxidase block over the sections for 10 minutes.
12. Wash the slides in phosphate buffer for 5 minutes x 2 changes.
13. Cover the sections with power block for 15 minutes.
14. The sections were drained (without washing) and appropriate primary antibody was applied over the sections and incubated for 45 minutes.
15. The slides were washed in phosphate buffer for 5 minutes x 2 changes.
16. The slides were covered with Super Enhancer for 30 minutes.
17. The slides were washed in phosphate buffer for 5 minutes x 2 changes.
18. The slides were covered with SS Label for 30 minutes.
19. Wash in phosphate buffer for 5 minutes x 2 changes.
20. DAB substrate was prepared by diluting 1 drop of DAB chromogen to 1ml of DAB buffer.
21. DAB substrate solution was applied on the sections for 8 minutes.
22. Wash with phosphate buffer solution for 5 minutes x 2 changes.
23. The slides are washed well in running tap water for 5 minutes.
24. The sections were counterstained with Hematoxylin stain for 2 seconds (1 dip).
25. The slides are washed in running tap water for 3 minutes.
26. The slides are air dried, cleared with xylene and mounted with DPX.

ANNEXURE V

ER, PR scoring

Intensity is assigned

0 = none,

1 =weak,

2 = intermediate,

3 = strong,

Proportion as

0 = none,

1 = <1/100,

2 = 1/100 to 1/10, (1-10cells/100 cells)

3 = 1/10 to 1/3, (11-33.3 cells /100 cells)

4 = 1/3 to 2/3, (34-66.7 cells /100 cells)

5 = >2/3. (>67 cells/100 cells)

The scores are added together to obtain a total score that can range from 0 to 8.

Tumors scoring 2 or less – ER negative and have a negligible chance of response.

STAINING PATTERN AND HER2 NEU SCORING

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

CK 5/6 SCORING

Score 1: 25% of the tumour tissue shows positivity.

Score 2: 26-50% of cells were positive.

Score 3: 51-75% showed positivity.

Score 4: 76-100% was positive.

S.NO	HPE NO	Age	Sex	Side	P/D	TL	Size	HT	G	AL	LVI	LYI	Nec	SK	LNI/LND	M	ST	TG	F UP	ER	PR	H2N	CK	Ki67	MC	T OP
1	2174/11	50	F	R	MRM	UOQ	4	MET		FCD	P	A	A	A	I/9	FREE	IIb	S+6+RT	A&H	3+3	3+2	2+	1+	HI	LB	S+3+PB+RT
2	3144/11	52	F	R	MRM	CQ	3	PAP		FCD	A	P	A	A	O/9	FREE	IIA	S+6	A&H	3+2	4+3	0	0	LO	LA	S+HT+3
3	5098/11	45	F	L	MRM	CQ	6	MET		FCD	P	A	A	A	II/8	FREE	IIIA	3+S+RT	D	1+1	1+1	0	3+	HI	BA	3+S+PB+RT
4	5223/11	54	F	L	MRM	LOQ	3	LOB		DCIS	P	A	A	A	III/9	FREE	IIB	S+3+RT	A&H	4+3	3+3	0	0	LO	LA	S+HT+RT
5	5451/11	54	F	R	MRM	CQ	7	MET		DCIS	P	P	A	A	V/5	POST	IIIA	3+S+3+RT	A&H	3+2	3+2	1+	1+	LO	LA	S+HT+RT
6	6973/11	43	F	L	MRM	UOQ	3	APO		FCD	P	P	A	A	VIII/11	FREE	IIIA	S+3+RT	A&H	3+3	3+2	3+	1+	LO	L+H	S+PB+HC+RT
7	7448/11	54	F	L	MRM	UOQ	4	PAP		FCD	A	P	A	A	II/7	FREE	IIB	S+3+RT	A&H	3+3	2+3	0	0	LO	LA	S+HT
8	7692/11	58	F	L	MRM	CQ	10	MET		FCD	A	P	P	P	II/12	FREE	IIIA	3+S+3+RT	REC	0+0	0+0	0	2+	HI	UC	S+3+PB+RT
9	7709/11	45	F	L	MRM	UOQ	5	MED		FCD	A	P	A	A	O/12	FREE	IIA	S+3	A&H	3+3	3+2	2+	1+	HI	LB	S+3+PB+RT
10	7764/11	70	F	R	MRM	CQ	4	MUC		FCD	P	P	A	A	I/9	FREE	IIB	S+3+RT	A&H	0+0	0+0	0	3+	LO	BA	S+HT+PB+RT
11	7891/11	53	F	R	MRM	UOQ	5	MET		FCD	A	P	A	A	I/9	FREE	IIB	S+3	A&H	4+2	1+1	0	0	LO	LA	S+HT
12	1353/12	44	F	R	MRM	CQ	3	APO		FCD	A	P	A	A	II/9	FREE	IIB	S+3	A&H	1+1	1+1	0	3+	HI	BA	S+PB+RT
13	1523/12	41	F	L	MRM	UIQ	3	PAP		FCD	A	P	A	A	O/9	FREE	IIA	S+3	A&H	2+3	4+3	0	0	LO	LA	S+HT
14	1696/12	56	F	R	MRM	UOQ	3	MED		FCD	A	P	A	A	II/10	FREE	IIB	3+S	REC	0+0	0+0	0	4+	HI	BA	S+3+PB+RT
15	3467/12	61	F	L	MRM	LOQ	2	LOB		FCD	P	P	A	A	I/7	FREE	IIA	S+3+RT	A&H	3+3	4+2	0	0	LO	LA	S+HT+RT
16	4653/12	65	F	R	MRM	UOQ	6	MUC		FCD	A	P	A	A	O/9	FREE	IIB	3+S+3	A&H	3+3	3+3	0	1+	LO	LA	S+HT
17	5159/12	45	F	R	MRM	CQ	9	MUC		FCD	A	P	A	P	III/9	SKIN	IIIB	3+S+RT	A&H	3+3	3+3	0	1+	LO	LA	3+S+HT
18	8213/12	58	F	L	MRM	CQ	5	MED		FCD	P	P	A	A	III/21	FREE	IIB	3+S+3+RT	A&H	4+2	5+3	0	1+	LO	LA	S+HT+RT
19	8236/12	50	F	R	MRM	UOQ	6	MED		SA	P	P	A	A	XII/12	FREE	IIIC	3+S+3+RT	REC	3+3	4+3	3+	0	HI	L+H	S+HC+PB+RT
20	9235/12	48	F	L	MRM	UOQ	4	APO		FCD	P	P	A	A	II/7	FREE	IIB	S+3+RT	A&H	0+0	0+0	0	3+	HI	BA	S+3+PB+RT
21	9737/12	65	F	L	MRM	UOQ	5	MUC		FCD	P	P	A	A	O/7	FREE	IIA	S+3	A&H	4+2	4+3	0	1+	LO	LA	S+HT+RT
22	1069/13	62	F	R	MRM	UOQ	5	DC NST	I	FCD	A	P	A	A	II/13	POST	IIB	S+6+RT	A&H	1+1	1+1	0	2+	HI	UC	S+PB+RT
23	1868/13	52	F	R	MRM	UOQ	4	DC NST	III	DCIS	P	P	P	A	O/11	FREE	IIA	S+6+RT	A&H	1+1	1+1	1+	3+	HI	BA	S+3+PB+RT
24	1899/13	55	F	L	MRM	CQ	3	DC NST	III	FCD	P	A	A	A	IV/7	FREE	IIIA	S+6+RT	REC	4+3	3+3	3+	1+	HI	L+H	S+3+PB+RT
25	2351/13	35	F	L	MRM	CQ	5	DC NST	III	SA	p	A	A	A	IV/8	POST	IIIA	S+6+RT	A&H	0+0	0+0	3+	1+	LO	H	S+HC+RT
26	2778/13	45	F	R	MRM	CQ	5	DC NST	II	FCD	A	P	P	P	O/13	FREE	IIIB	S+6	A&H	0+0	1+1	0	4+	HI	BA	S+HC+3+PB+RT
27	2916/13	55	F	R	MRM	LOQ	5	LOB		DCIS	P	A	A	A	II/8	FREE	IIB	S+3+RT	A&H	3+3	2+3	0	1+	LO	LA	S+HT+RT
28	3428/13	40	F	L	MRM	CQ	8	DC NST	I	FA	A	P	A	A	I/7	FREE	IIIA	S+3+RT	A&H	2+1	1+1	3+	1+	HI	H	S+HT+HC+RT
29	3446/13	50	F	L	MRM	UOQ	3	DC NST	II	FCD	P	P	A	A	VII/10	LAT	IIIA	S+6+RT	D	5+3	3+3	3+	0	HI	L+H	S+HC+PB+RT
30	3765/13	65	F	R	MRM	UOQ	6	DC NST	I	FCD	A	P	A	A	VII/13	FREE	IIIA	S+6+RT	REC	3+1	2+1	2+	2+	HI	LB	S+HC+RT
31	4161/13	72	F	L	MRM	UOQ	3	DC NST	II	SA	P	P	A	A	I/7	FREE	IIB	3+S+3	A&H	5+3	2+3	0	1+	LO	LA	S+HT+RT
32	5379/13	55	F	L	MRM	UOQ	3	DC NST	III	FCD	P	P	A	A	O/12	FREE	IIA	S+6+RT	A&H	0+0	0+0	3+	1+	HI	H	S+HC+RT

33	5429/13	50	F	L	MRM	CQ	7	DC NST	I	FCD	P	P	A	A	O/11	POST	IIB	3+S+3+RT	A&H	1+1	0+0	2+	3+	HI	BA	S+3+PB+RT
34	5601/13	45	F	L	MRM		4	DC NST	III	FCD	P	P	A	A	VII/12	FREE	IIIA	S+6+RT	REC	0+0	0+0	3+	0	HI	H	S+HC+RT
35	5882/13	65	F	L	MRM	UOQ	6	LOB		DCIS	P	P	P	A	VII/15	POST	IIIA	S+6+RT	A&H	0+0	0+0	3+	0	HI	H	S+HC+RT
36	6377/13	48	F	L	MRM	UOQ	7	DC NST	I	DCIS	P	P	A	A	O/13	FREE	IIB	S+3	A	0+0	0+0	0	4+	HI	BA	S+PB+RT
37	6513/13	59	F	L	MRM	LOQ	2	DC NST	I	DCIS	P	P	A	P	O/13	FREE	I	S+3	REC	0+0	0+0	0	1+	HI	UC	S+PB+RT
38	6639/13	37	F	R	MRM	UOQ	5	DC NST	I	FCD	P	P	A	A	III/9	POST	IIB	S+6+RT	A&H	3+2	3+2	0	0	LO	LA	S+HT+RT
39	6957/13	50	F	L	MRM	LOQ	3	DC NST	II	SA	P	P	A	A	O/9	FREE	IIB	S+3	A&H	0+0	0+0	3+	1+	LO	H	S+HC
40	6959/13	54	F	L	MRM	UOQ	3	DC NST	II	FCD	P	P	A	A	O/14	FREE	IIA	S+3	A&H	0+0	0+0	0	3+	HI	BA	S+PB+RT
41	7004/13	46	F	L	MRM	UOQ	4	PAP		FCD	P	A	A	A	II/6	FREE	IIB	S+3+RT	A&H	4+3	3+3	0	0	LO	LA	S+HT+RT
42	7093/13	75	F	R	MRM	UOQ	4	MUC		DCIS	P	P	A	A	III/11	T AND	IIB	S+3+RT+3	A&H	3+3	3+3	0	1+	LO	LA	S+HT+RT
43	7153/13	42	F	R	MRM	UOQ	6	DC NST	I	DCIS	P	P	A	A	II/10	FREE	IIIA	3+S+T+RT	A&H	1+1	0+0	0	1+	HI	UC	S+3+RT
44	7160/13	35	F	L	MRM	UOQ	6	DC NST	III	FCD	P	P	P	A	V/11	FREE	IIIA	S+3+RT	A&H	3+3	3+2	2+	1+	HI	LB	S+3+PB+RT
45	7164/13	60	F	R	MRM	UOQ	3	APO		FCD	A	P	A	A	IV/9	FREE	IIIA	S+6+RT	A&H	4+3	4+3	3+	0	HI	L+H	S+HC+PB+RT
46	7173/13	60	F	L	MRM	UIQ	8	DC NST	II	FCD	P	P	A	A	O/9	FREE	IIB	3+S+3	A&H	5+3	0+0	0	1+	HI	LA	S+HT+3
47	7285/13	51	F	R	MRM	UOQ	3	DC NST	II	FCD	A	P	A	A	O/7	FREE	IIA	S+3	A&H	0+0	0+0	0	4+	HI	BA	S+PB
48	7433/13	26	F	R	MRM	CQ	5	MED		FCD	P	P	A	A	V/11	POST	IIIA	S+3+RT	D	0+0	0+0	0	4+	HI	BA	S+PB+RT
49	7478/13	67	F	L	MRM	UOQ	4	APO		FCD	P	P	A	A	II/6	FREE	IIB	S+3+RT	A&H	3+2	2+2	2+	1+	HI	LB	S+HT+RT
50	7552/13	63	F	L	MRM	UOQ	4	LOB		FCD	P	P	A	A	II/7	FREE	IIB	S+3+RT	A&H	3+3	4+2	0	1+	LO	LA	S+HT+RT
51	7608/13	42	F	R	MRM	UOQ	2	DC NST	I	FCD	A	P	A	A	O/7	FREE	I	S+3	A&H	3+3	4+2	0	0	LO	LA	S+HT+RT
52	7750/13	42	F	L	MRM	UOQ	3	DC NST	II	FCD	P	P	A	A	III/8	FREE	IIB	S+3	A&H	0+0	0+0	0	4+	LO	BA	S+PB+RT
53	7986/13	57	F	L	MRM	UIQ	3	PAP		FCD	A	P	A	A	O/8	FREE	IIA	S+6	A&H	4+3	2+3	0	0	LO	LA	S+HT
54	8034/13	32	F	R	MRM	LOQ	5	DC NST	I	FCD	A	P	A	A	O/7	FREE	IIA	S+3	A&H	0+0	1+1	1+	0	LO	UC	S+3+RT
55	8407/13	59	F	R	MRM	UOQ	4	DC NST	II	DCIS	A	P	A	A	IV/9	FREE	IIIA	S+3+RT	A&H	4+2	2+2	0	0	HI	LA	S+HT+RT
56	8469/13	48	F	L	MRM	UOQ	4	DC NST	III	FCD	P	P	P	A	III/8	POST	IIB	3+S+3+RT	REC	1+1	1+1	3+	0	HI	H	S+HC+RT
57	8552/13	67	F	L	MRM	UOQ	2	DC NST	III	DCIS	P	P	A	A	III/8	MED	IIA	S+3+RT	A&H	0+0	1+1	0	3+	HI	BA	S+PB+RT
58	8593/13	67	F	R	MRM	UOQ	7	DC NST	III	FCD	P	P	P	P	II/9	SKIN	IIIB	3+S+3+RT	REC	0+0	0+0	1+	4+	HI	BA	S+PB+RT
59	8781/13	62	F	R	MRM	UOQ	3	DC NST	I	FCD	P	P	P	A	III/8	POST	IIB	S+3+RT	A&H	1+1	0+0	0	4+	HI	BA	S+PB+RT
60	8828/13	42	F	L	MRM	UOQ	4	DC NST	III	FCD	P	P	A	A	IV/9	POST	IIIA	S+6+RT	REC	0+0	0+0	0	0	HI	UC	S+3+RT

KEY TO MASTER CHART

M	: Male
F	: Female
R	: Right
L	: Left
P/D	: Procedure Done
MRM	: Modified radical mastectomy
TL	: Tumor location
UOQ	: Upper Outer Quadrant
UIQ	: Upper inner quadrant
CQ	: Central Quadrant
HT	: Histological type
IDC NST	: Infiltrating ductal carcinoma – No special type
PAP	: Invasive papillary carcinoma
MET	: Metaplastic carcinoma
MED	: Medullary carcinoma
MUC	: Mucinous carcinoma
APO	: Apocrine carcinoma
LOB	: Lobular carcinoma
G	: Grade
AL	: Associated lesions
DCIS	: Ductal carcinoma in situ
FCD	: Fibrocystic disease
SA	: Sclerosing adenosis
LVI	: Lymphovascular invasion

LYI	: Lymphocytic infiltration
Nec	: Necrosis
SK	: Skin involvement
LNI/LND	: lymph nodes involved/ lymphnodes dissected
M	: Margins
P	: Present/positive
A	: Absent
N	: Negative
POST	: Posterior margin
LAT	: Lateral margin
MED	: Medial margin
SUP	: Superior margin
INF	: Inferior margin
ST	: Stage
ER	: Estrogen receptor
PR	: Progesterone receptor
H2N	: Her 2 neu
CK	: Cytokeratin 5/6
Ki67	: Ki67 labelling index
MC	: Molecular classification
LA	: Luminal A
LB	: Luminal B
H	: HER2
L+H	: Luminal + HER2 hybrid
BA	: Basal
UC	: Unclassified
TG	: Treatment given

T OP	: Treatment option
S	: Surgery
RT	: Radiotherapy
HT	: Hormonal therapy
3	: 3 drug regimen chemotherapy
6	: 6 drug regimen chemotherapy
HC	: Herceptin
PB	: Platinum based chemotherapy
F UP	: Follow up
A&H	: Alive and healthy
A&R	: Alive with recurrence
D	: Dead

INFORMED CONSENT FORM

Title of the Study: **"Comparison of Clinicopathological parameters and Molecular classification of breast carcinoma"**

Name of the Participant :

Name of the Principal Investigator: **Dr.K.Indumathi**

Name of the institution : Institute of Pathology, Madras Medical College, Chennai – 600 003.

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in "Comparison of Clinicopathological parameters and Molecular classification of breast carcinoma ".

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study.
4. I have been explained about my rights and responsibilities by the investigator.
5. I have informed the investigator of all the treatments I am taking or have taken in the past.
6. I have informed the investigator about any native/ alternative treatment taken for _____ months.
7. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.
8. I have understand that my identity will be kept confidential if my data are publicly presented
9. I have had my questions answered to my satisfaction.
10. I have decided to be in the research study.

I am aware that if I have any question during this study. I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

Name and Signature/ thumb impression of the Participant (or legal representative if participant is incompetent)

_____ _____ _____

Name Signature Date

Name and Signature of impartial witness required for illiterate patients):

_____ _____ _____

Name Signature Date

Name and Signature of the investigator or his representative obtaining the consent:

_____ _____ _____

Name Signature Date

ஆராய்ச்சி ஒப்புதல் படிவம்

ஆராய்ச்சி தலைப்பு

மார்பக புற்றுநோயின் உடற்பொருள்- நோய் குறியியல் கூறுபாடுகளையும் மூலக்கூறு வகைபாட்டையும் ஒப்பீடு செய்தல்

பெயர்	:	தேதி	:
வயது	:	உள் நோயாளி எண்	:
பால்	:	ஆராய்ச்சி சேர்க்கை எண்	:

இந்த ஆராய்ச்சியின் விவரங்களும் அதன் நோக்கமும் முழுமையாக எனக்கு தெளிவாக விளக்கப்பட்டது.

எனக்கு விளக்கப்பட்ட விஷயங்களை புரிந்துகொண்டு நான் எனது சம்மதத்தை தெரிவிக்கிறேன்.

எனது மார்பக புற்றுநோயால் பாதிக்கப்பட்ட திசுக்களை சதை பரிசோதனை செய்து கொள்ள சம்மதம்.

இந்த ஆராய்ச்சியில் பிறரின் நிர்பந்தமின்றி என் சொந்த விருப்பத்தின்பேரில் நான் பங்கு பெறுகின்றேன். இந்த ஆராய்ச்சியில் இருந்து நான் எந்நேரமும் பின் வாங்கலாம் என்பதையும் அதனால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் நான் புரிந்துகொண்டேன்.

நான் மார்பக புற்றுநோயால் பாதிக்கப்பட்ட திசுக்களை குறித்த இந்த ஆராய்ச்சியின் விவரங்களைக் கொண்ட தகவல் தாளைப் பெற்றுக் கொண்டேன்.

நான் என்னுடைய சுய நினைவுடனும் மற்றும் முழு சுதந்திரத்துடனும் இந்த மருத்துவ ஆராய்ச்சியில் என்னை சேர்த்துக்கொள்ள சம்மதிக்கிறேன்.

எனக்கு அறுவைச்சிகிச்சை செய்யப்பட்டு நோய்க்குறியியல் துறையில் சதைப் பரிசோதனைக்கு பயன்பட்ட மெழுகுக்கட்டிகளை வைத்து ஆராய்ச்சி மற்றும் சிறப்புப் பரிசோதனை செய்து கொள்ள சம்மதம் தெரிவிக்கிறேன்.

ஆராய்ச்சியாளர் கையொப்பம் பங்கேற்பாளர் கையொப்பம்

நாள் :
இடம் :