CORRELATION OF HISTOPATHOLOGICAL FEATURES AND IMMUNOHISTOCHEMISTRY IN THE STUDY OF BREAST CANCER

Dissertation

Submitted to

THE TAMILNADU Dr. M.G.R MEDICALUNIVERSITY

In partial fulfilment of the requirements for the award of the degree of

M.D PATHOLOGY

Branch III

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CERTIFICATE

This is to certify that the dissertation entitled **"CORRELATION OF HISTOPATHOLOGICAL FEATURES AND IMMUNOHISTOCHEMISTRY IN THE STUDY OF BREAST CANCER"** is a bonafide work done by **Dr. JEM KOLLAVANA RAJ** in partial fulfilment of the university rules and regulations for award of **M.D. Pathology [Branch-III]** under my guidance and supervision during the academic year 2014-2017.

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DECLARATION

I **Dr. JEM KOLLAVANA RAJ** hereby submit the dissertation **"CORRELATION OF HISTOPATHOLOGICAL FEATURES AND IMMUNOHISTOCHEMISTRY IN THE STUDY OF BREAST CANCER"** done in partial fulfilment for the award of the degree **M.D.PATHOLOGY [Branch-III]** in Sree Mookambika Institute of Medical Sciences, Kulasekharam. This is an original work done by me under the guidance and supervision of **Dr. JAYASREE P.V**

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Dr. Jem Kollavana Raj

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Introduction

INTRODUCTION

Breast Cancer is the most frequent cancer in women worldwide with 1.05 million new cases every year and represents over 20% of all malignancies noted in females.[1] More than 50% of breast cancer incidence occurs in developed countries.[1] Incidence of breast cancer is increasing in most of the countries. It is estimated that in 2001 there were approximately 80,000 new breast cancer cases in India. [1]

The population based cancer registry data from the various parts of the country, has revealed breast cancer as the commonest cancer among women in Delhi, Mumbai, Ahmadabad, Calcutta and Trivandrum. This malignancy accounts for 19–34% of all cancer cases among women nationally.[1]

Several clinical and morphological parameters such as histological type of tumour, tumour grade, bilateralism etc. have been established as the predictors of tumour behaviour in breast cancer patients. These prognostic factors are indicators of the inherent aggressiveness of the tumour as well as of the extent of the disease and based on these factors, treatment decisions are being taken up by the clinicians.[1]

Immunohistochemistry (IHC) is a method for localizing specific antigens in tissues or cells based on antigen-antibody recognition; it seeks to exploit the specificity provided by the binding of an antibody with its antigen at a light-microscopy level. IHC has a long history that dates back more than 70 years, when Coons first developed an immunofluorescence technique to detect corresponding antigens in frozen tissue sections.[2]

Estrogen and Progesterone receptors (ER, PR) and more recently HER-2/neu have with increasing importance influenced the management of breast malignancy.[3]

Tumours that are better differentiated are more likely to be ER and PR positive and have a relatively better prognosis. Survival and response to hormone therapy are more favourable among women with tumour positive for ER and PR[4]

This study attempts to correlate the histopathological features seen in a variety of cases of breast carcinoma at the institution and compare it with the Immunohistochemical profile of thesis cases.

Aims of the Study

AIMS AND OBJECTIVES

1. To study the histopathological and immunohistochemical patterns of breast cancer
2. To establish a correlation between the expression patterns of ER, PR, HER2/neu with the tumour histopathology in carcinoma breast.

Review of Literature

REVIEW OF LITERATURE

History

Some of the initial accounts of breast cancer dates back to “The Edwin Smith Surgical Papyrus” (3000-2500 BC), that mentions eight cases of breast afflictions. The author also specifically mentions that there was no known treatment for the breast tumors that were cool to touch and were bulging. [5]

A detailed account of breast cancer cases was described by Hippocrates. He described that these tumours in the breast became progressively harder. They eventually disseminated to other parts of the body, accompanied by shooting pain radiating from the breast to the neck and shoulder blades. The death was certain with the development of thirst and emaciation.[5]

The manuscript, “De Medicina”, written by Celsus, defined four stages of breast cancer. The first stage of inflammation, was progressively accompanied by carcinomatous change without involving the skin, this then further developed into carcinomatous change with prominent involvement of the skin. The final stage was an exophytic lesion which bleeds on touch, this was, called the “thymium” which looked similar to thyme flower.

In uncertain conditions, the tumour was treated using caustics and if symptoms disappeared, it was considered as inflammation. If it got worse,

then it was cancer. Treatment was often successful in certain cases of phyllodes tumours or tuberculosis.[5]

The nineteenth century witnessed remarkable advancements in pathology and effectiveness of surgery as well as precautions taken during surgery.[5]

The advent of microscope by Anton Van Leuwenhoek (1674-1723) triggered the leap into microscopic identification of structures and the Germans became forerunners in developing the compound achromatic microscope.[5] Johannes Muller was the first scientist to hypothesize that cancer was also composed of live cells.[5]

The advancements in the surgical field brought modified mastectomies into existence.

Initially there was much mistrust regarding biopsies, it was considered to promote and progress the carcinoma, but with newer techniques and understanding regarding carcinomas this mind set has been completely disregarded and biopsies together with ancillary testings have proved to be effective in diagnosing as well as having prognostic implications.[5]

The past 100 has seen drastic changes from the radical surgery to the more novel approaches of breast conservative surgeries as well as proper effective screening methods, long before it relatively harmless lesions develop into an irreversible carcinoma.[5]

Incidence and epidemiology of Breast Carcinoma

Statistics taken from the American Cancer Society for the year 2015-2016 descriptively shows that the American population has a high risk of developing breast cancer. It has statistically evaluated cases over the years and has come up with projections and extrapolations from which numerous inferences have been drawn. Some of these predictions include:[6]

- In 2015-16 period, an estimated 231,840 cases of breast carcinoma will be diagnosed.[6]
- Estimates show that 2015-16, about 40,290 women will die from breast cancer. [6]
- Lung cancer is the only cancer that is responsible for more cancer deaths in women. [6]
- Estimates reveal that in 2015-16, 2,350 men would be diagnosed with breast cancer. [6]

These are some of the findings from international studies, the Indian scenario is fast approaching a similar pattern as seen in western societies as younger women are being diagnosed with breast carcinoma, this is usually attributed to changes in lifestyle and incorporation of western trends into a previously non-secular culture.

Risk factors associated with Breast Carcinoma

Many risk factors for the development of breast carcinoma have been implicated, prolonged unopposed estrogen stimulation has been proposed as

one of the more important underlying causes of development of breast carcinomas.[7]

1 *Place of birth*: Developed countries show more cases of breast cancer than developing countries.[7]

2 *Family history*: Relative with breast carcinoma have an increased risk than that of the general population.[7]

3 *Menstrual and reproductive history.* Increased risk is seen in early menarche, late age at first birth, and menopause. [7]

4 *Exogenous estrogens:* Hormone replacement therapy and use of oral contraceptive drugs have been implicated incarcinoma breast. [7]

5 *Ionizing radiation*: Exposure to ionizing radiation conferred an increased risk to developing breast carcinoma.[7]

6 *Breast augmentation*: Controversial risk factor that has been studied extensively and studies have yet to find if there is a positive correlation with breast augmentation and risk of developing breast carcinoma. [7]

7 Exercise: Reduced risk is associated with premenopausal women who exercise regularly.[7]

8 *Diet*: High fat diet and obesity carry increased risk.[7]

9 *Others.* Association between breast carcinoma and meningioma has been repeatedly noted. [7]

CLASSIFICATION OF BREAST CARCINOMAS

The histopathological classification of breast carcinoma is based on the diversity of the morphological features of the tumors. In the version, endorsed by the WHO in 2003, includes about 20 major tumor types and 18 minor subtypes.[8] The 4th edition of the WHO Classification of tumors of the Breast is the most recent addition to this series, and provides a timely update to many new aspects of breast cancer classification that have occurred since the publication of the 3rd edition in 2003. [9]

WHO CLASSIFICATION OF TUMOURS OF THE BREAST [9, 10]

Precursor lesions

- Ductal carcinoma in situ
- Lobular neoplasia
    - Lobular carcinoma in situ
        - Classic lobular carcinoma in situ
        - Pleomorphic lobular carcinoma in situ
    - Atypical lobular hyperplasia
- Intraductal proliferative lesion
    - Usual ductal hyperplasia
    - Columnar cell lesions including flat epithelial atypia
    - Atypical ductal hyperplasia

Invasive carcinoma of no special type (NST)

- Pleomorphic carcinoma

- Carcinoma with osteoclast-like stromal giant cells
- Carcinoma with choriocarcinomatous features
- Carcinoma with melanotic features

Invasive lobular carcinoma

- Classic lobular carcinoma
- Solid lobular carcinoma
- Alveolar lobular carcinoma
- Pleomorphic lobular carcinoma
- Tubulolobular carcinoma
- Mixed lobular carcinoma

Tubular carcinoma

Cribriform carcinoma

Mucinous carcinoma

Carcinoma with medullary features

- Medullary carcinoma
- Atypical medullary carcinoma
- Invasive carcinoma NST with medullary features

Carcinoma with apocrine differentiation

Carcinoma with signet-ring-cell differentiation

Invasive micropapillary carcinoma

Metaplastic carcinoma of no special type

- Low-grade adenosquamous carcinoma

- Fibromatosis-like metaplastic carcinoma
- Squamous cell carcinoma
- Spindle cell carcinoma

Metaplastic carcinoma with mesenchymal differentiation

- Chondroid differentiation
- Osseous differentiation
- Other types of mesenchymal differentiation

Mixed metaplastic carcinoma

Myoepithelial carcinoma

Epithelial-myoepithelial tumors

- Adenomyoepithelioma with carcinoma
- Adenoid cystic carcinoma

Rare types

Carcinoma with neuroendocrine features

- Neuroendocrine tumor, well-differentiated
- Neuroendocrine carcinoma poorly differentiated (small cell carcinoma)
- Carcinoma with neuroendocrine differentiation

Secretory carcinoma

Invasive papillary carcinoma

Acinic cell carcinoma

Mucoepidermoid carcinoma

Polymorphous carcinoma

Oncocytic carcinoma

Lipid-rich carcinoma

Glycogen-rich clear cell carcinoma

Sebaceous carcinoma

Inflammatory carcinoma

Lobular neoplasia

- Lobular carcinoma in situ

Intraductal proliferative lesions

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

Microinvasive carcinoma

Intraductal papillary neoplasms

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

Benign epithelial proliferations

Adenosis including variants

- Sclerosingadenosis

- Apocrine adenosis
- Blunt duct adenosis
- Microglandularadenosis
- Adenomyoepithelialadenosis
- Radial scar / complex sclerosing lesion

Adenomas

- Tubular adenoma
- Lactating adenoma
- Apocrine adenoma
- Pleomorphic adenoma
- Ductal adenoma

Myoepithelial lesions

- Myoepitheliosis
- Adenomyoepithelialadenosis
- Adenomyoepithelioma
- Malignant myoepithelioma

Mesenchymal tumours

- Haemangioma
- Angiomatosis
- Haemangiopericytoma
- Pseudoangiomatous stromal hyperplasia
- Myofibroblastoma

- Fibromatosis (aggressive)
- Inflammatory myofibroblastic tumour
- Lipoma
- Angiolipoma
- Granular cell tumour
- Neurofibroma
- Schwannoma
- Angiosarcoma
- Liposarcoma
- Rhabdomyosarcoma
- Osteosarcoma
- Leiomyoma
- Leiomyosarcoma

Fibroepithelial tumours

- Fibroadenoma
- Phyllodes tumour
- Benign
- Borderline
- Malignant
- Periductal stromal sarcoma, low grade
- Mammary hamartoma

Tumours of the nipple

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

Malignant lymphoma

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

Metastatic tumours

Tumours of the male breast

- Gynaecomastia
- Carcinoma
    - Invasive
    - In situ

Breast carcinoma can be broadly categorized into in situ carcinoma and invasive (infiltrating) carcinoma. Breast carcinoma in situ is further sub-classified as either ductal or lobular; growth patterns and cytological features form the basis to distinguish between the two types. Ductal carcinoma in situ (DCIS) is considerably more common than its lobular carcinoma in situ (LCIS) and comprises of a heterogeneous group of tumors. DCIS has been further sub classified based on the architectural features of the tumor which

has given rise to five well recognized subtypes: Comedo, Cribiform, Micropapillary, Papillary and Solid. [11]

While this classification scheme has been a valuable tool, it relies solely on histology without utilizing newer molecular markers that have a proven prognostic significance.[11]

MOLECULAR CLASSIFICATION OF BREAST CARCINOMA

Studies have shown several molecular subtypes of breast cancer that were classified as: Basal-like, Her2/neuenriched, normal breast like, luminal subtype A and luminal subtype B [11]

A new subtype categorized as "claudin low" has also been incorporated. Claudin low subtype lack tight junction proteins including claudin 3 and E-cadherin, and were characterized by a low expression of luminal markers and a high expression of mesenchymal markers.

These subtypes of cancer were detected using microarray-based gene expression analysis.[11]

These subtypes display highly significant differences in predicting overall survival, as well as disease-free survival with the basal like (ER- /PR- /ErbB2-) subtype having the shortest survival.[11]

Molecular classification was able to stratify the ER+ population into several subtypes that, again, demonstrated a difference in patient survival.[11]

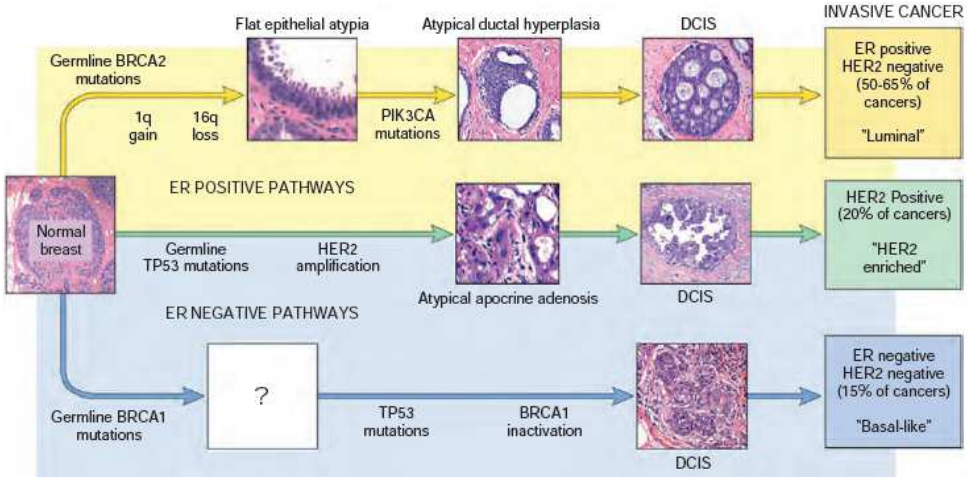

Fig.1 Major pathways of breast cancer development

FUNCTIONAL CLASSIFICATION OF BREAST CARCINOMA [11]

Breast cancer stem cells are getting widespread appeal because of the amount f research that has come in support saying that these stem cells are capable of initiating malignancies.[11]

The stem cell theory hypothesizes that there are only a few elements present in the tumour that is capable of initiating and propagating the progression of the tumour. While the remaining cells present in the tumour are of generally low malignant potential.[12]

Numerous classifications of breast carcinoma exist. Newer classifications are still being researched and more data is becoming available. These classifications give us an idea of how to incorporate newer methods into the histological classification, so as to improve the diagnosis as well as to ensure the physician has a clear understanding regarding the prognosis of each case.

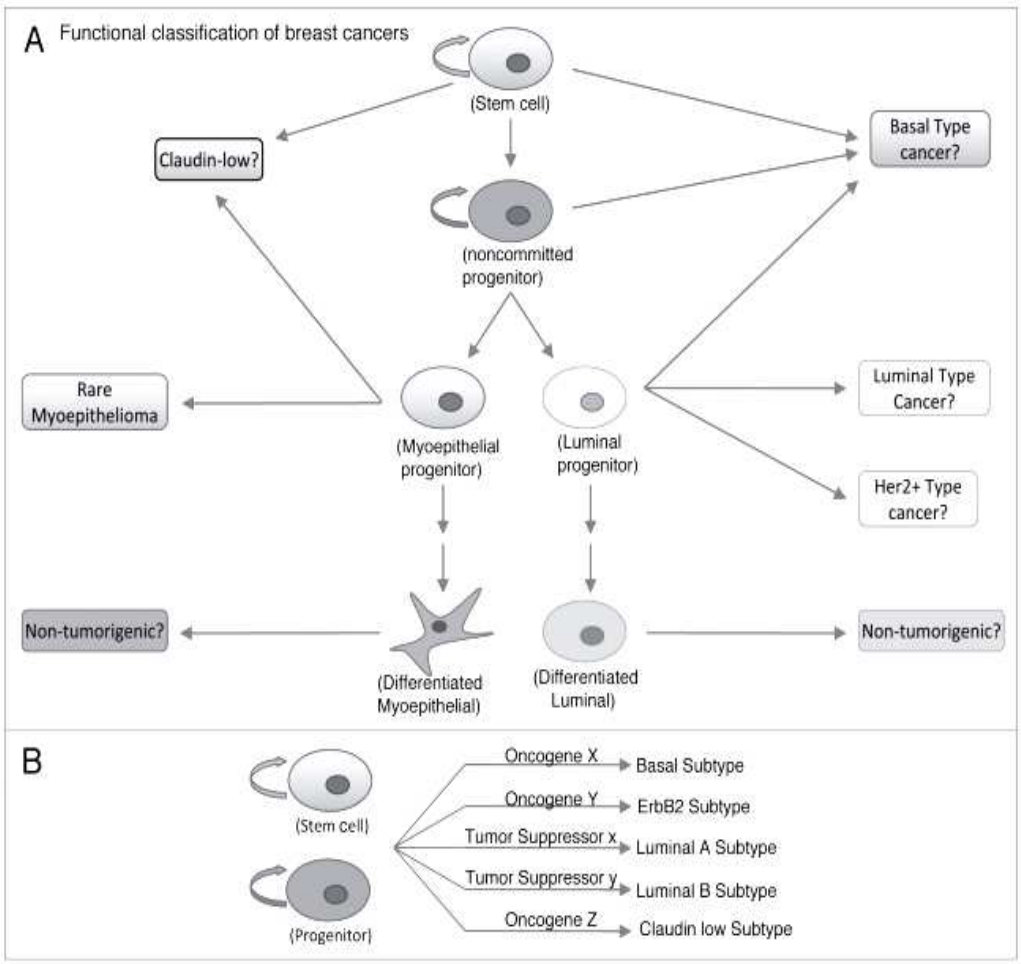

Fig.2 Molecular subtypes of breast cancer

HISTORICAL DEVELOPMENTS IN HISTOLOGICAL GRADING SYSTEM OF BREAST CARCINOMA

The grading of tumours is primarily based on the fact that the malignancy of a tumour must be related to its pathological pattern.

Von Hansemannwas the first to suggest this in 1893. Only in the early twentieth century were these observations systematized. [13]

In 1920, Broders was the first to use the method of grading and he published numerous papers with further improvement on this technique. [13]

Two features have been used to identify malignancy and thereby two techniques. Both includes intimate features of tumour cells and different methods of grading, including that of Broders, was based solely on this variable, Greenoughwas the first to apply this to breast cancer. [13]

Numerous factor based on morphology was introduces, and with it came many classifications of breast cancer. In 1925, Greenough introduced breast carcinoma grading. He used seven histological features to classify breast carcinomas into 3 grades. [13]

Scarff simplified the Greenough method and took in three factors namely; variation in nuclear size, tubule formation and hyperchromatism of nuclei.[13]

In 1957, Bloom and Richardson devised a simpler system which used only three of Greenough's variables: tubularity, pleomorphism, and nuclear hyperchromatism. [14]

This grading system was effective for prognosis. Modifications to enhance responsibility of scores resulted in the Nottingham Modification-Bloom-Richardson system (MBR), which has been approved by the World Health Organization and College of American Pathologists .[14]

The basic principle was adding up scores for the three variables. each of which is assigned from one to three points according to how much they vary from normal breast epithelium. A total score of 5 or less defines Grade I, 6-7 points Grade II, and 8-9 points Grade III. [14]

Factors in Breast Carcinoma Prognosis

The CAP Consensus Statement has proposed three categories of prognostic markers:[15]

Category I include factors of proven histological importance and are useful in clinical management namely the TNM staging, the histological grade, the histological subtype, the mitotic count and hormone receptor status. These factors hence become mandatory inclusions for breast pathology report.[15]

Category II factors are studied biologically and clinically but their importance need to be validated with more dependable studies. The factors are Human epidermal growth factor receptor2/neuroblastoma (HER2/neu), lymphovascular invasion, p53, proliferative markers (MIB-1).[15]

The Category III factors are not yet sufficiently studied to prove their prognostic value. They include DNA ploidy, microvessel density, EGFR, Bcl2, pS2 and Cathepsin D.[15]

The prognosis of breast carcinoma is related to number of clinical and pathological parameters. These are as follows:

1. *Age of patient*: Age less than 50 years is often associated with a better prognosis than those in older women. Survival rates usually tend to decline as age increases.[7]

2. *Size of tumour*: There is a good correlation between the diameter of the primary tumour and the incidence of nodal metastasis and the survival rate. As the size of primary tumour increases so does the risk of axillary lymph node metastases.[16] Tumour size has been incorporated into the TNM staging, the very stage of the disease depends on the size of the tumour. Tumours less than 2cms fall in the category of T1, further subdivisions result in T1a(0.1-≤0.5cm), T1b (>0.5-≤1cm), T1c (>1-≤2cm). The T2 category are tumours larger than 2 cms but no more than 5 cms in size. T3 category is for tumours larger than 5cms. T4 doesn't take size into consideration, it looks at spread beyond breast tissues irrespective of size.

3. *Site of Tumor*: The site of breast carcinoma is demarcated in conjunction to the quadrants of the breast. 17% in the central region, 50% in the upper outer quadrant, 15% in the upper inner quadrant, 10% in the lower outer quadrant, 5% in the lower inner quadrant, and 3% are diffuse (massive or multifocal). The marked difference in the carcinoma frequency depending on the quadrant, is usually associated to the amount of breast parenchyma in each quadrant.[7]Medial site tumors have a worse prognosis as compared to tumors located at outer sites.[18]

4. *Histological type*: There are four categories into which we can divide the prognostic outcome of different histological types; these are: excellent, good, poor and very poor prognosis. Invasive cribriform, tubulo-lobular, tubular and mucinous, have an above 80% survival at 10 years. Mixed ductal with special type, tubular mixed, atypical medullary and alveolar lobular carcinoma have good prognosis with a 60–80% 10-year survival. Invasive papillary, classic lobular and medullary cancers have worse prognosis. 10-year survival is below 50% with ductal, solid lobular, mixed ductal and lobular carcinoma.

   Infiltrating Micropapillary carcinoma is associated with an overall worse prognosis, as it is associated with relatively quick spread to lymph nodes.[17]

   Majority of cases constitute infiltrating ductal carcinoma making about 70% of all diagnoses. 10 years' survival drops to 30% in cases of Inflammatory carcinoma, making it one of the most aggressive as well as highly malignant breast carcinoma. [17]

5. *Histological grade*: is an important determinant of prognosis that allows risk stratification within a given tumour stage. Morphological assessment of the degrees of differentiation provide useful information in breast cancer. It has been recommended that all invasive breast carcinomas should be graded and the grading system used must be specified in the report. The most commonly used grading system is The

Nottingham Modification of Bloom Richardson grading (MBR grading), combines nuclear grade, tubule formation and mitotic rate.[17]

6. *Estrogen and Progesterone Receptor*: Immunohistochemistry has been used to detect the status of receptors in the nucleus. Women with hormone receptor-positive carcinomas have a better prognosis than women with hormone receptor-negative carcinomas. These methods have been most useful in predicting response to therapy.

7. *HER-2/neu*: is a transmembrane glycoprotein involved in cell growth control.[19] The significance of HER-2/neu oncogene as an adverse prognostic factor has been noted in many other cancers, such as ovary, lung, stomach, and pancreas. Amplification of the erb-b2 gene or over-expression of the erb-b2 protein has been detected in 10 to 30 per cent of breast cancers. [20]

8. *Types of Margin*: Tumours associated with pushing margins have a better prognosis than those with infiltrating margins [7]

9. *Tumour necrosis*: tumour necrosis is often associated with increased number of lymph node metastases and overall decreased survival rates, particularly it is extensive. This feature is usually associated with tumours of high histological grade.[7]

10. *BRCA 1 statu*s: A worse overall status has been noted in breast carcinomas with BRCA1 mutation carriers in the absence of adjuvant therapy. [7]

11. *Oral contraceptives& Pregnancy*: Breast carcinoma which occurs during pregnancy or lactation is usually an aggressive tumour with low expression of hormone receptors and equally high expression of HER2/neu and is associated with poor prognosis. However, no definitive proof has been found that previous use of oral contraceptive agents has an impact on overall prognosis and survival of breast carcinoma [7]

12. *Early diagnosis*: The relative survival rates for asymptomatic breast carcinomas are higher than those for clinically detectable carcinomas [7]

13. *Presence or absence of invasiveness*: Most women with adequately treated DCIS are often cured compared to about half of the invasive carcinomas which can metastasize locally or distantly at the time of diagnosis.[7]

14. *Stromal Reaction*: High degree of nodal metastases is seen in tumours with a presence of inflammatory reaction.[16]

15. *Skin invasion*: A decreased survival rate is seen in breast carcinomas with invasion to the underlying skin. [7]

16. *Nipple invasion*: Invasion of the nipple by carcinoma is associated with a higher incidence of axillary metastases. [7]

17. *Lymphatic tumour emboli*: There is an increased risk of tumour recurrence if there is presence of tumour emboli in the lymphatic vessels.[17]

18. *Microvessel density***:** Invasive breast carcinomas having a prominent vascular component in the surrounding stroma and behave in a more aggressive fashion than the others. Efforts have been placed to quantitate the 'density' of these vessels and to correlate this feature to other parameters, especially prognosis

19. *Blood vessel emboli*: There is high correlation between the presence of tumour emboli in the blood vessels, histological grade, tumour size, tumour type, status of lymph node, distant metastasis and poor prognosis.[7]

20. *Axillary lymph node metastases*: presence or absence of axillary lymph node involvement is a significant prognostic indicator for patients diagnosed with early stage breast cancer. The greater the number of lymphnodes involved, the poorer the prognosis. Sentinel node biopsy are usually the first lymphnodes that the tumour drains into, and this is usually dissected to assess the spread of the tumour and hence is an important determining factor in the prognosis as well as staging of the disease. [16]

21. *Metastases to Internal Mammary lymph node*: There is decreased survival rate for patients with involvement of internal mammary lymph nodes compared to those without the involvement of these nodes. [7]

22. *Distant metastases*: a cure is unlikely once distant metastases are present and is associated with poor prognosis.[7]

REVIEW OF IMMUNOHISTOCHEMISTRY

With the grand introduction in the early 1940s of immunostaining technology, it is fascinating to see how far Immunohistochemistry has developed over the years, and the applicability of immunohisto/cytochemical methods will undeniably continue to increase in many directions. [21]

Immunohistochemistry (IHC) is a method for locating specific antigens in tissues based on antigen antibody recognition. [22]

It exploits the specificity of the binding antibody with the antigen at a light microscopic level.[22]

IHC has a history, spanning more than half a century from 1940, where Coons developed an immunofluorescence technique to detect respective antigens in frozen tissue sections. However, only since the early 1990's has the method found general application in surgical pathology. A series of technical development led eventually to the wide range of IHC applications in use today. The enzymatic label (Horseradish peroxidase), developed by Avaremeas and by Nakane and colleagues, allowed visualization of the labelled antibody by light microscopy in the presence of a suitable colourogenic substrate system.[23]

Taylor(1974) successfully demonstrated antigens in formalin fixed paraffin embedded (FFPE) tissues. Critical issue in the development of immunoperoxidase technique was related to the need to achieve greater sensitivity. Greater sensitivity would facilitate staining of FFPE tissues from a

simple one step direct conjugate method to multiple step detection techniques such as peroxidase antiperoxidase(PAP), avidin-biotin conjugate (ABC), and biotin streptavidin(B-SA) methods and would eventually lead to amplification methods (such as tyramide) and highly sensitive polymer-based labelling system. [23]

Direct & Indirect detection systems:

Direct detection system incorporates the primary antibody to be conjugated directly to the label. The main advantage is that it requires one application of reagent and followed by appropriate chromogen substrate solution.

Indirect detection system involves a two-step method; firstly, secondary antibody reacts with the antigen bound primary antibody. Sensitivity was enhanced with the introduction of peroxidase enzyme complex. Further development and research went into the progression that resulted in Avidin- Biotin Complex method.[27]

As IHC has progressively improved, the use of more than one IHC stain is general practice in routine surgical pathology, especially in classification of tumours as well as diagnosis. IHC is also being used for demonstration and identification of predictive and prognostic markers. Huang introduced enzyme digestion as a pre-treatment for IHC staining to reveal antigens that have been changed due to fixation by formalin. Fraenkal- Conrat and co-workers were the pioneers of Antigen retrieval which was based on

their numerous biochemical tests, this technique were further improved and revised by Shi and co-workers in 1991.[24]

In stark contrast to enzyme digestion, the antigen retrieval technique is a relatively easier method that involves heating routinely processed paraffin sections at high temperature before IHC staining. The intensity of IHC staining was accentuated after pre-treatment by Antigen retrieval.

Antigen Retrival:

Antibody binding and detection by IHC requires treatment to undo formalin-induced changes. The last 2 decades has seen many new technological advancements for antigen, retrieval, these advancements have made it more acceptable to use immunohistochemistry in modern practice of pathology. [26]

World wide application of AR-IHC in pathology has validated the feasibility of AR-IHC and expanded its use in molecular morphology.[25]

Immunohistochemistry and Breast Carcinoma:

IHC has been of immense value in the diagnosis & prognosis of breast carcinoma.

The judicious use of immune histochemistry by applying a panel of immune markers and using accredited and universally accepted technical as well as interpretational standards can support morphologic assessment and

help in the accurate categorization of ambiguous breast lesions as well as detection of metastasis.[28]

Progesterone receptors & Estrogen Receptors are very powerful and extremely useful predictors. Hormonal treatment response in breast cancer is linked to the presence of ER and PR, receptor expression profile is clearly an important indicator which allows clinicians to better predict response to hormonal treatment of breast cancer.[29]

In 2014, Liu said that Immunohistochemistry has an important role in routine diagnostic breast pathology practice, especially in the evaluation of invasion, papillary lesions, and spindle cell lesions to exclude metaplastic carcinoma. Among the breast specific markers, she proposed that GATA3 is superior to others and should be used in a panel when working on metastases.[28]

BIOLOGICAL MARKERS IN NORMAL BREAST

Estrogen Receptor

ER was first identified in the 1960s when the development of radio labelled hormones made it possible to demonstrate the binding of estrogen to its receptor. ER is a nuclear transcription factor and normally involved in pathways controlling cell proliferation.[30] About 80% of all breast carcinomas have ER+ tumor cells[30] Estrogen stimulates growth of ER+ normal- and tumor cells. ER status, the protein expression, is a strong predictive marker for the

response to endocrine therapies i.e. tamoxifen and aromatase inhibitors (AIs).[31] Treatment with tamoxifen will reduce the effect of estrogen by blocking the ER. Tamoxifen, the anti-estrogen, has been used for treatment of breast cancer for about 40 years.

Aromatase is an enzyme that naturally converts the androgens: testosterone and androstenedione to estrone and estradiol in the peripheral tissue.[31] In postmenopausal women estrogens are mostly synthesized this way in contrast to the premenopausal women where most of estrogen is produced by the ovaries.[32] AI will thereby reduce the level of estrogen by inhibition of the aromatase enzyme. In addition, the AIs have been used since early 2000s. However, only about 50% of breast cancer patients with an ER+ expressing cancer will respond to endocrine therapy.[32]

Progesterone Receptor (PR)

PR is a nuclear receptor. PR expression is induced by ER activation.[33,63,64] The activity of progesterone in breast tissue is not clarified, however it is assumed that it induces lobular development.[34] Diverging results about proliferative activity of progesterone has been reported.[35] However, it has been clearly demonstrated that hormone replacement therapy (HRT) are associated with an increased risk of breast cancer. [36]

Additionally, PR negativity is shown to be a negative prognostic factor for breast cancer survival. [37, 38]

HER-2/neu

HER2, also known as HER2/neu or ErbB-2 is a protein coded by the gene ERBB2 which is present on the long arm of chromosome 17(17q21-q22).[39] HER2 belongs to the Epidermal Growth Factor (EGF) Receptor Tyrosine Kinases (RTK) family. Patients with cancers having protein over expression and/or gene amplification of HER2 have shown to have worse survival. [40,41,42]

HER2 content is analyzed either by; HER2 protein quantity, using IHC, a semi quantitative method (0, 1+, 2+ or 3+) or with measurements of HER2 gene copies, using an in situ hybridization method (ISH). In the latter case, a single-probe (detection of HER2 gene expression) or a dual-probe (detection of HER2 gene expression and chromosome 17) are used. [43] According to the National guidelines, (HER2+) is considered as over expression of the protein HER2 (3+) or amplification of the gene (HER2 copy number ≥5 or HER2/CEP17 ratio ≥ 2.0). [43]

HER2/CEP17 ratio of >2 is often indicative of gene amplification.

Approximately 10-30% of all primary breast cancers is HER2 positive,[40,44,45] particularly, the early studies reported HER2 over expression in the range of approximately 30% or more, likely for highly selected cohorts. Additionally, HER2 positivity in breast cancer is used for selection of patients sensitive for anti-HER2 directed therapies, e.g. trastuzumab, lapatinib, pertuzumab and trastuzumabemtansine .[46,47,48,49,50,51]

Other biomarkers:

Numerous biomarker expressions have been extensively studied in benign breast tissue.[52]

Anti-apoptotic protein bcl-2 is frequently expressed by normal breast epithelial cells.[53,54,55]

The S-100 protein expression is strong in normal myoepithelial cells and expressed variably in mammary epithelial cells.[56,57]

Epithelial cells also show variable expression for lactalbumin, casein, GCDFP-15, and c-kit (CD117).[58,59,60,61,62]

Epithelial cells usually express CK 7, 8, 18, and 19, whereas CK 5, 6, 14, and 17 are expressed in myoepithelial cells.

SCORING SYSTEM USED IN IMMUNOHISTOCHEMITRY

Estrogen and Progesterone receptors are expressed as nuclear positivity. They are scored by the summation values obtained from proportion of tumour cells showing positivity and intensity of the reaction. [65]

Following are a few scoring systems used for evaluation of ER and PR:

H-Score system

The H score system was one of the first scoring systems used initially for assessment of IHC.[66]

A score of 0-3 was given based on the following expression[65]:

0- No reaction

1-Weak reaction

2 - Moderate reaction

3- Strong reaction

Score calculation = % weakly positive cells x 1 + % moderately positive cells x 2+ % strongly positive cells x 3. [65]

Total score comes about 0-300 positive score: 51-300, A Negative score is 50 or less. The main disadvantage of this score is that it is time consuming, laborious and prone to discordance between observers and hence impractical for most pathologists.[66,67]

Quick Score system:

The quick score categories is based on intensity and the proportion of staining. [68]

The proportion of malignant cells staining positively throughout the section was termed category A and is assigned scores from 1 to 6 (1 =0-4%; 2= 5-19%; 3 = 20-39%; 4 = 40-59%; 5 = 60-79%; 6 = 80-100%). The whole section is then scanned at low power in order to gauge the general level of intensity throughout.[69] The average intensity, corresponding to the presence of negative, weak, intermediate, and strong staining, was given a score from 0 to 3 respectively.[69]

Allred Scoring system

Allred score is a semi quantitative system that utilizes the proportion of cells which appear positive (scale of 0-5) and intensity of staining (scale of 0-

3). The proportion and intensity are added to give total scores of 0 or 2 to 8. A score of 0 -2 is negative while 3 - 8 is positive. (See Appendix IV)[70,71,72]

Scoring of HER2/neu

The HER2 scoring method is a semi-quantitative system that is primarily based on how much of intensity of stain is seen in the tissues as well as product and percentage of membrane positive cells, giving a score range of 0–3+. [73] A score of 3+ are regarded as positive, and scores of 0/1+ as negative. Score of 2+ is taken as equivocal and further testing by FISH is needed. (Appendix V)[74,75,]

Study of Prognostic Index

This prognostic index was a means o develop a scoring system that was easy to use as well as having an altogether satisfactory evaluation of categorizing patients into a group that could stratify the outcome relating to these patient. This is how the Nottingham Index was formed. Its categorization in different prognostic groups based on Tumour size, Grade and Lymph nodes involved, gave a sense of simplicity to the overall analysis of the prognosis of the patient. (See Appendix III).

Materials & Methods

MATERIALS AND METHODS

Study Design: Cross Sectional Study

Study Setting: Department of Pathology, Sree Mookambika Institute of Medical Sciences.

Appropriate total duration of study: One year

Number of groups to be studied: 1

Detailed description of group: Patients diagnosed with malignant breast cancer who have undergone mastectomy, excision biopsies and tru cut biopsies (if sample is adequate)

Sample size of each group: One group with 56 samples

Total sample size of study: 56

Scientific basis of sample size used in study:

Sample size was calculated using the formula

$N = 4pq/d^2$

P = Prevalence

q = 100 – p

d = Allowable error (5-20%p)

Prevalence of ER & PR Positivity: 64% [12]

4 x 64 x 36 = 9216

20% of 64 = 12.8

$9216/ (12.8)^2$= 56 Samples

Sampling technique: Convenient Sampling.

Inclusion criteria:

Biopsy and mastectomy specimens from female and male patients of all ages with breast carcinoma will be studied.

Exclusion criteria:

Cases where limited surgery has been done, as in such cases all the parameters will not be available for assessment.

Cases where there is extensive tumour necrosis without sufficient viable tumour cells for accurate evaluation of the immunohistochemical results.

Cases where specimen sample seems inadequate for analysis.

Whether placebo used in the study: No

Drugs used if any: No

If Drugs/Medical devices used: No

If research proposal is a clinical trial: No

Parameters to be studied:

- Age
- Site of Lesion

- Type of Margins
- Nipple invasion
- Skin involvement
- Tumour size
- Lymph nodes affected (Site and Number)
- Lymphatic tumour emboli
- Blood vessel emboli
- ER status
- PR status
- Her/2 neu status
- Histological grading
- Resection margin
- Presence/Absence of invasiveness

Methods/Techniques/Instruments/Reagents/Kit(s) used to measure quantitative parameters along with their manufacturing source:

Tissue sampling

Light Microscope (LABOMED VISION 2000)

Immunohistochemistry done with NovoLink™ Polymer Detection System

Procedure in detail:

This study proposal was submitted before the Research Committee and Institutional Human Ethical Committee (IHEC) of Sree Mookambika Institute

of Medical Sciences (SMIMS), Kulasekharam, Kanyakumari District, Tamilnadu. The research proposal was approved by the Institutional Human Ethical Committee (IHEC) of SMIMS and the permission for doing the study in the institution was obtained. Reference Number: SMIMS/IHEC/2015/A/22.

The Certificate of approval for the same has been enclosed in annexure.

After getting approval from IHEC, a written informed consent was obtained from all volunteers. The detailed clinical history and results of relevant investigations done was collected from the patients' case files. For prospective cases, the details of the surgery was informed to the department and all necessary arrangements was taken to ensure that the specimens reach the department as soon as the surgery is complete. The mastectomy and lymph node dissection specimen was received in the Pathology department in 10% formalin. In every case the standard protocol for surgical grossing of radical mastectomy specimens was followed. The specimen was kept overnight with incisions made according to the protocol for faster fixation of the specimens. After a detailed specimen description, multiple sections were taken from the tumour, surgical margins, nipple and areola, non-neoplastic breast, and all the lymph nodes. The resection margin was painted with India Ink, to examine completeness of resection as well as if there is involvement of the resection margin. The tissue bits were processed in Leica automatic tissue processor and paraffin blocks were prepared Tissue sections of 4- 6µm

thickness were cut and stained by haematoxylin and eosin (H & E) for histopathological study. The sections was studied extensively and the results noted in the proforma, after which , 3-5µm sections were cut from a paraffin block of tumour tissue and taken on 3 glass slides coated with adhesive (Poly-L-Lysine) for immunohistochemistry (IHC) to detect ER,PR,HER2/neu. For retrospective cases, the histopathology reports, slides and paraffin blocks will be retrieved from the archives. Sections will be cut from the paraffin blocks in a similar manner. The technique for IHC will include antigen retrieval in Sodium Citrate buffer in a pressure cooker, blocking endogenous peroxidase with 3% hydrogen peroxide, incubating with primary mouse monoclonal antibody(NovoLink), linking with rabbit anti mouse secondary antibody(NovoLink), enzyme labelling with streptavidin- horseradish peroxidise (NovoLink), developing chromogen with deaminobenzidine(DAB) and counterstaining with haematoxylin.[13] Positive and negative controls will be run with each batch of slides. The H &E stained slides will be studied for the tumour histology, type, Modified Bloom-Richardson (MBR grade) (See Appendix I), lymph node metastasis etc. The immunostained slides will be examined for nuclear staining in case of ER, PR and membrane staining in case of HER2/neu.

Molecular subtyping will be done on all the cases and will be grouped into Luminal, HER2 enriched and basal type depending on expression of

markers studied. The relationship between various parameters and the expression of ER, PR, HER2/neu will be studied. (Appendix II)

The Nottingham prognostic index (NPI) is a widely used clinic-pathological staging system for breast cancer prognostication. It is based on tumor size in breast, node involvement and histopathological grading. [102]

The Nottingham Prognostic Index will be utilized to categorize all patients into the respective prognostic groups. (See Appendix III)

Statistical methods of analysis

1) Significance level decided before starting of study: 5%
2) Statistical test used in data analysis: Logistic Regression Analysis & Chi- Square test
3) Software to be used for statistical analysis:
    - Data will be entered in Microsoft Excel
    - Analysis will be done by SPSS Software Trial Version 20.0.

Observations & Results

RESULTS

The study was conducted from 2015 to 2016 and included 56 cases of breast carcinoma that was received at the Department of Pathology. This study primarily focused on patients that came to Sree Mookambika Institute of Medical Sciences and where diagnosed with breast cancer. These patients opted for either excision biopsy, tru cut biopsy and even modified radical mastectomy. All these patients who willing agreed and signed the consent form were included in this study.

Retrospective cases where followed up and consent was taken from the patient.

AGE DISTRIBUTION OF PATIENTS

The age of the patients ranged from the third decade to well above the 6th decade of life. The 34-42 age group included 5 patients (8.9%), 13 patients were in the 43-50 years age group (23.2%), 19 patients in the 51-60 age group (33.9%) and 19 patients in the above 60 years age category (33.9%). The youngest patient was 35 years old and the oldest patient was 78 years old.

Table 1: Distribution of breast carcinoma according to age

Age	Frequency	Percent
34-42	5	8.9
43-50	13	23.2
51-60	19	33.9
>60	19	33.9
Total	**56**	**100.0**

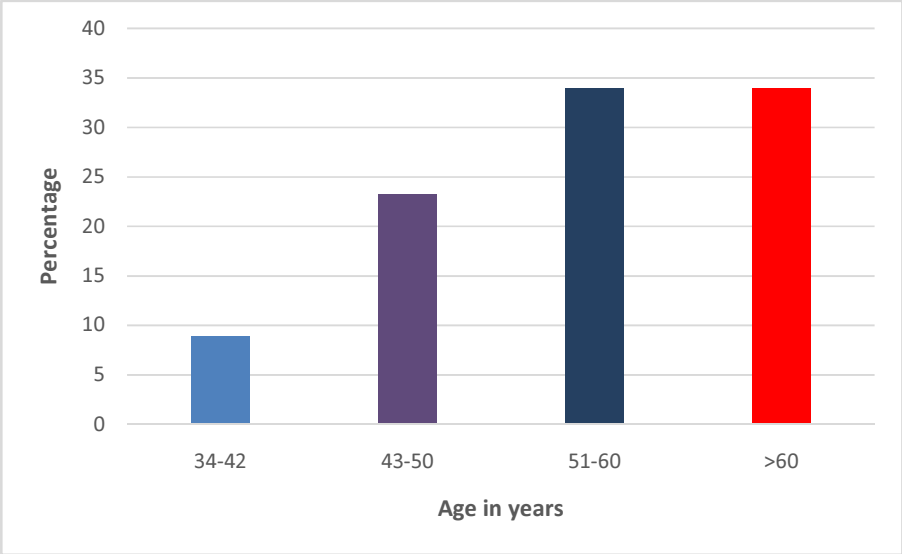

Fig. 3: Age wise distribution of carcinoma of the breast

SEX DISTRIBUTION OF PATIENTS

Out of the 56 patients diagnosed with breast carcinoma, 54 were female (96.4%) and 2 were male (3.6%).

Table 2: Distribution of Breast carcinoma according to sex

Sex	Frequency	Percent
Female	54	96.4
Male	2	3.6
Total	**56**	**100.0**

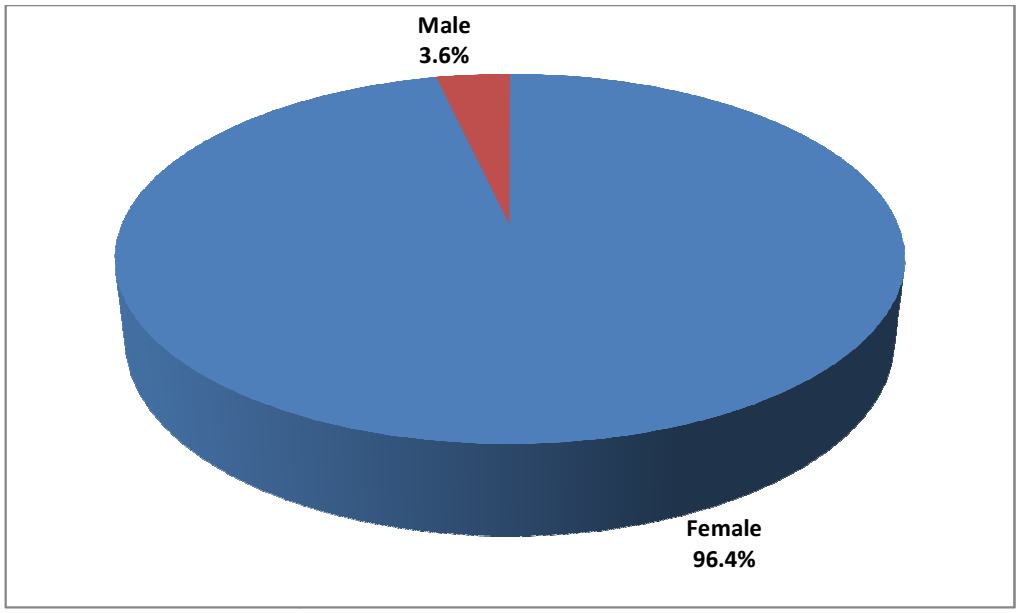

Figure 4: Sex distribution of carcinoma of the breast

TYPE OF SPECIMEN

Out of the 56 cases received, modified radical mastectomy accounted for 37 cases (66.1%), followed by 17 cases of Excision Biopsy (30.4%) and 2 cases of Tru-cut biopsy (3.6%). The tru cut biopsy 71was accepted because the patients were undergoing neoadjuvant therapy for palliative mastectomy.

Table 3: Distribution according to type of specimen received.

Type of Specimen	Frequency	Percent
Excision Biopsy	17	30.4
Modified Radical Mastectomy	37	66.1
Tru-Cut biopsy	2	3.6
Total	**56**	**100.0**

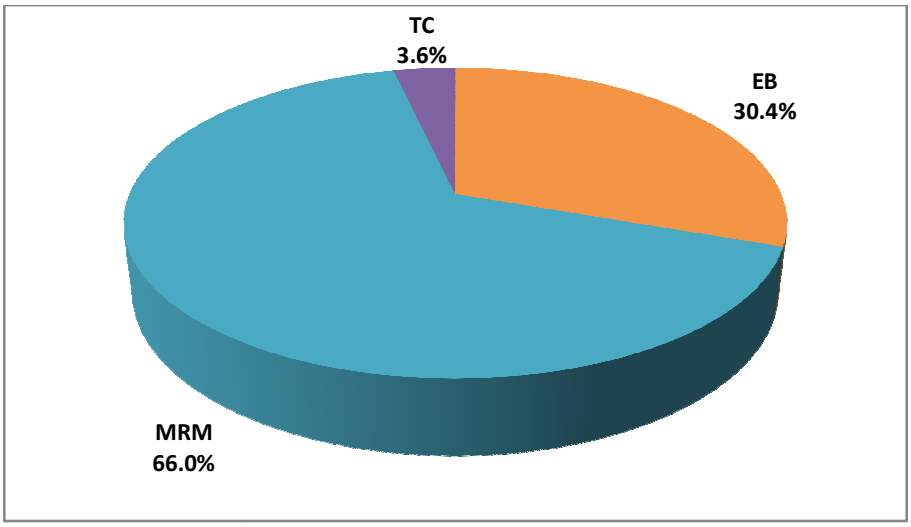

Figure 5: Distribution based on type of specimen received

LOCATION OF TUMOUR

Based on the data collected, in 36 cases, the tumour was located in the Upper Outer Quadrant (64.3%), 9 cases were in the Upper Inner Quadrant (16.1%), In 5 cases, the tumour location was found to be below the nipple (8.9%), the Lower Inner Quadrant and Lower Outer Quadrant accounted for 4 cases (7.1%) and 2 cases (3.6%) respectively.

Table 4: Distribution according to site of tumour

Site	Frequency	Percent
Below Nipple(BN)	5	8.9
Lower Inner Quadrant (LIQ)	4	7.1
Lower Outer Quadrant (LOQ)	2	3.6
Upper Inner Quadrant (UIQ)	9	16.1
Upper Outer Quadrant (UOQ)	36	64.3
Total	**56**	**100.0**

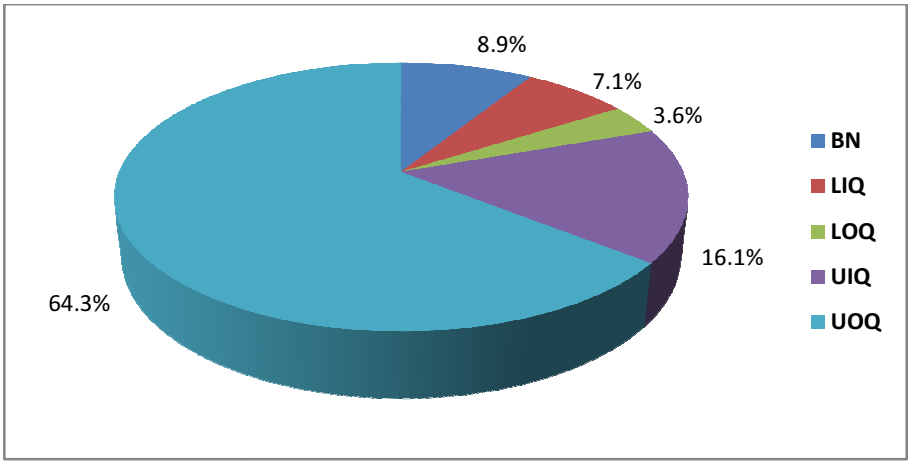

Figure 6: Distribution based on Site of tumour

TUMOUR SIZE

Tumour size was analysed in this study, 9 cases had a tumour size of <2cms (17%), 37 cases fell in the tumour size category of 2-5cms (69.8%), and 7 cases were in the >5cms tumour size category (13.2%).

Table 5: Distribution according to tumour size

Size of Tumour (cms)	Frequency	Percent
<2	9	17
2-5	37	69.8
>5	7	13.2
Total	**56**	**100.0**

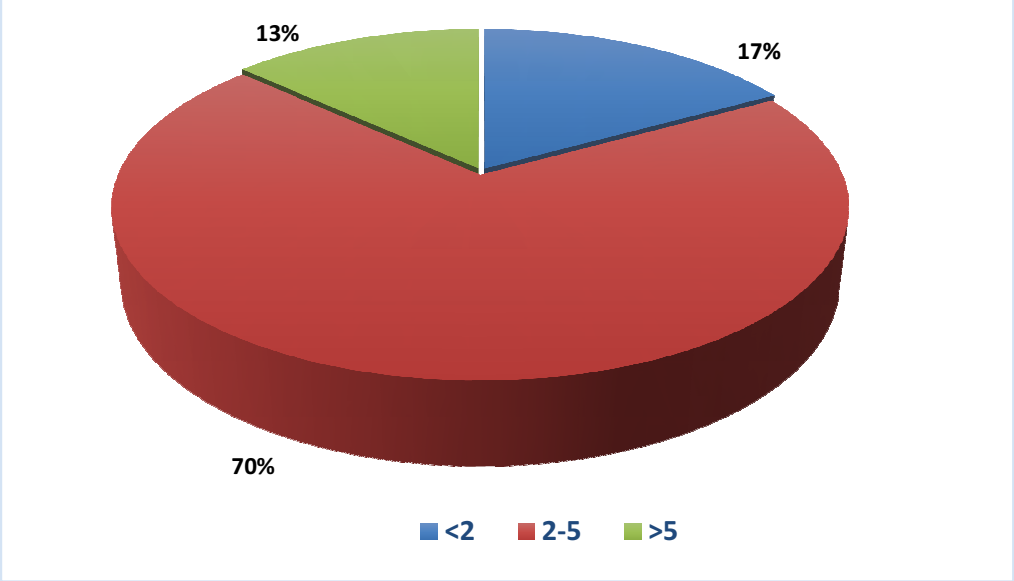

Figure 7: Distribution based on Size of tumour (cms)

MARGINS OF TUMOUR

Margins of the tumour were assessed based on being either infiltrating or pushing. In 6 cases (10.7%) the margins couldn't be analysed, due to not proper representation of tumour margins in excision biopsies 38 cases (67.9%) showed an infiltrating margin, whereas 12 cases (21.4%) had pushing margins.

TABLE 6: Distribution according to Margin of tumour

Margins	Frequency	Percent
Cannot Be Assessed (CBA)	6	10.7
Infiltrating	38	67.9
Pushing	12	21.4
Total	**56**	**100.0**

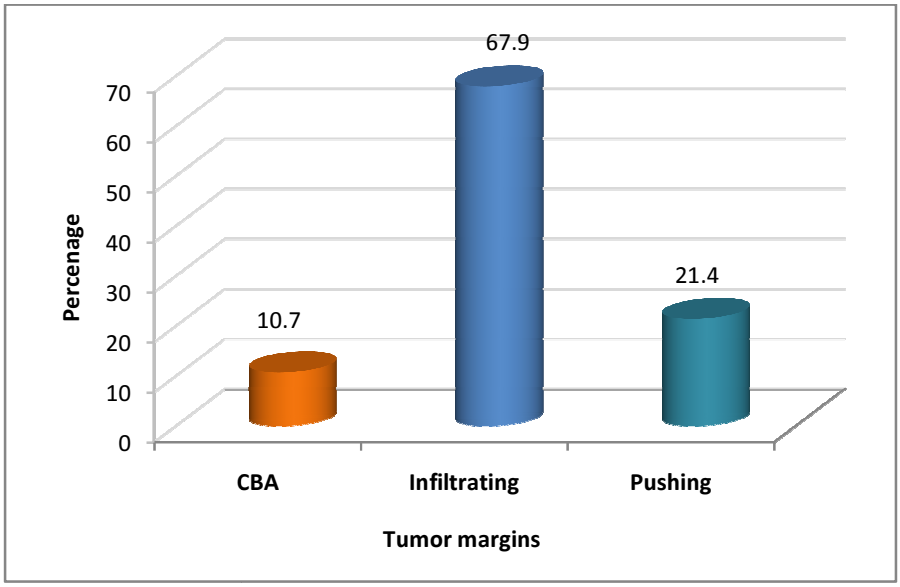

Figure 8: Distribution based on margin of tumour

NIPPLE INVASION

Invasion of the nipple was noted in 10 cases (17.9%), No invasion to the nipple was seen in 37 cases (66.1%) and in 9 cases (16.1%) invasion couldn't be assessed (CBA).

TABLE 7: Distribution of tumour infiltration into nipple

Nipple	Frequency	Percent
Cannot be Assessed (CBA)	9	16.1
No Invasion	37	66.1
Invasion Present	10	17.9
Total	**56**	**100.0**

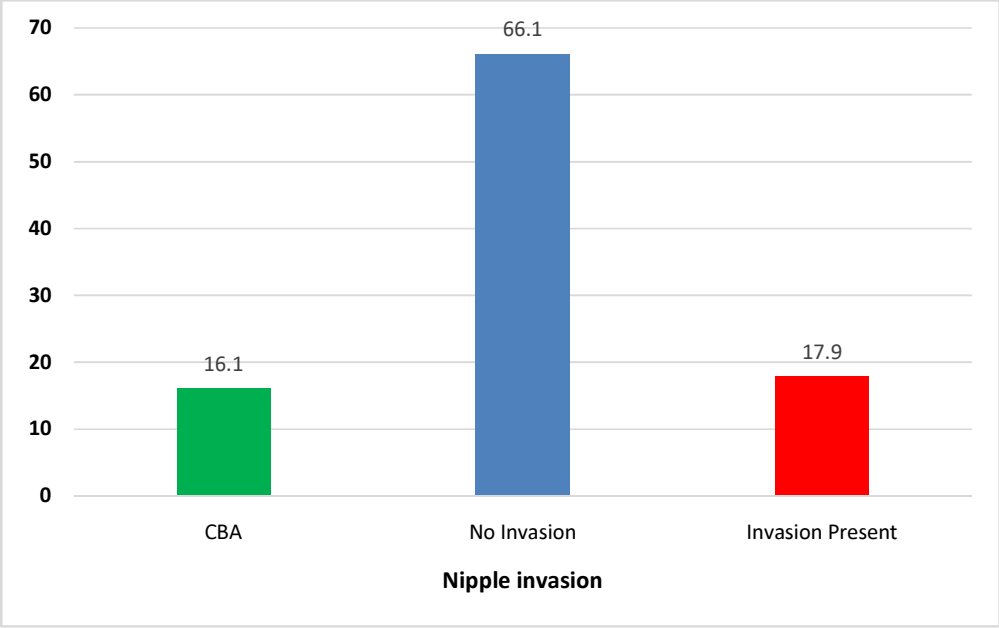

Figure 9: Distribution based on Nipple Invasion

SKIN INVASION

Skin invasion was seen in 9 cases (16.1%) and no invasion was noted in 39 cases (69.6%) and in 8 cases invasion could not be assessed (14.3%)

TABLE 8: Distribution of Skin invasion

Skin	Frequency	Percent
Cannot be assessed (CBA)	8	14.3
No Invasion	39	69.6
Invasion Present	9	16.1
Total	**56**	**100.0**

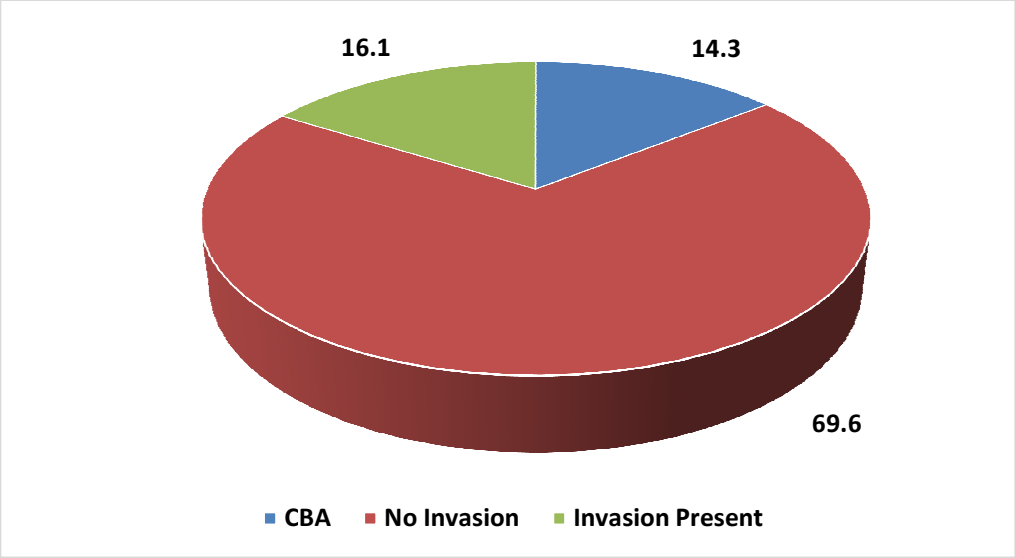

Figure 10: Distribution based on Skin Invasion

LYMPHOVASCULAR EMBOLISM

Lymphovascular embolism was seen in 37 cases (66.1%) out of 56 cases.

TABLE 9: Distribution of Lymphovascular Embolism

Lymphovascular Embolism	Frequency	Percent
Absent	19	33.9
Present	37	66.1
Total	**56**	**100.0**

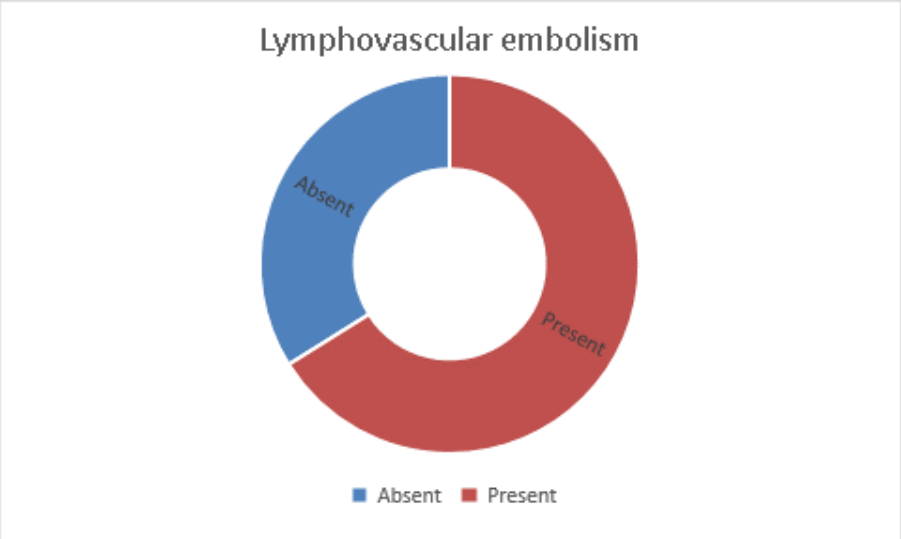

Figure 11: Distribution based on Lymphovascular embolism

PERINEURAL INVASION

Perineural Invasion was observed only in 10 cases (17.9%).

Table 10: Distribution of Perineural invasion (PNI)

Perineural Invasion	Frequency	Percent
Absent	46	82.1
Present	10	17.9
Total	**56**	**100.0**

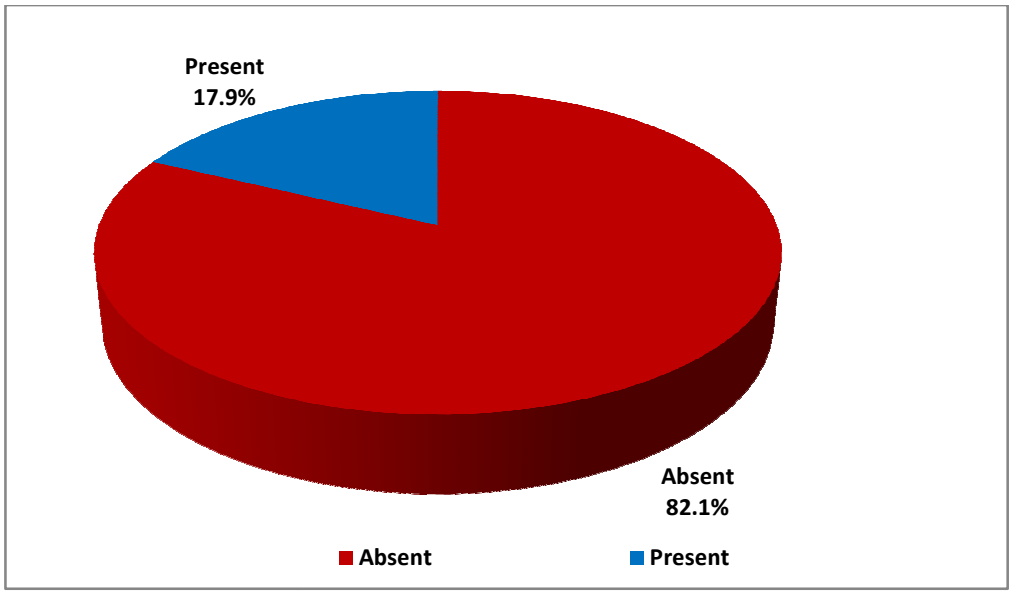

Figure 12: Distribution based on Perineural Invasion

PERINODAL EXTENSION

Perinodal extension was found to be present in 16 cases (28.6%).

TABLE 11: Distribution of Perinodal Extension (PNE)

PNE	Frequency	Percent
Absent	40	71.4
Present	16	28.6
Total	**56**	**100.0**

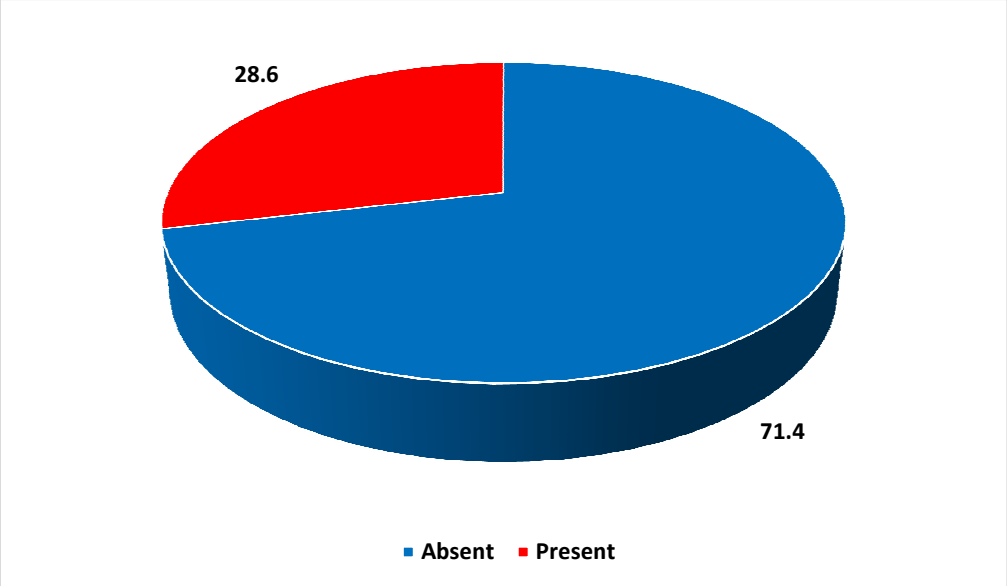

Figure 13: Distribution based on Perinodal Extension

LYMPH NODE METASTASES

The total number of Lymph nodes retrieved from all cases and how many showed metastases was analyzed. The results have been tabulated into the table below.

TABLE 12: Distribution of Lymph node status

Lymph node status	Frequency	Percent
Nx	10	18
N0	21	38
N1	9	16
N2	8	14
N3	8	14
Total	**56**	**100**

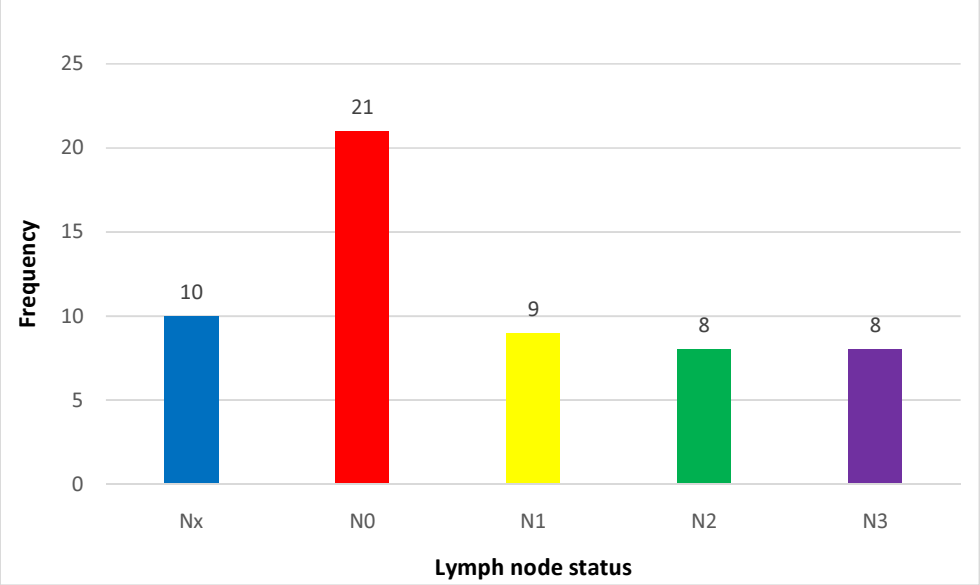

Figure 14: Histogram showing the distribution of Lymph node status

MODIFIED BLOOM RICHARDSON GRADING

Of the 56 cases studied, 2 cases were categorized as GRADE I (3.6%), a further 25 cases (44.6%) fulfilled the criteria of GRADE II and 29 cases (51.8%) was assigned to the category of GRADE III according to the Bloom Richardson grading system.

TABLE 13: Table showing distribution of Modified Bloom Richardson Grades

Modified Bloom Richardson Grade (MBR)	Frequency	Percent
I	2	3.6
II	25	44.6
III	29	51.8
Total	**56**	**100.0**

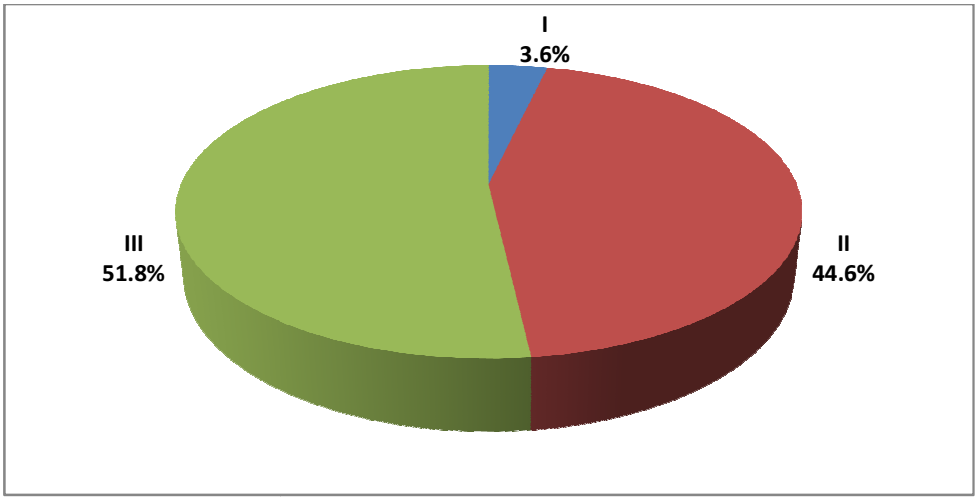

Figure 15: Distribution based on MBR Grade

HISTOPATHOLOGICAL DIAGNOSIS OF THE CASES

Of the 56 cases studied, 39 cases were diagnosed as Infiltrating Ductal Carcinoma: NOS (69.6%). Table 12 summarizes in detail the different types of carcinoma breast encountered in this study.

TABLE 14: Different types of Breast carcinomas diagnosed during study

Diagnosis	Frequency	Percent
Cribiform Carcinoma	2	3.6
IDC: Apocrine type	2	3.6
IDC: Micropapillary	2	3.6
IDC: Mixed ductal lobular Carcinoma	1	1.8
IDC: NOS	40	71.4
IDC: Scirrhous type	1	1.8
IDC:Micropapillary type	1	1.8
Infiltrating Lobular Carcinoma	2	3.6
Intraductal comedo Carcinoma	1	1.8
Medullary Carcinoma.	1	1.8
Mucinous Carcinoma	2	3.6
Papillary Carcinoma.	1	1.8
Total	**56**	**100**

TNM STAGING

All cases were further staged and Table 12 shows the findings:

TABLE 15: TNM staging of the cases

TNM	Frequency	Percent
T1cN0Mx	1	1.8
T1cN2aMx	1	1.8
T1micN0Mx	1	1.8
T1N0Mx	2	3.6
T2cN0Mx	1	1.8
T2N0Mx	14	25.0
T2N1aMx	1	1.8
T2N1cMx	2	3.6
T2N1Mx	3	5.4
T2N2Mx	5	8.9
T2N3aMx	2	3.6
T2NxMx	5	8.9
T3N1aMx	1	1.8
T3NxMx	2	3.6
T4aN1aMx	1	1.8
T4bN0Mx	1	1.8
T4bN3aMx	2	3.6
T4bN3AMx	1	1.8
T4N0Mx	1	1.8
T4N1Mx	1	1.8
T4N2aMx	2	3.6
T4N3aMx	2	3.6
TxN3Mx	1	1.8
TxNxMx	3	5.4
TOTAL	56	100.0

ESTROGEN RECEPTOR

Out of 56 cases, Estrogen Receptor (ER) positivity was noted in 29 cases (51.8%) and Estrogen Receptor (ER) was negative in 27 cases (48.2%).

Table 16: Distribution of Estrogen Receptors in breast cancer

Estrogen Receptor (ER)	Frequency	Percent
-	27	48.2
+	29	51.8
Total	**56**	**100.0**

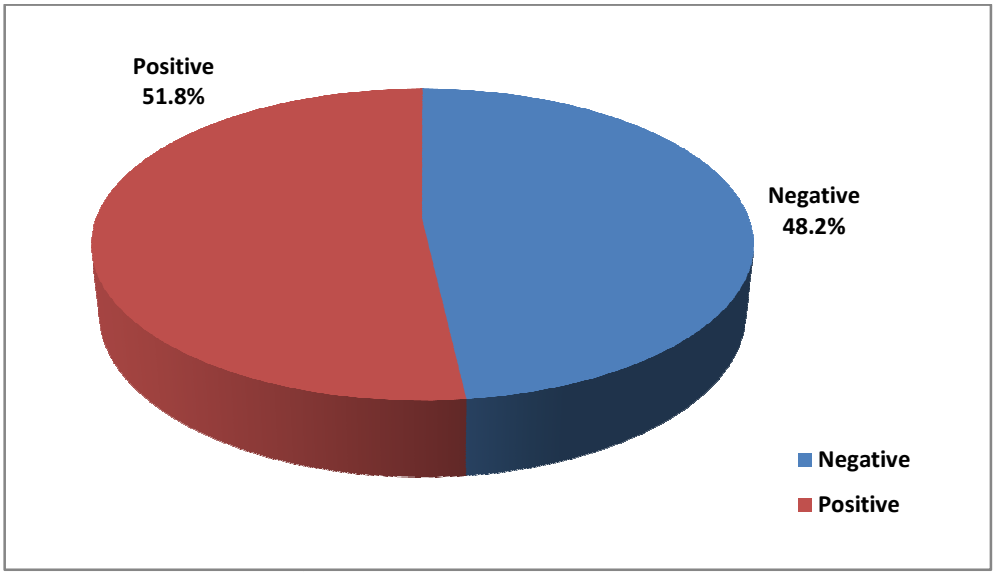

Figure 16: Distribution of ER receptors in breast carcinoma

PROGESTERONE RECEPTOR

Progesterone Receptor was positive in 27 cases (48.2%) and negative in 29 cases (51.8%).

TABLE 17: Distribution of Progesterone Receptor in Breast Carcinoma

Progesterone Receptor	Frequency	Percent
-	29	51.8
+	27	48.2
Total	**56**	**100.0**

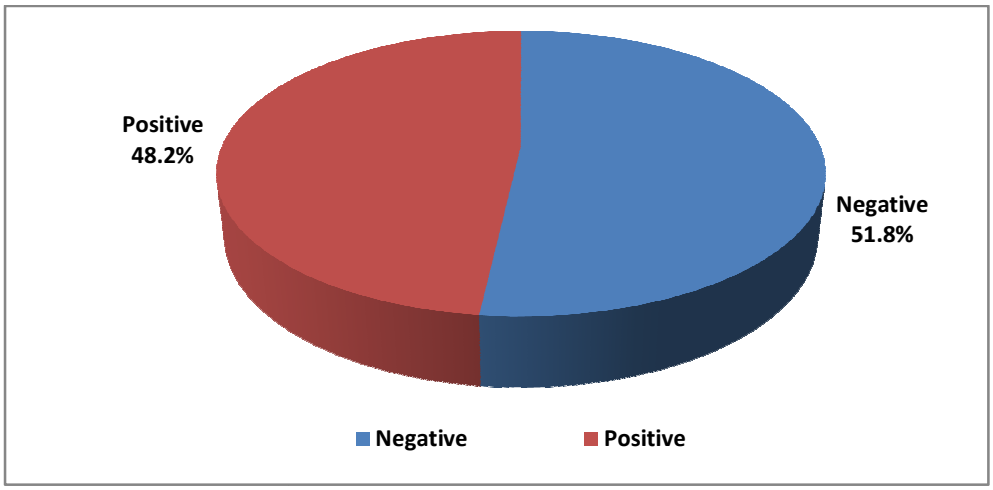

Figure 17: Distribution of ER receptors in breast carcinoma

CORRELATION OF ESTROGEN RECEPTOR WITH PROGESTERONE RECEPTOR

Estrogen Receptors and Progesterone receptors were assessed for all cases, a combination of these scores are presented in Table 14.

TABLE 18: Correlation of ER & PR Receptor Status

Category	Frequency	Percentage
ER+ PR+	22	39.3%
ER- PR+	5	8.9%
ER+ PR-	7	12.5%
ER- PR-	22	39.3%
TOTAL	**56**	**100**

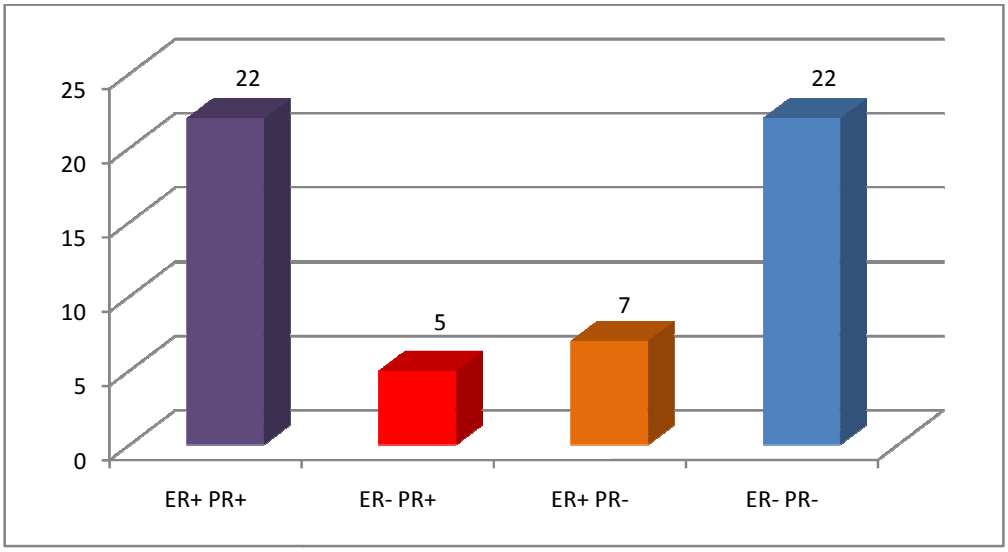

Figure 18: Correlation of ER & PR status

HER2 neu

Table 15 shows the distribution of HER2 neu among the cases we examined, Positivity was seen in 32 cases (57.1%) and Her 2 neu was negative in 19 cases (33.9%). An equivocal score was seen in 5 cases (8.9%).

TABLE 18. Distribution of HER2 neu.

Her2 neu	Frequency	Percent
-	19	33.9
+	32	57.1
EQ	5	8.9
Total	**56**	**100.0**

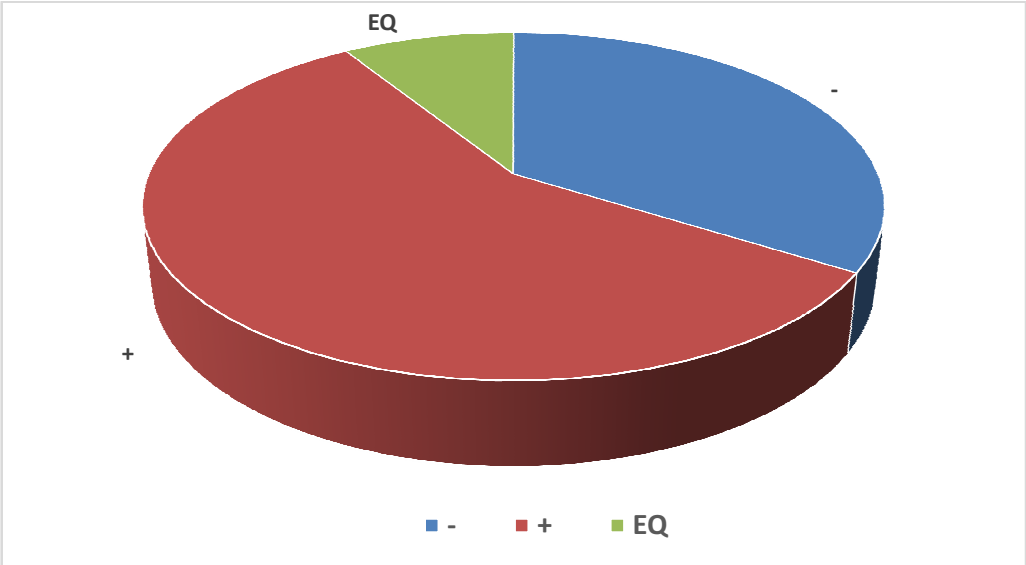

Figure 19: Distribution of HER2 - neu

MOLECULAR SUBTYPES OF BREAST CARCINOMA

Based on the observations on ER, PR and HER2neu, further classification into molecular subtyping was conducted. Table 16 shows the distribution.

TABLE 20: Distribution of Molecular subtypes based on ER, PR & HER2 neu expression.

Molecular Subtypes	Frequency	Percent
BASAL	14	25.0
HER2ENRICHED	37	66.1
LUMINAL	5	8.9
Total	**56**	**100.0**

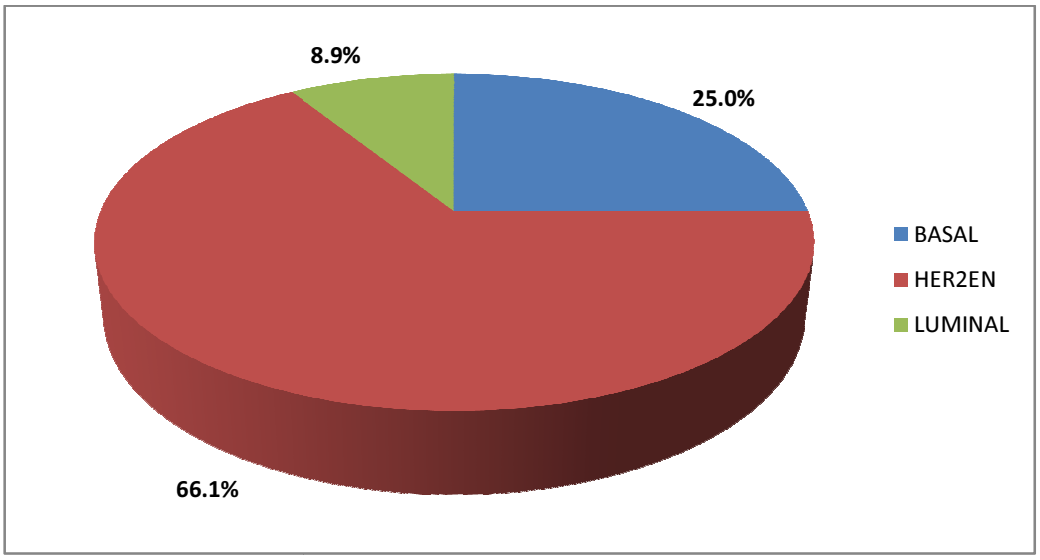

Figure 20: Representation of distribution of Molecular Subtypes

NOTTINGHAM PROGNOSTIC INDEX

Nottingham prognostic Index was used to analyse the 56 cases. 20 cases were grouped into the Moderate prognostic group II (35.7%). Table 18 shows this distribution clearly.

TABLE 20: Nottingham Prognostic Index Distribution

Nottingham Prognostic Index	Frequency	Percent
Cannot Be assessed (CBA)	8	14.3
Good Prognostic Group (GPG)	7	12.5
Moderate Prognostic Group I (MPG I)	5	8.9
Moderate Prognostic Group II (MPG II)	20	35.7
Poor Prognostic Group (PPG)	8	14.3
Very Poor Prognostic Group (VPG)	8	14.3
Total	**56**	**100.0**

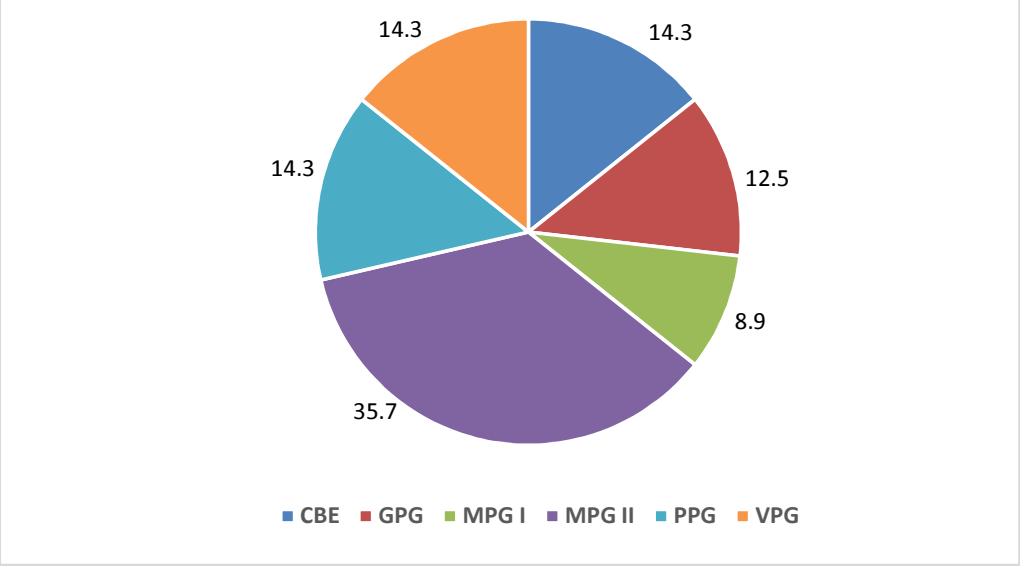

Figure 21: Pictorial representation of the different Prognostic groups.

Statistical analysis was done and found to be significant (p = 0.01) in regard to histological grade of the tumor and the type of margin of the tumor. Increase in grade of the tumour was associated with a more infiltrating pattern of tumour margin.

On further analysis it was shown that significant correlation was found (p= 0.001) with regard to Estrogen Receptor, Progesterone Receptor (p= 0.03) & HER2 neu expression (p= 0.019) and the grade of the tumor.

Molecular subtypes show strong correlation with the grade of tumours (p= 0.04) .Statistical correlation was noted with the Nottingham Prognostic Index and the various grades of the tumour (p <0.001).

All remaining variables including, Tumour size, Lymphovascular invasion, Perineural invasion, skin and nipple invasion showed no significant correlation with grade of the tumour.

Discussion

DISCUSSION

This study was conducted to understand the histological features and the immunohistochemical behaviour of various breast carcinomas.

Breast carcinoma is common in western and developed countries, statistics shows that one in 12 women will develop breast cancer in their lifetime.[84,85]

Breast carcinoma death rates and incidence has been known to increase with age , the age group of 40 years and above has been notorious for the sheer increase in incidence of breast carcinoma reported[85]

Worldwide, an estimated 1.4 million women were diagnosed with breast cancer in 2008 and 458,400 women died from the disease that same year.[86]

Breast cancer subtypes are biologically distinct and may have distinct etiologies.[86, 87] This includes cases that express estrogen and/or progesterone receptors and those that overexpress the tyrosine kinase human epidermal growth factor receptor-2 (HER2) due to amplification of its encoding oncogene ERBB2. [88]

In our study, we noted that 38 patients were above the age of 50 (76%), followed by the 43-50 year age group which had 13 patients (23.2%) and 5 patients in the 34-42 age group (8.9%). We did not encounter any patients below the age of 34 -42 years. The youngest patient in our study group was 35

years. These findings were similar to the study conducted by Sweeny et al[89] which also had more number of patients in the 5th -7th decade of life.

In our study, 54 were female (96.4%) and 2 were male (3.6%). Similarly, a higher proportion of female patients have been seen in studies conducted by Ly & Cook et. al [90, 91]

Our study, shows that Modified radical mastectomy (MRM) was the surgery of choice in 37 cases (66.1%), followed by 17 cases of Excision Biopsy (30.4%) and 2 cases of Tru-cut biopsy (3.6%). Studies conducted by Mudholkar et al [92] had more cases of excision biopsies as compared to Modified radical mastectomies.

Location of tumour in the breast also was found to be more common in the Upper outer quadrant (64.3%) than other sites. This finding also corrabrates perfectly with the Mudholkar et al [92]study, in which they observerd that the upper outer quadrant was the most frequent site for tumours of the breast.

Margins of the tumours were analysed and majority of cases showed an infiltrating pattern (67.9%), this was also consistent with studies done by Moore et al who also demonstrated that most of the cases in their study showed a more prominent infiltrating margin as compare to the pushing margins.

Invasion of the nipple and skin by spread of tumor cells has been noted in 17.9% and 16.1% of cases respectively in our study. Studies done by

Sanders et al [94] showed only fewer patients presented with nipple invasion and dermal changes.

Modified Bloom Richardson scoring was the grading system incorporated in our study. We analyzed all the 56 cases and found that the majority of cases (51.8%) were in Grade III category, wheras only 2 cases (3.6%) was in the Grade I category. An exhaustive study done by Albrektson[95] et al shows that majority of cases they encountered in their study also was of the High grade type (Grade III).Grossly the tumor size varied from 2 cm to 5cm. Cut section of most of the tumor showed gray white, ill-defined tumor mass, firm to hard in consistency

Histological Variants encountered in our study

Infiltrating Ductal Carcinoma NOS: This was the most commonly encountered subtype of breast carcinoma in our study. As well as studies done by Harris &Pinder et al.[96] Occasionally tumors show areas of hemorrhage and necrosis. Microscopically the neoplastic cells were arranged in diffuse sheets, nests and cords along with glandular and tubular differentiation. In few cases comedo - pattern of necrosis was seen.

All other subtypes of breast carcinoma have comparatively reduced frequency than Infiltrating ductal carcinoma: NOS. This was the main reason why literature shows that the rare cases of breast carcinomas are all the other types than Infiltrating Ductal Carcinoma: NOS.

The next main component of our study was a comparison of Immunohistochemical markers, which included Estrogen Receptor (ER), Progesterone Receptor (PR) & HER 2/neu.

ER was noted to be positive in 29 patients (51.8%) and PR was found to be positive in 27 patients (48.2) , these results are lower than the study conducted by Gupta et al [97] which showed an Positive hormone receptor expression for both ER and PR was noted in 96% breast lesions, the reason for this high value could be attributed to the combined incorporation of benign as well as malignant cases in their study group.

A prevalence of 32.6% for ER-positive and 46.1% for PR-positive breast cancers has also been documented in a study carried out in India by Desai et al [98].

This study was taken one step further by looking at the proportion of cases that expressed ER+PR+, ER-PR+, ER+PR-, ER- PR. The data we analysed for our study showed that 22 cases (39.3%) as positive for both ER & PR, whereas ER-/PR+ was positive in 5 cases (8.9%). The next highest frequency we noted in our study was the ER-PR- category which was seen in 22 cases (39.3%).

The Gupta study [97] showed that Fifteen out of 50 cases (30%) were ER+/PR+; 6 cases (12%) were ER-ve/PR+ve; 1 case (2%) was ER+ve/PR-ve and 28 cases (56%) were ER-ve/PR-ve.

This comparison itself shows that our values were higher than the literature from other studies.

Her2-neu was another parameter and marker that we incorporated into our study, we assessed the proportion of cases that were Her2-neu positive among the 56 patients. Her2-neu positivity was noted in 32 cases (57.1), negative in 19 cases (33.9%) and equivocal in 5 cases (8.9%)

Kumar et al[99] conducted a prospective study on 112 patients with diagnosed breast carcinoma at the Department of Surgical oncology at Banaras Hindu Univerisy between March 1997 and March 2000. He further assessed the expression of Her2-neu in these patients.

The results he obtained showed that the frequency of HER-2/neu over expression in the cohort of patients reported in the study (46.37%) is higher than the most studies is western countries showing 20–35% positivity among invasive breast cancer. [100]

With the combined ER, PR & HER2-neu combinations, we proceeded to subtype the different carcinomas based on their molecular subtype. 37 cases (66.1) were place in the Her 2 enriched category, which was then followed by the basal type (25%) and finally the Luminal type which had only 5 cases (8,9%).

Studies done by Hamza et al[102] show clearly that the NPI can accurately predict prognosis, and should be incorporporated into practice.

Nottingham Prognostic Index was used and 20 patients (35.7%) were found to be in the Moderate Prognostic group II. Of the total 56 patients, 16 patients were in the Poor & very Poor Prognostic group.

With all this information at hand we proceeded to find a correlation between Imunohistochemistry and histopathology.

Chi Square tests and correlation was done on many of the variables in this study and strong correlations was found between ER, PR & HER2-neu as compared to the grade of the tumour ($p<0.05$).

Other factors like the Nottingham Prognostic Index and Tumour grade also had an equal strong correlation, along with tumour margins.

Correlation with Previous studies

Sofi.et al.[3]analysed 132 cases which included more than 100 Modified Radical Mastectomies, 17 Local excisions 7 Quadrantectomies. The results obtained were :20 years old was the age of the youngest patient and oldest was 86 years old. 59.1% cases were ≤50 years. Left breast was involved in 50.8% cases. Upper Outer-42.9%, Central-19.3%,.Lump not associated with pain was most common symptom. Followed by nipple discharge, , 0.9% with metastatic deposits in neck nodes.

Pathak. et al.[4]conducted a study on 136 cases of breast carcinoma and performed IHC with ER and PR and compared it to grading of tumour. 96% of these were Infiltrating Ductal Carcinoma;NOS . The most common tumour

grade was grade II ER and PR were positive in 38 (27.94%) and 26 (19.11%) cases .Results obtained in our study shows ER and PR combined positivity was seen in 39.3% of cases.

Nikhara et al.[77] examined 43 cases of breast cancer, results obtained showed maximum numbers of cases were seen in the age group of 31- 40 years (30.23%) and 41-50 years (30.23%). Only 1 (2.32%) case occurred in a male and remaining 42 (97.67%) cases were females. The male to female ratio was 1:42. According to side of affected breast left breast was found to be more commonly involved, comprising of 23 cases (53.4%) including the only male case, right breast was involved in 20 cases (46.51%) while bilateral involvement was not seen in any case. Grade I tumours were seen in patients more than 60 years of age implying a better prognosis. Our study showed majority cases to be Grade III (51.8%) and only 2 patients in our study were males..

Ahmed et al.[78] conducted a study to assess if a association exists between ER, PR, Her/2neu, p53 as well as clinicopathological factors of ductal carcinoma in 137 Yemeni women. Expression of ER, PR, Her2/neu and p53 showed43.8%, 27%, 30.6% and 48.9%, respectively. A significant correlation between ER and PR expression with lymph node spread was found, This study shows that of hormones receptor positive breast cancer in Asian countries is lower than those in the western world. It should be noted that these relatively lower ER and PR in the present study is consistent with

those noted in other Asian and African countries. HER2/neu status correlates with those reported in other countries.

Zubeda et al.[79]study reported that 32% of the breast tumours from South Indian women are found to be positive for both ER and PR..North Indian women show about 40% positivityin ER& PR expression. Positivity for Her 2/neu was observed in 51% of cases of breast cancer.

Shetty et al.[80] conducted a study to analyse the different morphological parameters and correlate it with receptor status in Indian women. Sixty-seven percent of patients were 50 years or younger. Histological types were invasive ductal carcinoma, not otherwise specified accounting for the majority and they were all grade III. Estrogen receptor was positive in 36.5%, HER/neu was overexpressed in three cases; 50.0% were “triple” negative (estrogen receptor, progesterone receptor, HER/neu negative). Estrogen receptor (ER) andprogesterone receptor (PR) positivity decreased with increase in tumour grade. There was significant association between tumour size and ER positivity.

Lal et. al [81] studied the inverse relationship between HER-2/neu ,estrogen (ER) and progesterone (PR) receptors and correlated HER-2 status with Histologic features in 3,655 unselected invasive breast carcinomas. ER and PR expression were decreased significantly in HER-2+ tumours compared with HER-2– tumours (ER, 49.1% vs 78.17%; PR, 24.3% vs 53.13%). Even among HER-2+ tumours, the rate of ER or PR expression in

high-grade tumours was significantly decreased compared with intermediate grade tumours. HER-2 was positive in 10.87% of grade 2 and 27.84% of grade 3 ductal carcinomas and negative in all grade I ductal carcinomas. Although ER or PR expression is decreased in HER-2+ tumours, a substantial proportion of them still express ER or PR.

Jain et.al[82] did a study on 203 breast cancer patients to find ER, PR & HER2/neu reactivity in breast cancer. Their study concluded that Her2/neu over expression was found in patients with poor prognostic factors. Most of the patients who had HER2/neu over expression also were ER-or PR-. This was not the case in our study where ER & PR positivity was noted with HER2 positivity.

IMAGES

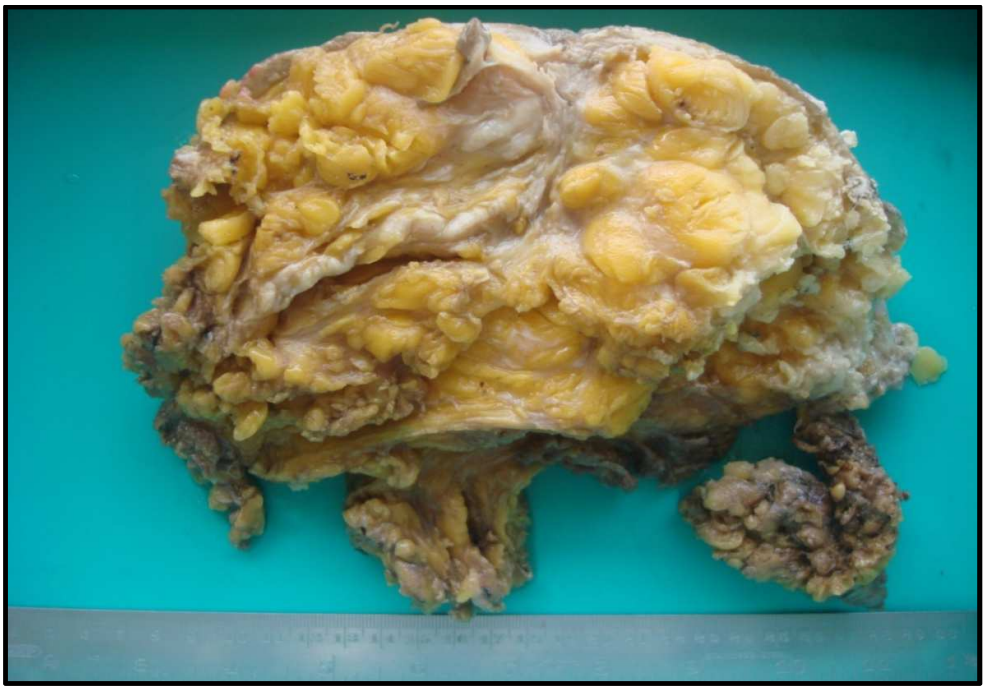

Fig: 22 Mastectomy specimen showing areas of fibrosis

Fig: 23 Areas of fibrosis

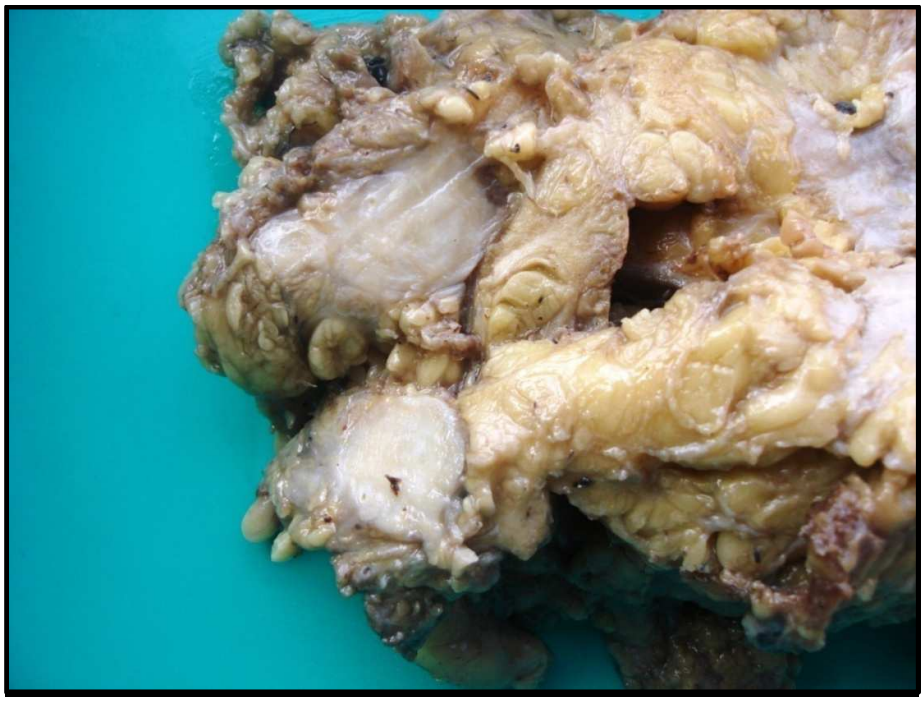

Fig: 24 Cut section of axillary pad of fat showing tumour

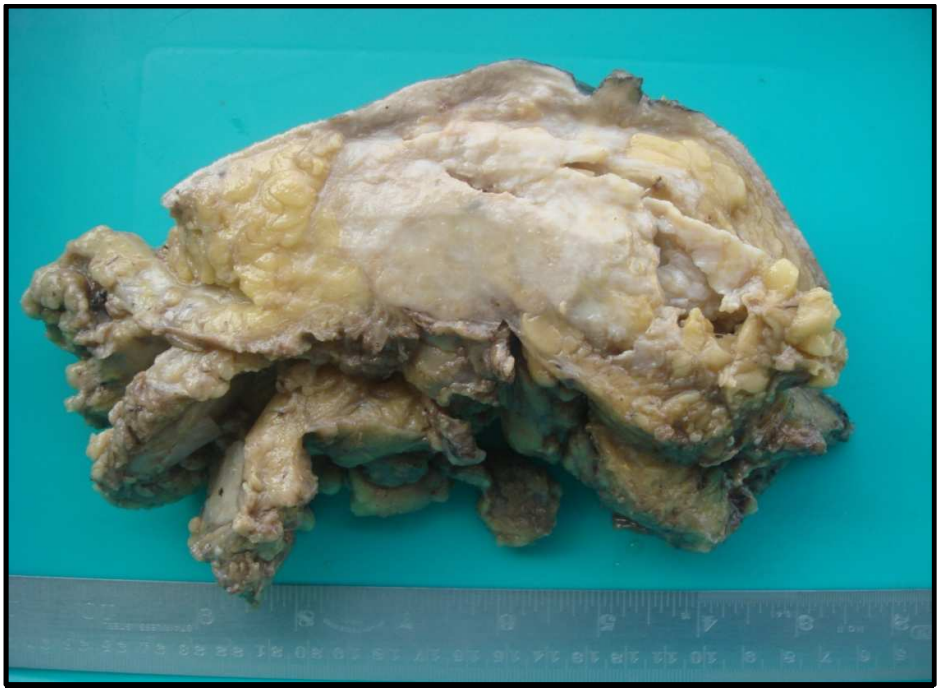

Fig: 25 Mastectomy specimen showing tumour

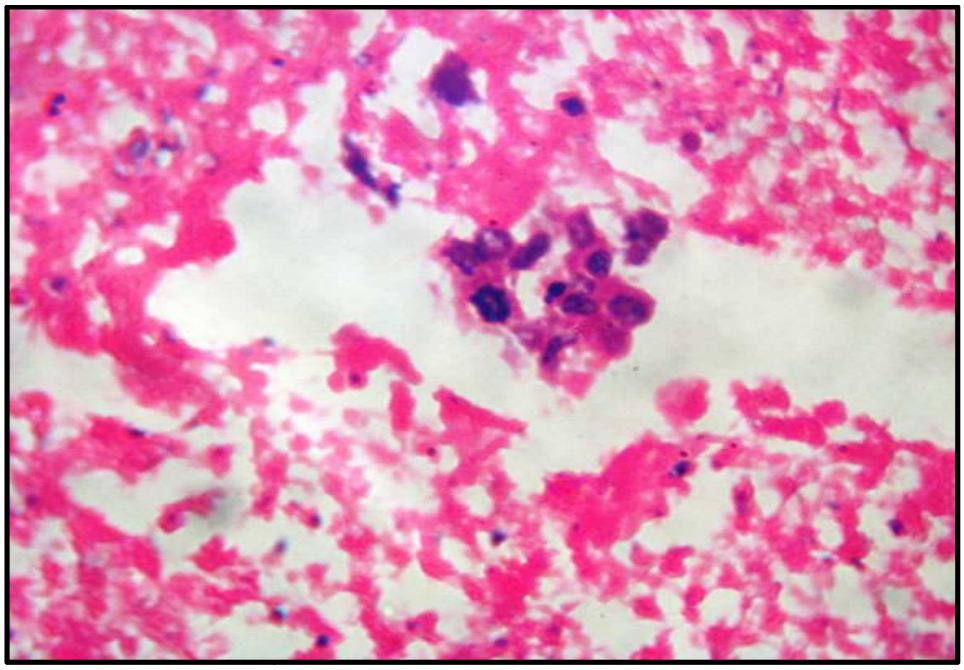

Fig: 26 Tumour cells showing nuclear pleomorphism and and hyperchromatism in a background of necrosis H&E 400x

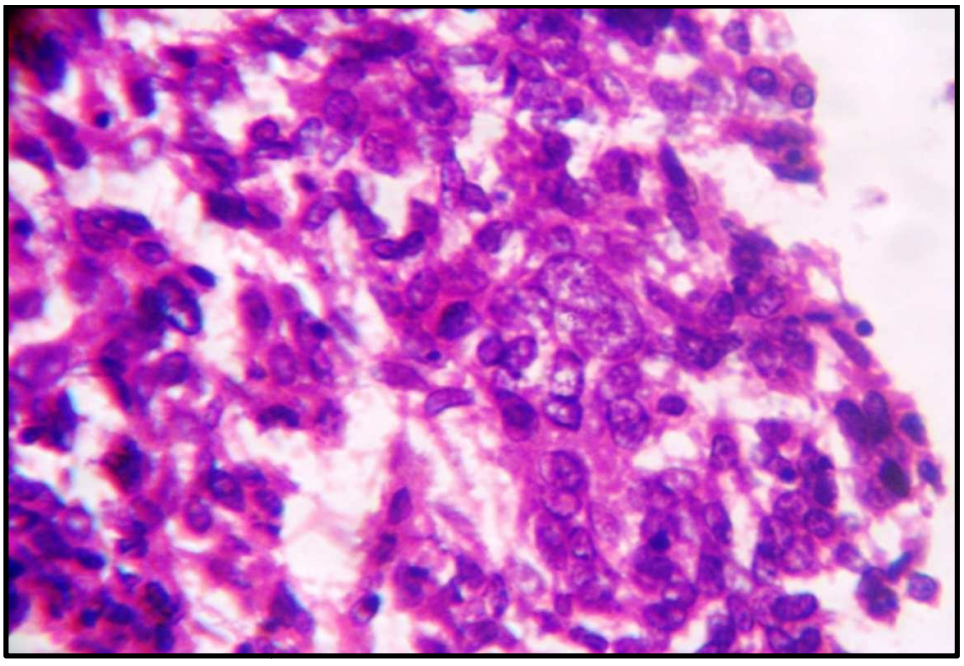

Fig: 27 Multinucleate giant cells and lymphocytic infiltrates H&E 100x

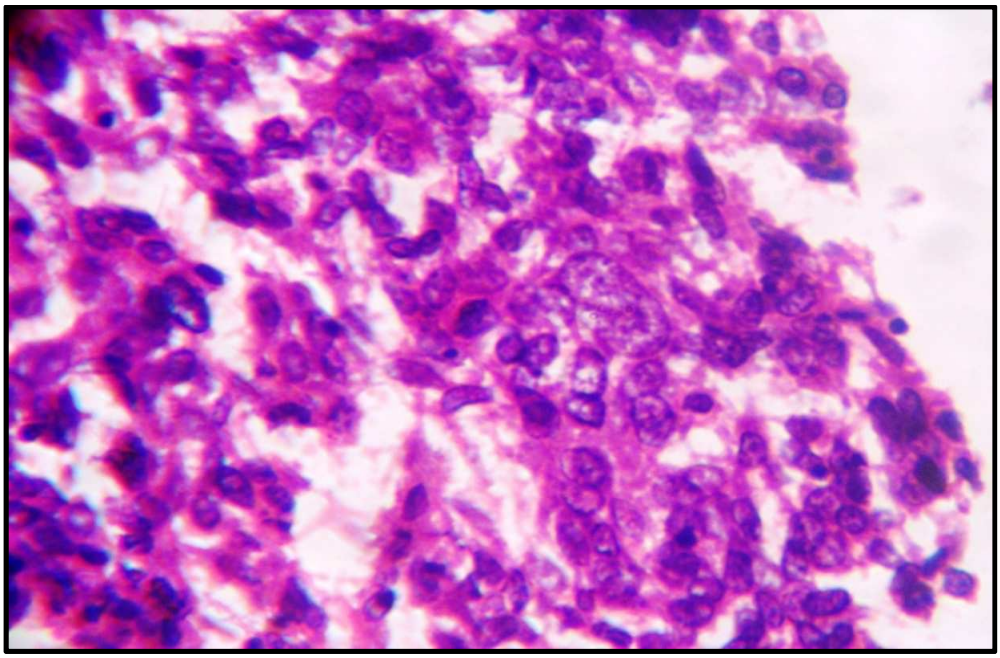

Fig: 28 Tumour cells showing hyperchromatism, pleomorphism and bizarre nuclei. H&E, 400x.

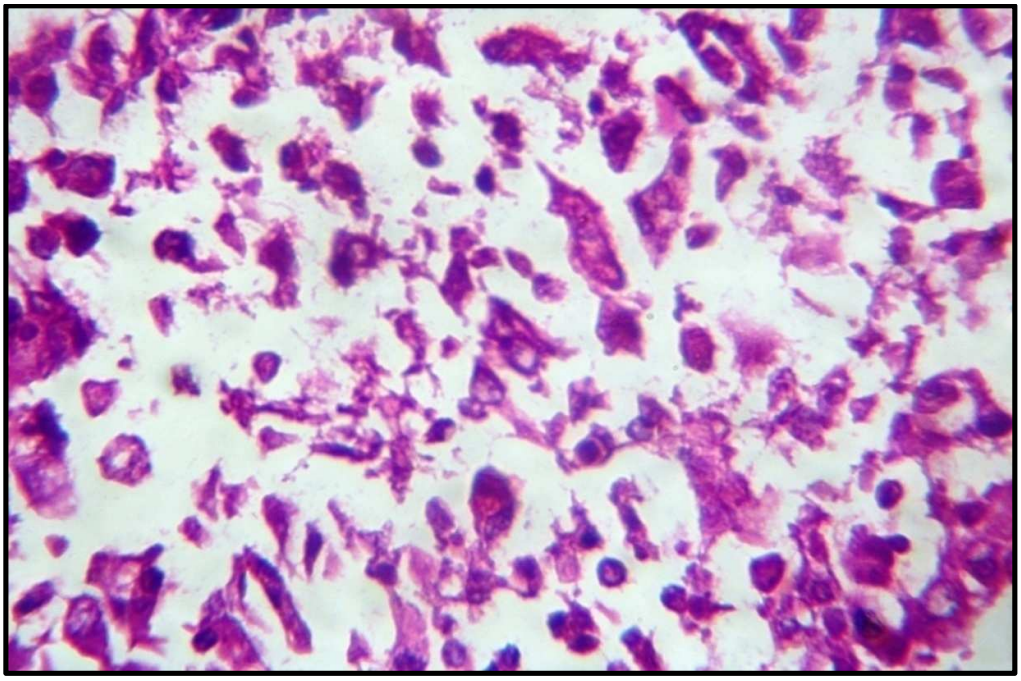

Fig: 29 Pleomorphic tumour cells scattered singly H&E 400x

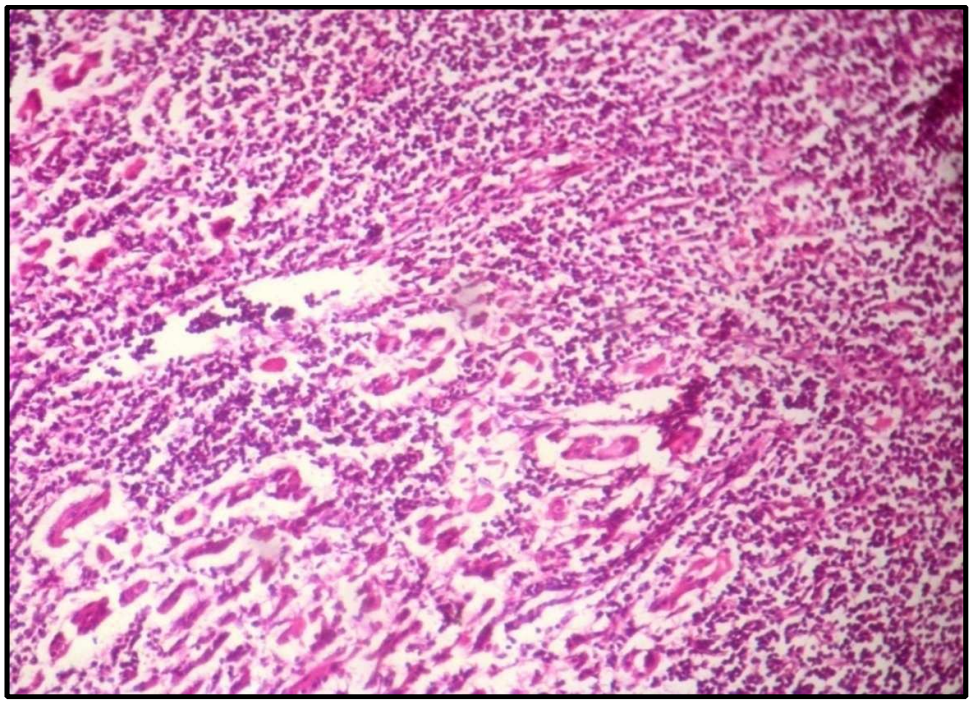

Fig: 30 Lymph node metastases H&E 100x

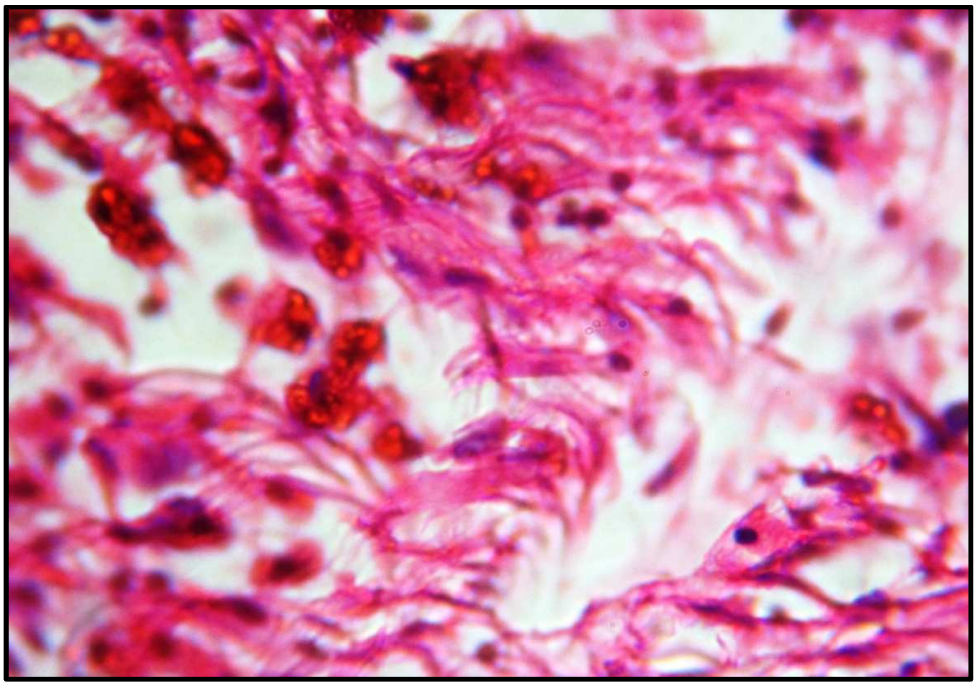

Fig: 31 Haemosiderin -laden macrophages H&E 400x

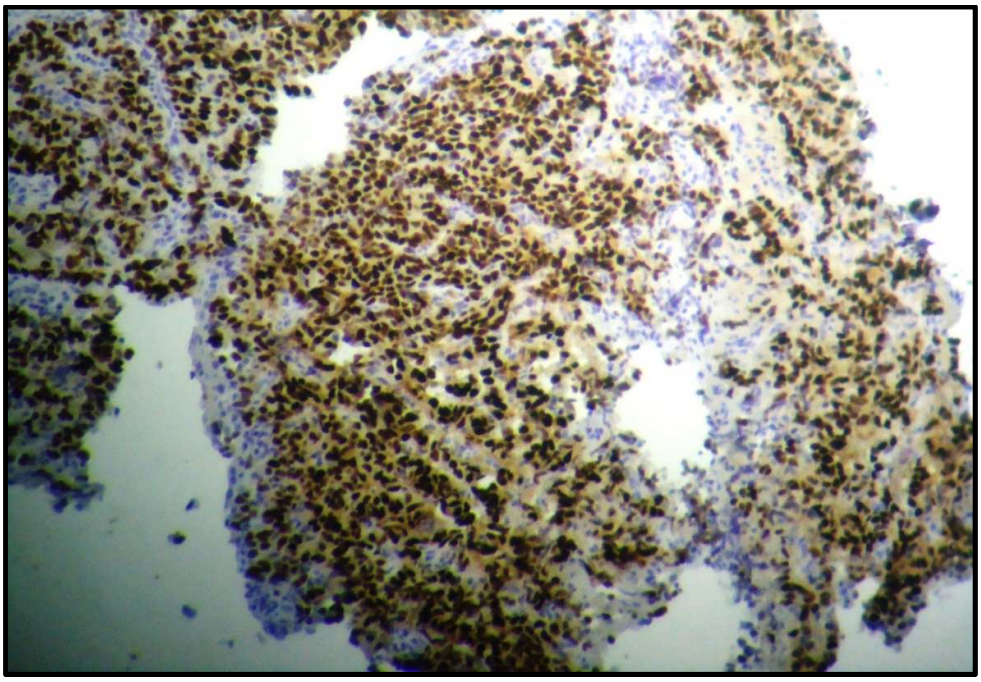

Fig: 32 Hormone receptor positivity ER 100x

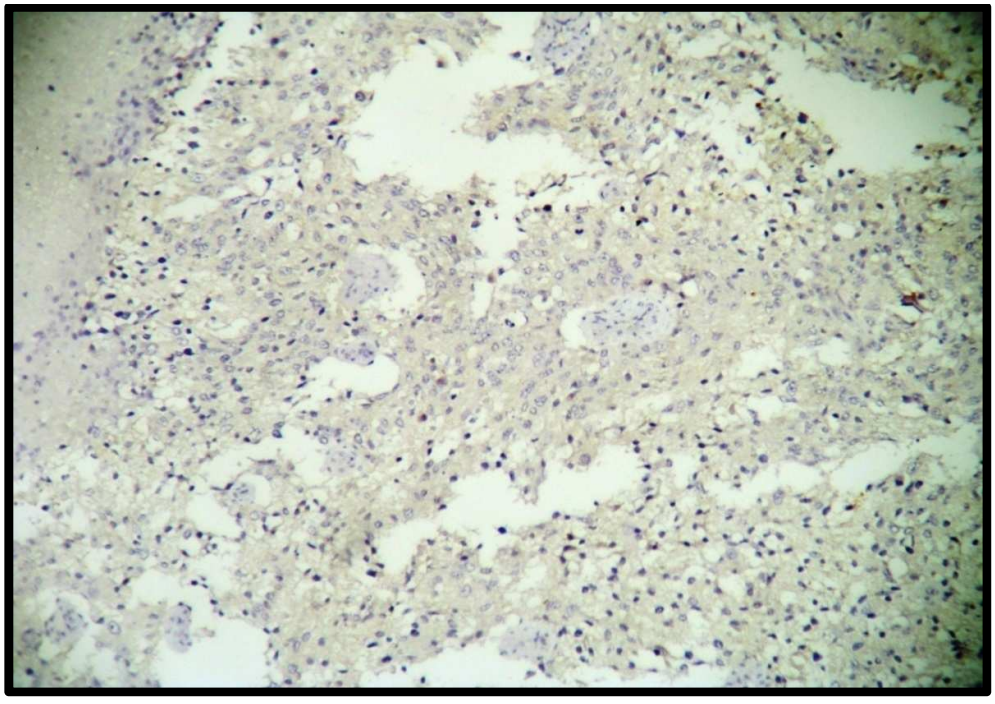

Fig: 33 Hormone receptor negativity ER 100x

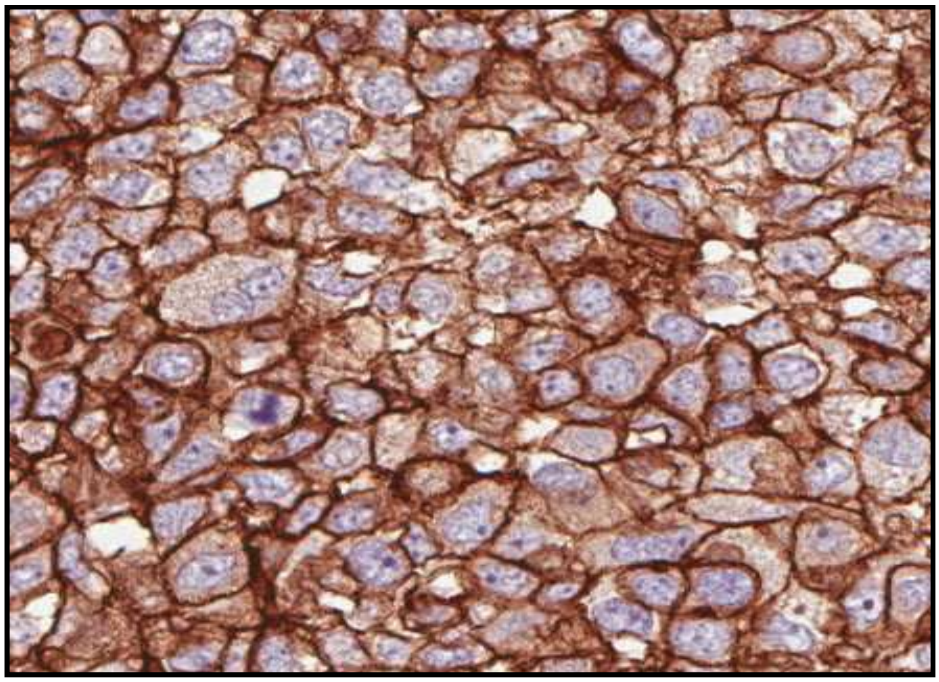

Fig: 32 HER2/neu membrane positivity(3+) 100x

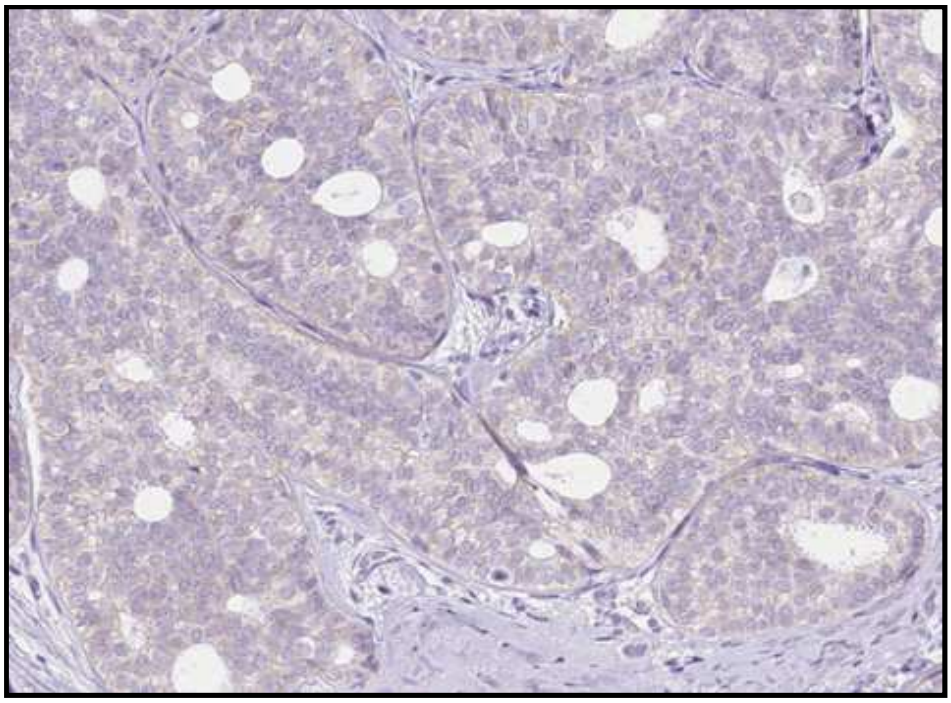

Fig: 33 HER2/neu negative(1+) 100x

Conclusion

CONCLUSION

This study was done in collaboration with Department of Surgery & Department of Pathology and the main aim was to analyse the different cases of breast carcinomas that presented at this institution as well as to perform immunohistochemical tests and arrive at a conclusive correlation.

We are all well aware that breast carcinoma cases are steadily rising, and this is no different in our part of the world. Increasing changes in lifestyle as well as innate hotspots of radiation have made this southern sector of India a well-established carcinoma belt.

Cases that have been chosen for this study have all been diagnosed with Breast carcinoma, what we have been trying to establish was to look at all the possible variables in these cases, then subject them to proper IHC evaluation, and then subtyping them based on their response to IHC. We further went ahead and put a prognostic index measurement, so that we can clearly analyse the way in which these cases are expected to respond to treatment.

This study has clearly shown that Infiltrating ductal carcinoma: NOS is still the most common histological type of breast carcinoma; it also shows that all the hormone receptors as well as HER2-neu have established a positive strong correlation with the different grades of tumours based on the Modified Bloom Richardson score.

Molecular subtyping was also done on all the cases analyzed by Immunohistochemistry.

Molecular subtyping was done so that increased understanding of the molecular heterogeneity that is intrinsic to the various subtypes of breast cancer will likely shape the future of breast cancer diagnosis, prognosis and treatment.

Advances in the field over the last several decades have been remarkable and have clearly translated into better patient care as evidenced by the earlier detection, better prognosis and new targeted therapies.

The future in diagnosis of breast carcinomas has a lot of potential. We have only just begun to understand the immense potential in the newer methods of using genetic methods as well as revolutionary molecular methods that have paved the way for easier diagnosis as well as treatment.

This study has reinforced the use of immunohistochemistry and molecular subtyping in diagnosis of breast carcinoma.

This has all motivated us to promote newer targeted therapies as well as regimes to ensure a more satisfactory as well as an improved survival rate in the patients who have suffered immensely from the burden of this devastating disease.

Summary

SUMMARY

- The present study was conducted at the Department of Pathology, Sree Mookambika Institute of Medical Sciences, Kulasekharam during the study period 2015 to 2016
- 56 cases of breast cancer, whose Modified radical Mastetomies, Excision biopsies and trucut biopsies with satisfactory material, was assessed for morphological and hormone receptor status evaluation.
- The maximum number of patients were in the age group 51-60 as well as > 60 years. The youngest patient was 35 years old and the oldest patient was 78 years old.
- The most common location of the tumour was the Upper outer quadrant.
- Among the various histological variants of breast carcinoma; Invasive Ductal carcinoma-NOS type constituted 69.6% cases.
- The most common TNM stage present was T2N0Mx which was seen in 25% of cases.
- Modified Bloom Richardson Score Grade III accounted for 51.8% of the cases.
- 24.4% of Lymph nodes examined in the cases showed metastatic extension of tumour cells.

- ER positivity was seen in 51.8% cases, PR was positive in 48.2% cases, combined ER+PR+ was seen in 39.3% cases. HER 2-neu positivity was seen in 57.1% cases.
- HER2 enriched was the most common molecular subtype and was observed in 66.1% of the cases. Basal type accounted for 25% of the cases studied.
- Moderate Prognostic Group II was the most common Prognostic index seen in our study with 35.7% cases falling in this category.
- Statistical analysis showed strong correlation between ER, PR & HER2 neu and the different histological grades of tumours.
- HER2-neu values observed in this study are higher than international correlates, but corresponds to studies conducted in India,(Zubeida et al) hence supporting the claim that HER2-neu positivity is higher in India than other western countries.
- Strong correlation was also noted with Nottingham prognostic Index and the grade of the tumour.
- Well established molecular subtypes are very important in targeted therapy and this information is very crucial for the patient as well as the physician; who can then make judicious use of this information and treat the patient better as well as suggest targeted therapy which can lead to better outcome in the prognosis of the disease.

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Appendix

SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES
KULASEKHARAM

RESEARCH COMMITTEE

CERTIFICATE

This is to certify that the Research Protocol Submitted

by Jem kollavana Raj

~~*Faculty*~~ */ Post Graduate from Department of* Pathology

Titled "Correlation of Histopathological features and Immunohistochemistry in the study of Breast Cancer"

is approved by the Research Committee.

Chair Person

Prof. & H.O.D.
Dept. of Bio-Chemistry
Sree Mookambika Institute of Medical Sciences
Kulasekharam 629 161

J.I.C

Convenor

Dr P.S. KRISHNAMURTHY

Date : 27/1/15

Sree Mookambika Institute of Medical Sciences
Kulasekharam (K.K District, TN) 629161

Phone No: 04651-280866, Fax No. 04651-280740

Institutional Human Ethics Committee

Registered under CDSCO with Reg No. ECR/446/Inst/TN/2013

Ref. No. SMIMS/IHEC/2015/A/22 **Date: 10th April 2015**

Certificate

This is to certify that the Research Protocol Ref. No. **SMIMS/IHEC/2015/A/22**, entitled "Correlation of Histopathological Features and Immunochemistry in The Study of breast Cancer" submitted by Dr. Jem Kollavana Raj, Postgraduate of Department of Pathology, SMIMS has been approved by the Institutional Human Ethics Committee at its meeting held on 13th of March 2015.

[This Institutional Human Ethics Committee is organized and operates according to the requirements of ICH-GCP/GLP guidelines and requirements of the Amended Schedule-Y of Drugs and Cosmetics Act, 1940 and Rules 1945 of Government of India.]

SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES IHEC Date: 10/4/15

Dr. Rema Menon. N
Member Secretary
Institutional Human Ethics Committee
Professor of Pharmacology and HOD
SMIMS, Kulasekharam [K.K District]
Tamil Nadu -629161

CONSENT FORM

PART 1 OF 2

INFORMATION FOR PARTICIPANTS OF THE STUDY

Dear Participants,

We welcome you and thank you for your keen interest in participation in this research project. Before you participate in this study, it is important for you to understand why this research is being carried out. This form will provide you all the relevant details of this research. It will explain the nature, the purpose, the benefits, the risks, the discomforts, the precautions and the information about how this project will be carried out. It is important that you read and understand the contents of the form carefully. This form may contain certain scientific terms and if you have any doubts or if you want more information, you are free to ask the study personnel or the contact person mentioned below before you give consent and also at any time during the entire course of the project.

1. Name of the Principal Investigator : **Dr. JEM KOLLAVANA RAJ**
Post graduate
Department of Pathology,
SMIMS, Kulasekharam.

2. Name of the Guide : **Dr. JAYASREE P.V.**
Professor
Department of Pathology,
SMIMS, Kulasekharam.

3. Name of the Co- Guide : **Dr. D. BALAJEE**
H.O.D and Professor
Department of Surgery
SMIMS, Kulasekharam.

4. Institute : **Sree Mookambika Institute of Medical Sciences**,
Kulasekharam,
Kanyakumari District
Tamil Nadu.

5. Title of Study:"CORRELATION OF HISTOPATHOLOGICAL FEATURES AND IMMUNOHISTOCHEMISTRY IN THE STUDY OF BREAST CANCER."

6. Background Information:

Breast cancer is the most common cancer in both developed and developing countries. In India, the number of breast cancer cases are increasing every year, which is mostly attributed to the change in lifestyle, adoption of a western lifestyle, together with changes in environmental factors as well as genetic susceptibility; all of which have contributed to an increase in number of patients diagnosed with breast cancer.[5] Diagnosis along with assessment of prognostic factors is also very important in understanding the aggressiveness of the cancer.

7. Aims and objectives:

1) To correlate the various prognostic factors related to breast cancer.
2) To study the immunohistochemical profile for ER, PR and Her2/neu in patients with breast cancer

8. Scientific justification of the study:

The percentage of women affected by breast cancer is on the rise, and it is of paramount importance that the prognostic factors related to breast cancer are carefully examined and correlations between these factors be made. This study also focuses primarily on the factors which determine the outcome of breast cancer along with an in-depth study of ER, PR and Her/2 neu expression.

9. Procedure for the study.

The detailed clinical history and results of relevant investigations done will be collected from the patients' case files for all the participants included in this study. For prospective cases, the mastectomy and lymph node dissection specimen will be received in the Pathology department in 10% formalin. In every case the standard protocol for surgical grossing of radical mastectomy specimens will be followed. After a detailed specimen description, multiple sections will be taken from the tumour, surgical margins, nipple and areola, non-neoplastic breast, and all the lymph nodes. Tissue sections will be made and stained with haematoxylin and eosin

(H & E) for histopathological study. In retrospective cases, the histopathology reports, slides and paraffin blocks will be retrieved from the archives and similar tissue processing and staining will be done. Immunohistochemistry (IHC) is an integral part of this study; tissue bits required for IHC will be taken from the specimen and will be subjected to testing using ER, PR and HER 2/neu.

10. **Expected Risk for the participant**: Nil

11. Expected benefits of research for the patient:

This study will give the patient a clear understanding on their prognostic aspect of their disease.

12. Maintenance of Confidentiality:

All your study records will be kept confidential. Your personal identity will not be revealed in any publication or release of results. Study records will be kept indefinitely for analysis and follow up**.**

13. Why have I chosen to be in this study?

You are coming under my inclusion criteria, which is why I have chosen you for my study.

14. How many people will be in the study? : 56

15. Agreement of compensation to the participant (In case of study related injury):

Any adverse event as experienced due to the study will be treated as per hospital guidelines

16. **Anticipated prorated payment, if any, to the participant(s) of the study**: No

17. Can I withdraw from the study at any time during the study period: Yes

18. If there is any new findings/ information would I be informed? Yes

19. Expected duration of the participants participation in the study Once

20. Any other pertinent information? None

21.Who do I contact for further information?

For any study related queries, you are free to contact

Dr. JEM KOLLAVANA RAJ

. **Post graduate student,**

Department of Pathology,

SMIMS,

Kulasekharam.

9751260886

Email ID:**kollamana@gmail.com.**

Place: Kulasekharam *Signature of Principal Investigator*

Date:

Signature of Participant

CONSENT FORM.

PART 2 OF 2

PARTICIPANTS CONSENT FORM

The details of the study have been explained to me in writing and the details have been fully explained to me. I am aware of the results of the study may not be directly beneficial to me but will help in the advancement of medical sciences. I confirm that I have understood the study and had the opportunity to ask questions. I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason giving any reason, without the medical care that will normally be provided by the hospital being affected. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). I have been given an information sheet giving details of the study. I fully consent to participate in the study titled "CORRELATION OF HISTOPATHOLOGICAL FEATURES AND IMMUNOHISTOCHEMISTRY IN THE STUDY OF BREAST CANCER."

Serial no/Reference no:

Name of Participant: **Address of Participant:**

Contact no of Participant:

Signature/Thumb impression of the participant/Legal guardian

Witness

1.

2.

Date:

Place: Kulasekharam

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DEPARTMENT OF PATHOLOGY

Case Record Sheet

STUDY TITLE: "CORRELATION OF HISTOPATHOLOGICAL FEATURES AND IMMUNOHISTOCHEMISTRY IN THE STUDY OF BREAST CANCER."

PROFORMA

NAME	:	
DATE	:	
BIOPSY No	:	
AGE (in years)	:	{18-25}/{26-33}/{34-42}/ {43-50}/{51-60}/{> 60}
SEX	:	MALE / FEMALE
AGE AT FIRST SYMPTOMS	:	{18-25}/{26-33}/{34-42}/{43-50}/ {51-60}/{> 60}
MEDICAL HISTORY	:	HYPERTENSION / DIABETES / OTHER CHRONIC CONDITIONS
SURGICAL HISTORY	:	PREVIOUS SURGERY: Y / N IF YES, SPECIFY.....
COMPLAINTS	:	PAIN / TENDERNESS / SWELLING / RASH / DISCHARGE FROM NIPPLE
ANY OTHER SYMPTOMS	:	
PREVIOUS INVESTIGATIONS CONDUCTED	:	FNAC/MAMOGRAM/BIOPSIES
CURRENT INVESTIGATIONS	:	IMMUNOHISTOCHEMISTRY USING ER, PR & HER2/NEU.

GROSS FINDINGS

TYPE OF SPECIMEN	:	MRM/SM/EXCISION BIOPSY
OVERALL SIZE OF SPECIMEN	:	____ X ___ X ___ CMS
WEIGHT OF SPECIMEN	:	GMS
SITE OF THE TUMOUR	:	UOQ / LOQ / UIQ / LIQ/BELOW NIPPLE UNILATERAL/BILATERAL/ : MULTICENTRIC/MULTIFOCAL
TUMOUR SIZE	:	____ X ___ X ___ CMS
TOTAL NUMBER OF LYMPH NODES	:	INVOLVED/DETECTED
TUMOUR MARGINS	:	PUSHING, INFILTRATING.
NIPPLE INVOLVEMENT	:	Y / N
SKIN INVOLVEMENT	:	Y / N
ANY OTHER RELEVANT FINDINGS	:	

MICROSCOPIC FINDINGS

MICROSCOPIC DESCRIPTION OF SPECIMEN :

TYPE	:	
ASSOCIATED INTRA DUCTAL CARCINOMA	:	Y / N, If Y; Area involved__
LYMPHOVASCULAR EMBOLISM	:	Y / N
PERI NEURAL INVASION	:	Y / N
PERINODAL EXTENSION	:	Y / N
ASSOCIATED BREAST DISEASE	:	
POSTERIOR RESECTION MARGIN	:	
MBR GRADE	:	
PATHOLOGICAL STAGING	:	T_____N_____M_____

IMMUNOHISTOCHEMISTRY

ER& PR using Allred Scoring

PROPORTION SCORE (PS)			**INTENSITY SCORE (IS)**			**TOTAL SCORE (TS)**
% of nuclei with positive staining (count 200 tumour cells)	PS		Nuclear staining Intensity	NS		
	ER	**PR**		**ER**	**PR**	
No Staining	0	0	No Staining	0	0	TS = PS + IS Possible Total Score Values: 0,2,3,4,5,6,7,8
0-1%	1	1	Weak	1	1	
2-10%	2	2	Moderate	2	2	
11-33%	3	3	Strong	3	3	**TOTAL SCORE (ER) =**
34-67%	4	4				
68-100%	5	5				**TOTAL SCORE (PR) =**

Interpretation of Total Score: ER: _____ PR: _____

0-2 NEGATIVE 3-8 POSITIVE

HER 2 NEU

POSITIVE (3+)	>30% cells show complete homogenous membrane staining
EQUIVOCAL (2+)	≤30% of cells show strong complete membrane staining OR 10% of cells showing weak or moderate heterogeneous complete membrane staining.
NEGATIVE (0-1+)	In any percentage of cells, there is no staining or weak incomplete membrane staining

MOLECULAR SUBTYPING OF INVASIVE BREAST CANCER

LUMINAL TYPE **(ER +ve, PR+ve , HER2 -ve)**	**HER-2 ENRICHED** **(ER +/-ve, PR+/-ve , HER2 +ve)**	**BASAL LIKE** **(ER -ve, PR-ve , HER2 -ve)**

NOTTINGHAM PROGNOSTIC INDEX

NPI = [0.2 x S] + N + G

S is the size of the lesion in centimetres

N is the number of lymph nodes involved:

No metastases to lymph node- **1**

Metastases in 1-3 lymph nodes- **2**

Metastases in 4 or more lymph nodes- **3**

G is the grade of tumour: Grade I =**1**, Grade II =**2**, Grade III =**3**

NPI SCORE: _____

PROGNOSTIC GROUP	**NPI SCORE**
EXCELLENT PROGNOSTIC GROUP (EPG)	**2.08-2.4**
GOOD PROGNOSTIG GROUP	**2.42-3.4**
MODERATE PROGNOSTIC GROUP I (MPG I)	**3.42-4.4**
MODERATE PROGNOSTIC GROUP II (MPG II)	**4.42-5.4**
POOR PROGNOSTIC GROUP (PPG)	**5.42-6.4**
VERY POOR PROGNOSTIC GROUP (VPG)	**6.42-6.8**

LIST OF ABBREVIATIONS

AR	-	Antigen Retrieval
ASCO- CAP	-	American Society of Clinical Oncology- College of American Pathologists
CISH	-	Chromogenic in- situ hybridization
DSM	-	Deep Surgical Margin
ER	-	Estrogen Receptor
EPG	-	Excellent Prognostic Group
FISH	-	Fluorescent In-Situ Hybridization
Fibro	-	Fibrosis
GPG	-	Good Prognostic Group
Her2/neu	-	Human epidermal growth factor receptor2/neuroblastoma
H & E	-	Haematoxylin& Eosin
HPF	-	High Power Field
IDC	-	Infiltrating Duct Carcinoma
IHC	-	Immunohistochemistry
ILC	-	Infiltrating Lobular Carcinoma
LVI	-	Lymphovascular Invasion
MPG I	-	Moderate Prognostic Group I
MPG II	-	Moderate Prognostic Group II
Necro	-	Necrosis
No.	-	Number
NOS	-	Not Otherwise Specified

NPI	-	Nottingham Prognostic Index
MBR	-	Modified Bloom Richardson Grading
PR	-	Progesterone Receptor
PPG	-	Poor Prognostic Group
Sl No.	-	Serial Number
SR	-	Stromal Reaction
TDLU	-	Terminal Duct Lobular Unit
VEGF	-	Vascular Endothelial Growth Factor
VPG	-	Very Poor Prognosis
CBA	-	Cannot be assessed

MODIFIED BLOOM RICHARDSON GRADING SYSTEM

Feature	Score
Tubule formation	
Most tumors (>75%)	1
Moderate degree (10-75%)	2
Little or none (<10%)	3
Nuclear pleomorphism	
Small, regular uniform cells	1
Moderate increase in size and variability	2
Marked variation	3
Mitotic counts**	
0-7	1
8-16	2
>17	3
Olympus BX-40 microscope	
Objective	X 40
Field diameter (mm)	0.55
Field area (mm^2)	0.239

*According to Elston and Ellis (1991; 1998)

**Assessed as number of mitoses per 10 fields at the tumor periphery.

MOLECULAR SUBTYPING OF BREAST CANCER

LUMINAL TYPE **(ER +ve, PR+ve , HER2 -ve)**	**HER-2 ENRICHED** **(ER +/-ve, PR+/-ve , HER2 +ve)**	**BASAL LIKE** **(ER -ve, PR-ve , HER2 -ve)**

NOTTINGHAM PROGNOSTIC INDEX

NPI = [0.2 x S] + N + G

S is the size of the lesion in centimetres

N is the number of lymph nodes involved:

No metastases to lymph node- **1**

Metastases in 1-3 lymph nodes- **2**

Metastases in 4 or more lymph nodes- **3**

G is the grade of tumour: Grade I =**1**, Grade II =**2**, Grade III =**3**

NPI SCORE: _____

PROGNOSTIC GROUP	**NPI SCORE**
EXCELLENT PROGNOSTIC GROUP (EPG)	**2.08-2.4**
GOOD PROGNOSTIG GROUP	**2.42-3.4**
MODERATE PROGNOSTIC GROUP I (MPG I)	**3.42-4.4**
MODERATE PROGNOSTIC GROUP II (MPG II)	**4.42-5.4**
POOR PROGNOSTIC GROUP (PPG)	**5.42-6.4**
VERY POOR PROGNOSTIC GROUP (VPG)	**6.42-6.8**

ALLRED SCORING SYSTEM FOR ER & PR

ER& PR using Allred Scoring

PROPORTION SCORE (PS)			**INTENSITY SCORE (IS)**			**TOTAL SCORE (TS)**
% of nuclei with positive staining (count 200 tumour cells)	PS		Nuclear staining Intensity	NS		
	ER	**PR**		**ER**	**PR**	TS = PS + IS Possible Total Score Values: 0,2,3,4,5,6,7,8
No Staining	0	0	No Staining	0	0	
0-1%	1	1	Weak	1	1	
2-10%	2	2	Moderate	2	2	
11-33%	3	3	Strong	3	3	**TOTAL SCORE (ER) =**
34-67%	4	4				
68-100%	5	5				**TOTAL SCORE (PR) =**

Interpretation of Total Score: ER: _____ PR: _____

0-2 NEGATIVE 3-8 POSITIVE

HER2/NEU SCORING SYSTEM

POSITIVE (3+)	>30% cells show complete homogenous membrane staining
EQUIVOCAL (2+)	≤30% of cells show strong complete membrane staining OR 10% of cells showing weak or moderate heterogeneous complete membrane staining.
NEGATIVE (0-1+)	In any percentage of cells, there is no staining or weak incomplete membrane staining

MASTERCHART

S.No	Age	Sex	TOS	Site	T.Size (cms)	LN	Margins	Nipple	Skin	LVE	PNI	PNE	MBR	TNM	ER			PR			Her2 neu	Mol. Subtype	NPI	Diagnosis
															PS	IS	Res	PS	IS	Res				
1	>60	F	MRM	LOQ	3.5x2.5x4	42379	Pushing	Y	N	Y	N	N	I	T2N1Mx	4	1	+	4	2	+	+	HER2EN	GPG	IDC: Micropapillary
2	43-50	F	MRM	BN	3x2x2	42688	Infiltrating	Y	Y	Y	N	Y	II	T4bN3AMx	3	1	+	1	1	-	+	HER2EN	PPG	IDC: Apocrine type
3	>60	F	MRM	UIQ	2.5x2.5x2	0/21	Infiltrating	N	N	N	N	N	II	T2N0Mx	5	3	+	3	2	+	+	HER2EN	GPG	Mucinous Ca
4	51-60	F	EB	UIQ	1x1x1	0/10	Pushing	-	-	N	N	N	III	T1N0Mx	3	2	+	0	0	-	-	LUMINAL	GPG	IDC: NOS
5	43-50	F	MRM	UOQ	4.5x3x2	42443	Infiltrating	N	N	Y	N	N	III	T2N1cMx	4	2	+	4	2	+	-	LUMINAL	VPG	IDC: NOS
6	51-60	F	EB	UOQ	2.6x1.6x2	42543	Pushing	-	-	N	N	Y	II	T2N2Mx	1	1	-	0	0	-	+	HER2EN	PPG	IDC: NOS
7	43-50	F	MRM	UOQ	5.5x5x5	42509	Pushing	N	Y	Y	Y	N	II	T4N2aMx	2	2	+	5	3	+	EQ	HER2EN	PPG	Cribiform Ca.
8	43-50	F	MRM	UIQ	5x3x3	11355	Infiltrating	N	N	N	N	N	II	T3N1aMx	5	2	+	0	0	-	+	HER2EN	MPGII	Infiltrating Lobular Ca.
9	51-60	F	MRM	UOQ	8x3x3	42718	Infiltrating	Y	Y	Y	N	Y	II	T4bN3aMx	0	0	-	0	0	-	+	HER2EN	VPG	IDC:NOS
10	43-50	F	EB	UOQ	5x3.2x2.4	0/0	Infiltrating	N	N	Y	Y	N	III	T2NxMx	0	0	-	0	0	-	-	BASAL	MPG II	IDC:NOS
11	>60	F	MRM	UOQ	3x2.5x2.5	0/8	Infiltrating	N	N	Y	Y	Y	III	T2N0Mx	0	0	-	0	0	-	-	BASAL	MPG II	IDC:NOS
12	34-42	F	MRM	UOQ	4.3x3x2	14/16	Infiltrating	N	N	Y	Y	Y	III	T2N3aMx	0	0	-	0	0	-	-	BASAL	VPG	IDC:NOS
13	43-50	F	MRM	UOQ	4.5x4x3	0/22	Infiltrating	N	N	Y	N	N	III	T2N0Mx	0	0	-	0	0	-	-	BASAL	MPG II	IDC:NOS
14	34-42	F	EB	UOQ	2.5x1.5x0.5	0/0	-	N	N	Y	N	N	III	T2NxMx	0	0	-	1	2	+	+	HER2EN	MPG II	IDC:NOS
15	51-60	F	EB	UIQ	6x3x1	0/0	-	-	-	Y	N	N	III	T3NxMx	1	1	-	1	2	+	+	HER2EN	MPG II	IDC:NOS
16	51-60	F	EB	UOQ	2.6x1.6x2	42543	Pushing	N	N	Y	N	Y	II	T2N2Mx	0	0	-	0	0	-	-	BASAL	PPG	IDC:NOS
17	51-60	F	TC	UOQ	-	-	-	-	-	N	N	N	III	TxNxMx	5	3	+	5	2	+	+	HER2EN	-	IDC: NOS
18	>60	F	MRM	LIQ	4x2.5x2	0/25	Infiltrating	N	N	N	N	N	III	T2N0Mx	0	0	-	0	0	-	+	HER2EN	MPG II	IDC:NOS
19	>60	F	EB	UOQ	1.6x1.7x2	0/13	Pushing	N	N	Y	N	N	II	T1cN0Mx	5	2	+	5	2	+	+	HER2EN	GPG	IDC: Apocrine type
20	51-60	F	MRM	UOQ	3x1.8x2	42422	Infiltrating	N	N	N	N	N	II	T2N1aMx	5	2	+	5	2	+	+	HER2EN	MPG II	IDC: Scirrhous type
21	43-50	F	MRM	UOQ	3x3.5x2	42483	Infiltrating	N	N	Y	N	Y	III	T2N2Mx	3	2	+	3	2	+	-	LUMINAL	VPG	IDC:NOS

22	>60	F	MRM	UOQ	2x2x2	42384	Infiltrating	N	N	Y	N	N	III	T2N1Mx	2	1	+	0	0	-	-	BASAL	MPG II	IDC:NOS
23	>60	F	EB	UOQ	-	-	Infiltrating	-	Y	Y	N	N	II	TxNxMx	5	3	+	5	3	+	+	HER2EN	-	IDC:NOS
24	>60	M	MRM	BN	1.5x1.5x1	42447	Pushing	Y	N	Y	N	N	II	T4aN1aMx	5	3	+	5	3	+	+	HER2EN	MPG I	IDC: Micropapillary
25	51-60	F	MRM	UOQ	1.5x1.8x1	0/22	Infiltrating	N	N	Y	N	N	II	T2cN0Mx	5	3	+	5	3	+	EQ	HER2EN	GPG	Medullary Ca.
26	>60	F	MRM	UIQ	2.5x2.5x2	0/20	Infiltrating	Y	N	N	N	N	II	T1micN0Mx	1	1	-	1	1	-	+	HER2EN	MPG I	Intraductalcomedo Ca
27	>60	F	MRM	BN	9.5x9x7	0/11	Infiltrating	N	Y	Y	N	N	II	T4bN0Mx	0	0	-	0	0	-	-	BASAL	MPG II	IDC:NOS
28	>60	F	MRM	UOQ	4x4x3.5	42384	Infiltrating	Y	Y	Y	N	Y	III	T4N1Mx	1	1	-	4	3	+	+	HER2EN	MPG II	IDC:NOS
29	>60	F	MRM	LIQ	3x2.5x2	0/13	Infiltrating	N	N	Y	N	N	II	T2N0Mx	5	2	+	4	2	+	EQ	HER2EN	MPG I	IDC:Micropapillary
30	51-60	F	MRM	BN	8x3x3	42718	Infiltrating	Y	Y	Y	N	Y	III	T4bN3aMx	5	2	+	0	0	-	-	BASAL	VPG	IDC: NOS
31	>60	F	MRM	UOQ	3.5x2x0.5	42381	Infiltrating	N	N	Y	N	N	III	T2N1Mx	2	1	+	4	2	+	+	HER2EN	MPG II	IDC: NOS
32	51-60	F	MRM	LOQ	3x2.5x2	0/18	Infiltrating	N	N	Y	Y	N	II	T2N0Mx	2	2	+	1	1	-	EQ	HER2EN	MPG I	IDC: NOS
33	51-60	F	MRM	UOQ	1.5x1x1	28/31	Infiltrating	N	N	N	N	Y	II	TxN3Mx	5	3	+	5	3	+	-	LUMINAL	MPG II	IDC: NOS
34	51-60	F	MRM	UIQ	.2x2.1x3	0/14	Pushing	N	N	N	N	N	III	T2N0Mx	0	0	-	0	0	-	-	BASAL	MPG II	IDC: NOS
35	51-60	M	MRM	UOQ	3x2x2	18/29	Infiltrating	N	N	Y	N	Y	III	T4N3aMx	1	1	-	1	1	-	+	HER2EN	VPG	IDC: NOS
36	>60	F	MRM	BN	3x3x2	0/14	Infiltrating	Y	N	N	N	N	II	T4N0Mx	5	3	+	4	3	+	-	LUMINAL	MPG I	Papillary Ca.
37	43-50	F	MRM	UOQ	3x3x2	0/23	Infiltrating	Y	N	N	N	N	III	T2N0Mx	1	1	-	1	1	-	+	HER2EN	MPG II	IDC: NOS
38	51-60	F	MRM	UOQ	1.6x1.2x1.2	42497	Infiltrating	Y	N	Y	N	Y	II	T1cN2aMx	4	2	+	5	3	+	+	HER2EN	MPG II	IDC: NOS
39	>60	F	EB	UOQ	1x0.8x0.5	0/0	Infiltrating	N	Y	Y	N	N	II	T4N3aMx	5	2	+	5	3	+	+	HER2EN	-	IDC: NOS
40	43-50	F	EB	UOQ	4x2.5x2	0/0	Infiltrating	N	N	N	N	N	I	T2NxMx	5	2	+	5	3	+	+	HER2EN	-	Infiltrating Lobular Ca
41	34-42	F	EB	UOQ	2.8x1.6x2.5	0/18	Infiltrating	N	N	Y	Y	N	II	T2N0Mx	0	0	-	0	0	-	-	BASAL	PPG	IDC: Mixed ductal lobular Ca
42	43-50	F	EB	UOQ	5x3.2x2.4	0/0	Infiltrating	N	N	Y	Y	N	III	T2NxMx	0	0	-	0	0	-	-	BASAL	-	IDC: NOS
43	>60	F	MRM	UOQ	3x2.5x2.5	0/8	Infiltrating	N	N	Y	Y	Y	III	T2N0Mx	0	0	-	0	0	-	-	BASAL	MPG II	IDC: NOS
44	34-42	F	MRM	UOQ	4.3x3x2	14/16	Infiltrating	N	N	Y	Y	Y	III	T2N3aMx	0	0	-	0	0	-	-	BASAL	VPG	IDC: NOS
45	43-50	F	MRM	UOQ	4.5x4x3	0/22	Infiltrating	N	N	Y	N	N	III	T2N0Mx	0	0	-	1	2	+	+	HER2EN	MPG II	IDC: NOS
46	34-42	F	EB	UOQ	2.5x1.5x0.5	0/0	-	N	N	Y	N	N	III	T2NxMx	1	1	-	1	2	+	+	HER2EN	-	IDC: NOS
47	51-60	F	EB	UIQ	6x3x1	0/0	-	-	-	Y	N	N	III	T3NxMx	0	0	-	0	0	-	-	BASAL	-	IDC: NOS

48	51-60	F	EB	UOQ	2.6x1.6x2	42543	Pushing	N	N	Y	N	Y	II	T2N2Mx	5	3	+	5	2	+	+	HER2EN	PPG	IDC: NOS
49	51-60	F	TC	UOQ	-	-	-	-	-	N	N	N	III	TxNxMx	0	0	-	0	0	-	+	HER2EN	-	IDC: NOS
50	>60	F	MRM	LIQ	4x2.5x2	0/25	Infiltrating	N	N	N	N	N	III	T2N0Mx	0	0	-	0	0	-	+	HER2EN	MPG II	IDC: NOS
51	>60	F	MRM	UIQ	2.5X2.5X2	0/21	Infiltrating	N	N	N	N	N	II	T2N0Mx	5	3	+	3	2	+	+	HER2EN	GPG	Mucinous Ca
52	51-60	F	EB	UIQ	1x1x1	0/10	Pushing	-	-	N	N	N	III	T1N0Mx	3	2	+	0	0	-	+	HER2EN	GPG	IDC: NOS
53	43-50	F	MRM	UOQ	4.5x3x2	42443	Infiltrating	N	N	Y	N	N	III	T2N1cMx	4	2	+	4	2	+	+	HER2EN	VPG	IDC: NOS
54	51-60	F	EB	UOQ	2.6x1.6x2	42543	Pushing	-	-	N	N	Y	II	T2N2Mx	1	1	-	0	0	-	+	HER2EN	PPG	IDC: NOS
55	43-50	F	MRM	UOQ	5.5x5x5	42509	Pushing	N	Y	Y	Y	N	II	T4N2aMx	2	2	+	5	3	+	EQ	HER2EN	PPG	Cribiform Ca
56	>60	F	MRM	LIQ	4x2.5x2	0/25	Infiltrating	N	N	N	N	N	III	T2N0Mx	0	0	-	0	0	-	+	HER2EN	MPG II	IDC: NOS