CORRELATION OF ER, PR AND HER-2/neu WITH HISTOLOGICAL VARIANTS OF BREAST CARCINOMA

Dissertation submitted in
Partial fulfillment of the regulations required for the award of

M.D. DEGREE
in
PATHOLOGY - BRANCH III

The Tamil Nadu

Dr. M.G.R. Medical University

Chennai
March -2010

DECLARATION

I, **Dr. R.D. Puvitha** solemnly declare that this dissertation entitled **"CORRELATION OF ER, PR AND HER-2/neu WITH HISTOLOGICAL VARIANTS OF BREAST CARCINOMA"** done by me at Coimbatore Medical College, Coimbatore during the period from May 2007 – June 2009 under the guidance and supervision of **DR. R. VIMALA, M.D.,** Professor and Head, Department of Pathology, Coimbatore Medical College, Coimbatore.

This dissertation is submitted to The Tamilnadu Dr.M.G.R. Medical University, Chennai towards the partial fulfillment of the requirement for the award of M.D. Degree in Pathology to be held in March 2010.

I have not submitted this dissertation on any previous occasion to any University for the award of any degree.

Place**:**

Date**:** **Dr. R.D. PUVITHA**

CERTIFICATE

This is to certify that the dissertation entitled **"CORRELATION OF ER, PR AND HER-2/neu WITH HISTOLOGICAL VARIANTS OF BREAST CARCINOMA"** is a record of bonafide work done by **DR. R.D. Puvitha**, Post graduate student in the Department of Pathology, Coimbatore under the supervision of **DR. R. VIMALA, M.D.,** Professor and Head, Department of Pathology, Coimbatore Medical College and submitted in partial fulfillment of the regulations of the Tamilnadu Dr.M.G.R. Medical University towards the award of M.D. Degree in Pathology.

Dr. V. KUMARAN M.S.M.Ch.,
Dean, Coimbatore Medical College
Coimbatore

Dr. R. VIMALA, M.D.
Professor & Head,
Department of Pathology
Coimbatore Medical College,
Coimbatore

ACKNOWLEDGEMENT

I would like to express my gratitude to **DR.V.KUMARAN M.S. M.Ch**., Dean, Coimbatore Medical College, Coimbatore for permitting me to carry out this study.

I express my sincere gratitude and heart felt thanks to my respected Professor and guide **DR.R.VIMALA, M.D**., Professor & Head, Department of Pathology, Coimbatore Medical College, Coimbatore for her expert guidance, encouragement and constructive criticism throughout this work.

I am grateful TO DR. M.MURTHY, M.D., Additional Professor and DR.C.LALITHA,M.D., Additional Professor, Department of Pathology for their constant support and encouragement throughout the course of this study.

I express my gratitude to all the teaching staff of the department for their tremendous support and valuable suggestions.

I am indebted to all the technical staff of the department for their immense help in conducting this study.

Also, I wish to record my thanks to my family and friends for the enduring patience and moral support throughout the study period.

CONTENTS

LIST OF TABLES

LIST OF CHARTS

LIST OF COLOUR PLATES

INTRODUCTION

Carcinoma afflicts all the communities worldwide. Breast carcinoma is a major health concern and a leading cause of death among women.[1] It causes 3, 76,000 deaths in a year worldwide and every year 9, 00,000 new cases are diagnosed[4]. Among the Indian women carcinoma of the breast and cervix account for 60% of total cases, of which breast carcinoma accounts for 10.4% [1]. A study conducted by WHO revealed that Chennai has the highest incidence among all leading centres in India accounting for 26/1, 00,000 women [7]. The mean age of occurrence is 42 years.[3, 4]

The approach to the management of breast carcinoma has undergone enormous changes over the last 20 years [13]. Today, the choice of conservative and reconstructive surgery is more popular than mastectomy. Such changes are accompanied by increasing range of systemic, hormonal and cytotoxic drugs used in both adjuvant and neoadjuvant settings [6, 13.] Prognosis and management of breast carcinoma are influenced by the classic variables such as histological type and grade, tumor size, lymph node status, Estrogen, Progesterone receptor status and HER-2/neu overexpression .[11]

Identification of biomarkers plays a paramount role in the treatment, management and prognosis of breast carcinoma. Determination of hormonal status is an important primary assessment at the time of diagnosis of breast carcinoma. Testing for Estrogen and Progesterone receptor status is critical to plan optimal treatment for breast cancer.[1, 2]

Estrogen receptor is a well established predictive and prognostic factor in breast cancer. Patients with ER-positive/PR-positive tumors have a better prognosis than patients with ER-negative/PR-negative tumors. Hormone receptor test is done routinely in these cases since hormone treatment has fewer side effects and it prevents recurrence in 25% of cases.[1]

HER2/neu status became clinically relevant with the demonstration that HER-2/neu

positive tumors have a worse prognosis than HER2/neu negative tumors.[2] It has been recognised that HER2/neu overexpression served as both a marker of aggressive disease and a target for treatment. HER2/neu status not only predicts poor outcome, but predicts sensitivity to treatment with Trastuzumab (Herceptin), a humanized monoclonal anti- HER2/neu antibody .[13, 14]

With these prognostic implications, the need for accurate and precise assessment of ER, PR, and HER2/neu expression in breast carcinoma is critical in the determination of patients appropriate for treatment with these drugs. Immunochemistry is an important tool in precise histopathological diagnosis. Immunohistochemistry (IHC) is the most commonly used method of testing for ER, PR, and HER2/neu status.[2]

Survival and response to hormone therapy are most favourable among women who are receptor positive, intermediate for tumors discordant on receptor status and least favourable for receptor negative patients. The interrelationship of Estrogen, Progesterone receptor status and HER-2/neu overexpression has an important role in management of breast carcinoma. It has been shown that patients with HER-2/neu overexpression do not respond to tamoxifen therapy, while they serve as a candidates for trastuzumab therapy.[3, 15, 18]

AIM OF THE STUDY

1. To statistically evaluate the occurrence of breast lesions in patients attending Comibatore Medical College hospital.
2. To evaluate the distribution of Estrogen, Progesterone receptors and HER-2/neu in breast malignancies by using immunohistochemical method.
3. To correlate the receptor expression in various histological types of breast carcinoma.
4. To find out the correlation of Estrogen, Progesterone receptors and HER-2/neu status with other possible variables.

REVIEW OF LITERATURE

INCIDENCE AND EPIDEMIOLOGY

Carcinoma afflicts all the communities worldwide. Approximately 10 million people are diagnosed with carcinoma and more than 6 million people die of the disease every year.[4] Breast carcinoma is the most common non-skin malignancy in women. It causes 3, 76,000 deaths in a year worldwide and every year 9, 00,000 new cases are diagnosed. [5, 6]

Among the Indian women cancer cervix and breast carcinoma accounts for 60% of total cases, of which breast carcinoma incidence is 10.4%. A study conducted by WHO [7] (1999) denotes that Chennai accounts for the highest incidence among all the leading centres in India (ie) 26/1, 00,000 women. The mean age of occurrence is 42 years. [4, 6]

There has been a sharp increase in the detection of breast carcinoma, owing to the widespread use of mammography. [3, 7] However the mortality from breast carcinoma is beginning to fall, presumably because of earlier diagnosis and improved therapy.[13]

RISK FACTORS

The frequency of the disease has prompted an intensive study of risk factors. It has been proposed that the common denominator for most of these factors is strong and prolonged estrogen stimulation operating in a genetically susceptible background. [4, 6,9,10,11]

1. Country of birth: Incidence of breast carcinoma is more in developed countries than in developing countries.
2. Family history: Women who have first degree relatives with breast carcinoma have a risk two to

three times that of the general population.

3. Menstrual and reproductive history: Increased risk is correlated with early menarche, nulliparity, age at first birth and late menopause owing to hyper estrogenic stimulation [9,10]
4. Breast biopsies: Increased risk is associated with prior biopsies showing atypical hyperplasia.
5. Race and socio economic group: Incidence of breast carcinoma is high among the high socio- economic group. The risk of developing invasive carcinoma within the next 20 years at age 50 is 1 in 26 for Asian / Pacific islanders.[11]

Additional risk factors as follows are also recognized, but there is a lack of definitive correlation. [4, 6,7, 9,10]

1. Estrogen exposure: Post menopausal hormone replacement therapy and use of oral contraceptive drugs are associated with increased risk of breast cancer. Oophorectomy reduces the risk of breast carcinoma by 75%.
2. Radiation exposure: Those who are exposed to therapeutic radiation and atom bomb survivors have a high incidence of breast cancer.
3. Carcinoma of contralateral breast and endometrium: Increased risk is associated with the above mentioned factors.
4. Geographic influence: Breast cancer incidence rates are increased about four times in the United States and Europe than in other countries.
5. Diet: High fat diet and obesity carry increased risk.
6. Exercise: Reduced risk is associated with premenopausal women who exercise regularly.
7. Breast feeding: The longer the women breast feed their children the greater is the reduction in the risk of breast carcinoma.

ETIOLOGY AND PATHOGENESIS [9,10]

The major risk factors for the development of breast carcinoma are hormonal and genetic (family history). Breast carcinoma can therefore be divided into 1) Sporadic breast carcinoma. 2) Hereditary breast carcinoma.

HEREDITARY BREAST CARCINOMA:

About 25% familial cancers can be attributed to two highly penetrant autosomal dominant genes BRCA1, and BRCA2, located in 17q21 and 13q12.3 respectively. BRCA1 and BRCA2 do not show sequence homology. They function on similar pathways and interact with multiprotein complexes. Both act as tumour suppressor genes that confers the risk of malignancy. Loss of these function leads to breast carcinoma perhaps due to intermittent proliferation of breast epithelium. BRCA1 interacts with Estrogen receptor and is involved in X- Chromosome activation. BRCA1 associated breast cancers are most common in medullary carcinoma (67%) and mucinous carcinoma (55%), BRCA2 mutation does not have a distinct morphologic appearance. [12, 13, 17]

SPORADIC BREAST CARCINOMA:

The major risk factors for sporadic breast cancer are related to hormone exposure, gender, age at menarche and menopause, reproductive history and exogenous estrogens. These carcinomas are associated with post menopausal women and they overexpresses the estrogen receptor. [10,12]

WHO HISTOLOGICAL CLASSIFICATION OF TUMOURS OF THE BREAST [17]

Epithelial tumours

Invasive ductal carcinoma, not otherwise specified

Mixed type carcinoma

Pleomorphic carcinoma

Carcinoma with osteoclastic giant cells

Carcinoma with choriocarcinomatous features

Carcinoma with melanotic features

Invasive lobular carcinoma

Tubular carcinoma

Invasive cribriform carcinoma

Medullary carcinoma

Mucinous carcinoma and other tumours with abundant mucin

Mucinous carcinoma

Cystadenocarcinoma and columnar cell mucinous carcinoma

Signet ring cell carcinoma

Neuroendocrine tumours

Solid neuroendocrine carcinoma

Atypical carcinoid tumour

Small Cell/oat cell carcinoma

Large cell neuroendocrine carcinoma

Invasive papillary carcinoma

Invasive micropapillary carcinoma

Apocrine carcinoma

Metaplastic carcinomas

Pure epithelial metaplastic carcinomas

Squamous cell carcinoma

Adenocarcinoma with spindle cell metaplasia

Adenosquamous carcinoma

- Mucoepidermoid carcinoma
- Mixed epithelial/mesenchymal metaplastic carcinomas

Lipid-rich carcinoma

Secretory carcinoma

Oncocytic carcinoma

Adenoid cystic carcinoma

Acinic cell carcinoma

Glycogen-rich clear cell carcinoma

Sebaceous carcinoma

Inflammatory carcinoma

Lobular neoplasia

- Lobular carcinoma in situ

Intraductal proliferative lesions

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

Microinvasive carcinoma

Intraductal papillary neoplasms

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

Benign epithelial proliferations

- Adenosis including variants
  - Sclerosing adenosis
  - Apocrine adenosis
  - Blunt duct adenosis
  - Microglandular adenosis
  - Adenomyoepithelial adenosis
- Radial scar / complex sclerosing lesion
- Adenomas
  - Tubular adenoma
  - Lactating adenoma
  - Apocrine adenoma
  - Pleomorphic adenoma
  - Ductal adenoma

Myoepithelial lesions

- Myoepitheliosis
- Adenomyoepithelial adenosis
- Adenomyoepithelioma
- Malignant myoepithelioma

Mesenchymal tumours

- Haemangioma
- Angiomatosis
- Haemangiopericytoma
- Pseudoangiomatous stromal hyperplasia
- Myofibroblastoma
- Fibromatosis (aggressive)
- Inflammatory myofibroblastic tumour

- Lipoma
- Angiolipoma
- Granular cell tumour
- Neurofibroma
- Schwannoma
- Angiosarcoma
- Liposarcoma
- Rhabdomyosarcoma
- Osteosarcoma
- Leiomyoma
- Leiomyosarcoma

Fibroepithelial tumours

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

Tumours of the nipple

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

Malignant lymphoma

Diffuse large B-cell lymphoma

Burkitt lymphoma

Extranodal marginal-zone B-cell lymphoma of MALT type

Follicular lymphoma

Metastatic tumours

Tumours of the male breast

Gynaecomastia

Carcinoma

Invasive

In situ

MICROSCOPIC TYPES OF BREAST CARCINOMA:

The two key determinations to make in the morphologic study of breast carcinoma are 1) whether the tumour is confined to the epithelial component of the organ (in situ carcinoma) or has invaded into the stroma (invasive carcinoma).

2) whether it is ductal or lobular type. [14,15,16]

INVASIVE CARCINOMA:

Tumours included in this category are all those in which stromal invasion is detectable, whether an insitu component is identifiable or not. Invasive tumours can be divided into two major categories: ductal type and lobular type. [14, 15]

INVASIVE DUCTAL CARCINOMA –NOS TYPE:

Rosen (1975) [18] accounts that this type constitutes 65-80% of mammary carcinomas. WHO

classification of Invasive ductal carcinoma -NOS type tumour is one of exclusion: "Invasive ductal carcinoma is most frequently encountered malignant tumour, not falling into any other categories of invasive mammary carcinoma".[17]

Microscopically WHO classification requires a non-specialized pattern in over 50% of the tumour area to be classified as NOS- type. Typically combinations of infiltrative margins, trabecular, diffuse sheet like, acinar and nested patterns are noted. Histological grading of the tumour depends on tubular formation, pleomorphism and mitotic count. They are graded into well differentiated, moderately differentiated and poorly differentiated grades. [14, 15]

Predictive value of Estrogen receptor is evaluated by quantitative immunohistochemical analysis in breast carcinoma.[19,23] Hormone receptor studies of IDC-NOS type shows 70-80% Estrogen, Progesterone positivity and 15-30% HER-2 / neu positivity [14,15,16].

INVASIVE LOBULAR CARCINOMA: (ILC)

Foote and Stewart (1946) [20] introduced the term Infiltrating Lobular carcinoma. Tavassoli (1992) [21] described that ILC of breast composed of small cells with linear growth pattern. Over 90% of the carcinoma should have a lobular morphology to be classified in this group. This type represents 4.9-15% of all invasive breast carcinomas. [22]

There is some evidence that increased risk of lobular carcinoma occur in postmenopausal women and it is frequently multicentric when compared with other sub-types. [16,22]

Microscopically the cell type characteristic of invasive lobular carcinoma is non-cohesive

with relatively round or oval eccentrically placed nuclei with small nucleoli and small amount of cytoplasm. The classical pattern of single file and targetoid arrangements were noted. [14,17]

Dixan J M (1985) [22] conducted receptor assay that reveals Estrogen, Progesterone receptor positivity in 67 -92% of cases, [14,15] E-Cadherin negative [15] and HER-2/neu negativity. [21]

MUCINOUS CARCINOMA:

In 1852 Robinson RR has described about gelatinous carcinoma of breast. The incidence ranges from 0.8 -6% probably reflecting the applied diagnostic criteria.[17] The tumor type is characterized by a mucinous morphology in over 90% of the tumour. WHO classification describes "large amount of extracellular mucin sufficient to be visible both grossly and microscopically surrounding the tumour cells". [17]

Diab SG (1999) [26] in his study concluded Estrogen receptor positivity in 73-95%, progesterone receptor positivity 79-84% and HER-2/neu negativity of mucinous carcinoma.

Mucinous carcinoma tends to be negative for HER-2/neu and for EGFR [24,25]

MEDULLARY CARCINOMA:

Geschicker C F (1945) described about high grade tumor as a different entity in breast carcinoma. Grossly the tumor appears as a well circumscribed mass and soft in consistency. [14] WHO defined as "well circumscribed carcinoma composed of poorly differentiated cells with scant stroma and prominent lymphocytic infiltration". Despite the high grade of tumor it carries relatively good prognosis. [17]

Immunohistochemical studies of Estrogen expression are found to be negative [14,27,28] and they also exhibit characteristic immunophenotype (i.e) p53 positive, HER-2 / neu negative [27]

NEUROENDOCRINE CARCINOMA;

In 1975 Coombes RC has described neuroendocrine carcinoma in his study. In 1977 Cubilla AL et al [30] described primary carcinoid tumor of breast in eight patients. WHO defines this type of carcinoma with neuroendocrine marker positivity noted in more than 50% of the cell population. [17] A study confirmed that 10-18% breast carcinoma showed evidence of neuroendocrine differentiation. This type has an infiltrative morphology with component cells arranged in nests, sheets or trabecular formation and peripheral palisading of cell groups. [14,15,17]

Immunohistochemical studies by LeeAK[31] show neuroendocrine marker positivity in 82% of the cases. However 67% show Estrogen receptor positivity and 56% exhibits Progesterone receptor positivity. [30, 32]

HER-2/neu overexpression is found to be negative in this type of tumour [32]

PAPILLARY CARCINOMA:

Foote and Stewart [20] (1946) defined it as "A rare carcinoma whose invasive pattern predominantly is in the form of papillary structures."

Estrogen, Progesterone receptor positivity and HER-2/neu negativity are characteristic of this tumour. [31,33]

Zekiglu.O.ErhanY et al (2004)[34] conducted immunohistochemical analysis of papillary carcinoma and interpreted receptor positivity in upto 89% of cases and HER-2/neu in 27 % of cases. [33,34]

CARCINOMA WITH METAPLASIA:

In 1984 Kaufman M.W [35] described special edition of mammary carcinoma with

pseudosarcomatous metaplasia. WHO (2003) publication defines metaplastic carcinoma breast as "a heterogeneous group of neoplasm with spindle cells, squamous cells or with mesenchymal differentiation" [17].

Oberman HA (1987) [36] et al found hormone receptors and HER-2/neu found to be negative. Wargotz ES et al (1989) [37] showed they are positive for keratin, EMA and S100 [15].

PROGNOSTIC AND PREDICTIVE FACTORS [9,10,12,17]

Prognosis is determined by the pathologic examination of the primary carcinoma and the axillary lymph nodes. Major prognostic factors are the strongest predictors of death from breast carcinoma and are incorporated into the American Joint committee cancer (AJCC) staging system. Predictive factors are used to determine the likelihood of response to a particular therapy. [12]

1. Invasive carcinoma or in situ disease:

By definition, in situ carcinoma is confined to the ductal system and cannot metastasize. In contrast, at least half of invasive carcinomas will have metastasized locally or distantly at the time of diagnosis**.** [15]

2. Distant metastases:

Once distant metastases are present, cure is unlikely, although long- term remissions and palliation can be achieved, especially for women with hormonally responsive tumors. Favoured sites for dissemination are the lungs, bones, liver, adrenals, brain and meninges. [6,13]

3. Lymph node metastases:

Axillary lymph node status is the most important prognostic factor for invasive carcinoma in

the absence of distant metastases. The sentinel node is highly predictive of the status of the remaining nodes. Sentinel node biopsy can spare women the increased morbidity associated with a complete axillary dissection. Macrometastases (>0.2 cm) are of proven to be prognostic importance. [9,15]

4. Tumor size:

The size of the carcinoma is the second most important prognostic factor and is independent from lymph node status. [16]

5. Locally advanced disease:

Tumors invading into skin or skeletal muscle are frequently associated with concurrent distant disease. [13]

6. Inflammatory carcinoma:

Women presenting with the clinical appearance of breast swelling and skin thickening have a particularly poor prognosis. [3]

MINOR PROGNOSTIC FACTORS:

In this group, minor prognostic factors can be used to decide among chemotherapy regimens and/or hormonal therapies. Three of these factors estrogen receptor, progesterone receptor, and HER2/neu-are most useful as predictive factors for response to specific therapeutic agents. [13,18]

1. Histologic subtypes:

The 30-year survival rate of women with special types of invasive carcinomas (tubular, mucinous, medullary, lobular, and papillary) is greater than 60% compared with less than 20% for women with cancers of no special type. [18]

2. Tumor grade:

The most commonly used grading system to assess the degree of tumor differentiation (Scraff Bloom Richardson) combines nuclear grade, tubule formation, and mitotic rate.

3. Estrogen and Progesterone Receptors:

Current assays use immunohistochemistry to detect the receptor status in the nucleus. [19] Women with hormone receptor-positive cancers have a slightly better prognosis than do women with hormone receptor-negative carcinomas. The evaluation of hormone receptors is most valuable to predict response to therapy. [39]

4. HER-2/neu:

HER2 (human epidermal growth factor receptor 2) or c-erb B2 is a transmembrane glycoprotein involved in cell growth control. HER2/neu is overexpressed in 20% to 30% of breast carcinomas. Overexpression of HER-2/neu is associated with a poor prognosis. Transtuzumab is a humanized monoclonal antibody to HER-2/neu developed to specifically target tumor cells and it is hoped to spare normal cells. [24]

5. Lymphovascular invasion (LVI):

Tumor cells may be seen within vascular spaces (either in lymphatic or in small capillaries surrounding tumours). This finding is strongly associated with the presence of lymph node metastasis and is a poor prognostic factor. [38]

6. Proliferative rate:

Proliferation can be measured by flow cytometry (as the S-phase fraction), by thymidine labelling index, by mitotic counts, or by immunohistochemical detection of cellular proteins (e.g.,cyclins, Ki-67) produced during the cell cycle. Tumours with high proliferation rates have the worst prognosis. [12]

7. DNA content:

The amount of DNA per tumour cell can be determined by flow cytometric analysis or by image analysis of tissue sections. Aneuploid tumours are those with abnormal DNA indices and have a slightly worse prognosis.

Despite the numerous prognostic indicators currently in use or under investigation, it is impossible in an individual case to predict the outcome. [14]For this reason, there are continuing searches for better or more refined biologic markers of prognosis and more effective treatment modalities. Current therapeutic approaches include local and regional control using combination of surgery (mastectomy or breast conservation) and postoperative radiation and systemic control using hormonal treatment or chemotherapy or both. Newer therapeutic strategies include inhibition (by pharmacologic agents or specific antibodies) of membrane-bound growth factor receptors (e.g., HER-2/neu), stromal proteases, and angiogenesis. [1,2]

IMMUNOHISTOCHEMISTRY (IHC)

Immunohistochemistry has over the years evolved into a revolutionary diagnostic tool for the pathologist. The necessity for some special training method can be recognized from the fact that surgical pathology is a subjective discipline. Despite the presence of various diagnostic criteria, there is overlapping among different entities and dissimilarities among the same entity. This when compounded with subjective disparity among the pathologists, reproducibility of diagnosis become difficult.[40]

This prompted the development of special staining technique to stain cells of particular lineage, marking the beginning of histochemistry in the mid nineteenth century. Francois Vincent Raspard was the first botanist to use immunochemistry. Advent of aniline dyes revolutionized immunochemistry from 1862 to 1929. [41]

With the development of immunology, a new method of staining was developed incorporating immunologic techniques. Here antibodies labelled with special stains are used to identify special

antigen in the tissue. The antigen –antibody reaction imparts a particular colour to the cells.

Immunochemistry is an important adjuvant method in histopathologic diagnosis. The origin of Immunohistochemistry techniques lies in the pioneering work of Albert Coons, starting in 1941. He described his first attempts to label antibodies directly with fluorescent isocyanate. Later the indirect technique was introduced by Nakane and Pierce in 1966, in that an unlabeled antibody is followed by second antibody or substrate. Various stages of development of Immunohistochemistry from peroxidase –antiperoxidase (1970), Alkaline phosphatase labelling (1971), Avidin-biotin technique (1977, 1979) and to two layer dextrin polymer technique (1993) carries both advantages and disadvantages for each techniques. [5]

DETECTION SYSTEM:

Subsequent to the development of specific antibodies to the antigens, next step for the immunochemist was to develop techniques to visualize the antigen-antibody reaction complex.

Two methods employed for this:

1) Direct method
2) Indirect method

DIRECT METHOD:

In this the primary antibody is conjugated directly to the label. Most popular direct conjugates are those which are labelled with a fluorochrome, horse radish peroxidase and alkaline phosphatase. The advantage of this method is that it is simple to use as it requires one application of reagent followed by appropriate chromogen substrate solution. [5]

INDIRECT METHOD:

The sensitivity of immunochemical stains was significantly improved with the development of an indirect technique [55]. This is a two step method in which labelled secondary antibody reacts with the

antigen bound to primary antibody. Further increase in sensitivity over the indirect technique was achieved with the introduction of peroxidase enzyme complex. Subsequent development exploited the strong affinity of Avidin for Biotin and resulted in Avidin- Biotin Complex method. [5]

CURRENT TECHNIQUES:

Enhanced polymer one step staining method (Epos) is a new direct technique reported by Pluzek et al in 1993.[42] In this, a large number of primary antibody molecules and peroxidase enzymes are attached to a dextran polymer "back bone". This method is rapid, can be used for frozen sections and is sensitive enough to demonstrate small amounts of antigen.

Dextran polymer conjugate –two step visualization system is a new indirect system based on dextran technology employed in the Epos system. This method offers greater sensitivity than the traditional indirect system. It is less time consuming than the three stage Avidin –Biotin system and does not read with endogenous biotin [5].

ANTIGEN RETRIEVAL IN IMMUNOHISTOCHEMISTRY (AR-IHC)

The following technique can retrieve many masked antigen in routinely processed tissue.

1. Proteolytic enzyme digestion.
2. Microwave antigen retrieval.
3. Pressure cooker antigen retrieval.
4. Microwave and trypsin antigen retrieval.

PROTEOLYTIC ENZYME DIGESTION:

Pre-treating formalin-fixed, routinely processed paraffin sections with proteolytic enzymes to unmask certain antigenic determinants was described by Huang et al (1976).[44] Before the advent of heat pretreatment, proteolytic digestion was an essential requirement in antigen retrieval. The most commonly used enzymes are trypsin and protease. Enzyme digestion somehow breaks down formalin

cross-linking and unmasks the antigenic sites. It still has an important place in demonstrating immunoglobulins and complements in renal biopsies, but it has limited value due to problems like over digestion, under digestion and antigen destruction.

MICROWAVE ANTIGEN RETRIEVAL:

This is a relatively new and revolutionary technique. Microwave oven heating retrieves many antigens thought previously to be either lost or destroyed by routine histological processing techniques. It involves the boiling of cleaned formalin –fixed paraffin sections in various solutions and allows rapid and uniform heating. Antibodies and such as the proliferation markers anti-Ki67 and MIB-I which are unstable in frozen section work well after heat pre-treatment on paraffin wax sections[5].

PRESSURE COOKER ANTIGEN RETREIVAL:

Replacing the microwave oven with the Pressure cooker has proved to have some advantages. Microwaving of larger numbers of slides using bigger containers does suffer from inconsistencies. Miller et al[45] in 1995 compared and proved that pressure–cooking method does not suffer from such inconsistencies; also it is less- time consuming.

PIT FALLS OF HEAT PRE-TREATMENT:

Care should be taken not to allow the sections to dry after heating, as this destroys antigenicity. Damage of nuclear details is seen in poorly fixed tissue. Fibres and fatty tissues tend to detach from the slides while heating. Not all antigens are retrieved by heat pretreatment and also some antigens show altered staining pattern with some primary antibodies eg PGP9.5. [46]

USES OF IMMUNOHISTTOCHEMISTRY IN BREAST PATHOLOGY [5,43]

- Assessment of Estrogen and Progesterone status by using specific antibodies to the receptor proteins.

- Assessment of HER-2/neu protein overexpression by using specific antibodies to the HER-/2 neu protein
- Distinguishing in situ from invasive carcinoma by using antibodies to myoepithelial markers (e g. Actins, calponin, smooth muscle myosin heavy chain, $_{P}63$) and basement membrane proteins (eg type IV collagen, laminin)
- Assessment of metastatic lesions of possible breast origin by using antibodies to ER, GCDFP, CK 7/ CK 20 and other marker.
- Evaluation of spindle cell lesions (metaplastic carcinoma Vs mesenchymal lesions).
- Distinguishing ductal from lobular in situ carcinoma by using antibodies to E- cadherin.

HORMONE RECEPTORS

In 1950s Elwood V Jensen [47] identified Estrogen Receptor, further in 1996 Kuiper identified Estrogen receptor β gene. [48] Estrogen and Progesterone receptors are localized in the nuclei of approximately 7% of epithelial cells of normal breast tissue. They are found in higher proportion in the lobular than the ductal cells. [48] Shoker at al [49] reported the tendency of contiguous Estrogen receptor positive pattern of valuable size in lobular unit. Variation in expression during menstrual period was also reported. [50]

Estrogen and Progesterone receptors belong to super family protein. They are nuclear transcription factors that are involved in breast development, growth, differentiation and tumorigenesis [5,48]. Estrogen receptor regulates the expression of other genes such as Progesterone & bcl2 [48]. Thus Progesterone receptor is an indicator for intact Estrogen receptor functional pathway. There are two

forms of Estrogen receptor referred to as Estrogen receptor alpha & Estrogen receptor beta encoded by 6p25.1 and 14q respectively. [51] Estrogen receptor alpha is found in endometrium, breast cancer cells, ovarian stroma and hypothalamus. Estrogen receptor beta distribution is seen in kidney, brain, bone, heart and lungs [48,49].

Walker D (1999) described that Estrogen receptors are regarded as cytoplasmic receptor in unliganded state. Since they are steroid receptors, they do not require membrane bound receptors for their activation. During activation Estrogen receptor rapidly diffuses into the cytoplasm, it migrates from cytosol to nucleus, then dimerisation of the receptor occurs and subsequently it binds into HREs (Hormone Response Elements). The DNA – receptor complex activates MAPK/P13K pathway and induces cell proliferation. Two hypotheses have been proposed for its action. One hypotheses states that Estrogen receptor induces transcription activity by alternative RNA splicing of Estrogen receptor alpha subunit thereby inducing rapid uncontrolled proliferation and accumulation of genetic mutation. The other hypotheses states that it acts by producing by genomic waste. [48,51]

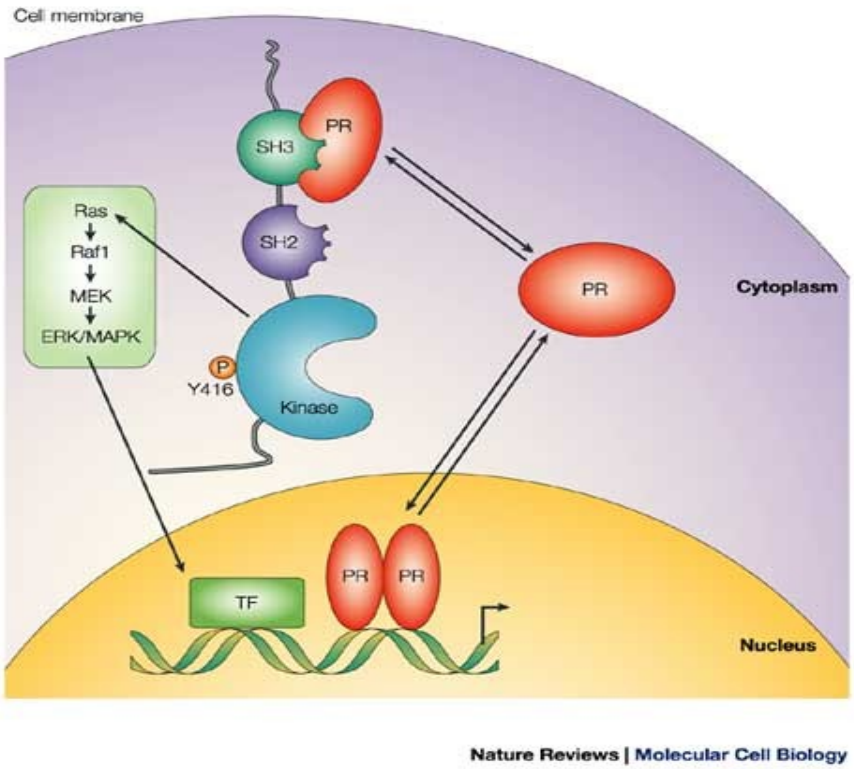
Hormone receptors are well established biomarkers in breast carcinoma and their assessment helps in predicting the response to endocrine therapy. [52, 53,54] Receptors can be assessed by either Ligand Binding Assay (LBA) on frozen sections or by immunohistochemistry on paraffin sections. [23] No laboratories offer LBA, but immunohistochemistry method is done worldwide. This assay is found to be superior to LBA. [55]

PROGESTRONE RECEPTOR :

Progesterone receptor is an intracellular steroid receptor that specifically binds to progesterone. Progesterone receptor is encoded by a single gene PGR 11q22 and regulated by Estrogen receptor. [49] Estrogen receptor is always necessary to induce Progesterone receptor. Binding of hormone to the receptor induces structural changes whereby inducing cell proliferation. Estrogen and Progesterone

receptor share a common structural and functional organization. WHO consensus development conference (1980) found that Progesterone receptor expression is a predictive marker. Hence it is estrogen regulated; most Progesterone receptor positive carcinomas are Estrogen receptor positive also. [54,56]

Clarks et al[57] (1988) demonstrated Progesterone receptor by immunohisto- chemistry in formalin fixed paraffin embedded section. It is seen visually in the same cells which are positive for Estrogen receptor. Scoring system is the same as adopted for Estrogen receptor [56]



SCORING SYSTEM:

Estrogen and Progesterone receptors express nuclear positivity. They are scored by the proportion of tumour cells showing positivity and intensity of the reaction. Both are summated to give a total score.

1. H – SCORE SYSTEM:

Goulding et al[58] (1995) used a semiquantitative method. It is based on the proportion of tumour cells showing different degree of reactivity. A score of 0-3 was given based on the following expression.

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

Total score comes about 0-300.Using H-score the positive score defined 51-300 and for negative upto 50 or less. The main disadvantage of this score is that it is time consuming and hence impractical for most pathologists.[81]

2. QUICK SCORE SYSTEM:

Barnes et al[59] (1998) used another scoring method called **Quick score** .A score for the proportion of stained cells and intensity of staining as follows:-

Intensity of Staining **Proportion of Staining**

This comes to a maximum score of 8. Score of more than 2 is considered as positive. [71] Advantage of this score is that it correlates into probability of response to endocrine therapy. [5]

3. Allred [60] scoring system followed by Nidal et al [61], Leake et al. [62]

The Intensity score, scored as mentioned above and the Proportion score as follows.

0	No positive cells
1	1-25% of positive cells
2	26-50% of positive cells
3	51-75% of positive cells
4	76-100% of positive cells

This gives total score about 0-7.The lower cut-off of less than 10% cells is considered to be positive. [61]

HER-2 /neu RECEPTORS

Her-2/neu (c-erb B -2) is an oncogene that encodes a transmembrane glycoprotein with tyrosinekinase activity. The proto-oncogene is encoded with 17q 11.2 –q12. [24] HER-2/neu belongs to EGFR family [63] Overexpression in breast carcinoma leads to recurrence and worst prognosis [24,25] It can be assayed by Florescent In situ Hybridization (FISH) for gene amplification and Immunohistochemical method for protein over expression. In over 90% gene amplification is associated with protein expression. Food and Drug Administration approves two tests, one is Hercep test for immunohistochemistry and another is Pathvysion for FISH. [54.] When compared to each other immunohistochemistry is relatively easy to perform and is less expensive. [63,64]

If the immunotest gives 1+ or 2+ it can be confirmed with FISH. Reliability for both the tests depends on laboratory experience [54,64]

SCORING SYSTEM [65]

Staining pattern	score	HER-2/neu expression
No staining or membrane staining in <10% of cells	0	Negative
A faint staining only a part of membrane > 10 % of cells	1+	Negative
A weak to moderate complete staining in > 10% tumour cells	2+	Weakly positive
A strong complete membrane staining > 10% tumour cells	3+	Strongly positive

BENEFITS OF ESTROGEN AND PROGESTERONE RECEPTOR ASSESSMENT IN BREAST CARCINOMA :[52,53,54]

1. Estrogen and Progesterone receptors are weak prognostic factors but strong predictive factors for tumor behaviour in breast carcinoma.
2. Estrogen and Progesterone receptor positive tumours are benefited by hormone therapy in 50-60% of cases.
3. Estrogen and Progesterone receptor positive tumours are associated with reduced risk of recurrence and mortality.
4. Estrogen and Progesterone receptor negative tumors are not found to respond to endocrine therapy.

MATERIAL AND METHODS

This is a descriptive study of cases of breast carcinoma during the period of May 2007 – June 2009 in the Department of Pathology, Coimbatore Medical College, Coimbatore. Three hundred and sixty eight breast specimens have been received in our department. during this period, out of this 233 cases are proven to be malignant.

A detailed history regarding age, parity, socio-economic status, family history, menstrual history, lactational history, and previous biopsy reports are reviewed in all cases. A detailed history regarding neo-adjuvant therapy is also noted.

Patients with unilateral presentation and newly diagnosed patients with no previous neo-adjuvant therapy are included in the present study. Patients with bilateral breast malignancies, other associated malignancies and those who have undergone neo-adjuvant therapy are excluded from the study.

All the mastectomy specimens received are properly sliced and fixed in 10% formalin for about 16-18 hours. Detailed gross examination pertaining to overall size of the specimen, appearance of skin with measurements of scars or incisions, appearance of nipple and areola, margins, nodal status are carefully noted. The specimens are cut into thin slices and examined .The tumor size, consistency and distance from cut margins are noted.

The representative sections are taken from the tumor, nipple, cut margins, deep margins and palpable lymph nodes. The tissue slices are processed in various grades of alcohol and xylol and subsequently embedded in paraffin. Paraffin sections of 4 µm thickness are subjected to haemotoxylin and eosin staining [24]. Histological assessment of tumor grade is done by Modified Richardson – Bloom Scoring system (Annexure -II). Nodal status and margins involvement are recorded in each case.

IMMUNOHISTOCHEMICAL ANALYSIS OF HORMONE RECEPTOR:

Immunohistochemical analysis of hormone receptor assay is done in paraffin embedded tissue blocks, by using the Supersensitive Polymer HRP system based on non-biotin polymeric technology that makes use of two major components, Super enhancer and poly- HRP reagent. The retrieved antigen is bound to primary antibody and then detected by the addition of secondary antibody conjugated with horse radish peroxidase polymer and DAB substrate. The score was calculated after adequate color development which can be more readily visualized under a light microscope.

The step by step procedure is included in Annexure III.

SCORING SYSTEM:

Immunohistochemically stained slides are evaluated for the presence of reaction, cellular localization (nuclear or cytoplasm), pattern of staining (focal or diffuse) and intensity of reaction in individual tumor cells (Strong or weak). Scoring for Estrogen &Progesterone receptors is done by using Quick score system [59] and for HER-2/neu according to the guideline published by Ellis et al.[65]

The statistical analysis is performed with Statistical Package For Social Science(SPSS) software version 11.The Pearson chi-square test is used to compare the possible correlation studies like Estrogen, Progesterone receptors and HER-2/neu with tumor size, nodal status, histological variants and grades.

The study is conducted after obtaining the ethical approval from the Ethical Review Committee of Coimbatore Medical College, Coimbatore.

OBSERVATION AND RESULTS

INCIDENCE OF BREAST TUMORS

In the two year study period from May 2007 – June 2009, there were 27,638 specimens received in our department. Of these, breast tumors accounted for 368 cases, the percentage being 9.4%.

The number of benign and malignant breast tumors were 135 and 233 respectively. Thus, the distribution of benign breast tumors was 36.65% and that of malignant breast tumors was 63.35%.

Table I shows the year wise distribution of breast tumors. During the study period of 2007-2008, benign tumors were 46 and malignant tumors were 72. Subsequently in the year 2008-2009, benign tumors were 89 and malignant tumors were 161.

TABLE1. DISTRIBUTION OF BENIGN AND MALIGNANT BREAST TUMORS.

S.No.	Year	Benign tumors	Malignant tumors
1.	2007 – 2008	46	72
2.	2008 – 2009	89	161
	Total no of cases	**135**	**233**

TABLE 2. AGE WISE DISTRIBUTION OF THE TUMORS.

	Age group	Benign tumors	Malignant tumors

S.No.	(In years)		
1.	Less than 20 Yrs	24	-
2.	21-30 Yrs	58(57%)	12
3.	31-40 Yrs	30	32
4.	41-50 Yrs	14	105 (63.33%)
5.	51-60 Yrs	7	46
6.	60 and above	2	38
	Total No. of cases	**135**	**233**

Table 2 shows the distribution of breast tumors according to age. Benign tumors had a peak incidence in the age group 21-30 years, where as the malignant tumors had a peak incidence in the age group 41-50 years.

TABLE 3. DISTRIBUTION OF HISTOLOGICAL VARIANTS IN BREAST CARCINOMA

S.No.	Histological Variants	No. of Cases	Percentage
1.	Invasive Ductal Carcinoma - NOS type	26	79%
2.	Invasive Lobular Carcinoma	1	3%
3.	Mucinous Carcinoma	2	6%

4.	Papillary Carcinoma	1	3%
5.	Metaplastic Carcinoma	1	3%
6.	Neuroendocrine Carcinoma	1	3%
7.	Medullary Carcinoma	1	3%
	Total No. of Cases	**33**	**100%**

Table 3 shows the distribution of histological variants in breast carcinoma. Among the 33 cases, 26 (79%) were Invasive Ductal Carcinoma NOS type, 2 (6%) were Mucinous carcinoma and one each in other special sub – types including Lobular carcinoma, Papillary carcinoma, Metaplastic carcinoma, and Neuroendocrine carcinoma.

TABLE 4.DISTRIBUTION OF HISTOLOGICAL GRADE IN INVASIVE DUCTAL CARCINOMA NOS TYPE

S.No.	Histological Grade	No. of Cases	Percentage
1.	Grade I	1	4%
2.	Grade II	23	88.5%
3.	Grade III	2	7.6%
	Total No. of Cases	**26**	**100%**

Table 4 shows the distribution of histological grading in breast carcinoma according to Modified Bloom-Richardson scoring system (Annexure II).Only 26 cases were included for grading, in that 23 cases (88.4%) were in grade II, 2 cases (7.6%) were in grade III and one case (4%) in grade I.

TABLE 5.SCORING SYSTEM FOR HORMONAL RECEPTORS

Of the total 233 cases in the study period, cases were analyzed on the basis of inclusion and exclusion criteria. Among these, 33 specimens were randomly selected for immunohistochemical analysis. All the cases were processed, immunohistochemically analyzed and carefully examined under the light microscope. Dark brown colour of the nuclei was interpreted as positive score for Estrogen and Progesterone receptor. For scoring Quick score system was adopted. A total score of more than 2 was considered as positive.

Dark brown membrane positivity was scored for HER -2/neu. Intensity of staining, percentage of cells positivity were included and scored. A score of complete membrane positivity in >10% of the tumor cells were considered as positive.

ER, PR, HER-2/neu SCORING TABLE

S.No	HPE NO	TYPE OF CARCINOMA	ER			PR			HER-2 /neu
			PS	IS	TS	PS	IS	TS	
1	297/08	IDC-NOS GrII	2	0	2	1	1	2	3+
2	553/08	IDC-NOS GrII	5	3	8	5	3	8	2+
3	587/08	IDC-NOS GrII	1	1	2	1	1	2	3+
4	594/08	IDC-NOS GrII	1	0	2	1	1	1	3+
5	819/08	IDC-NOS GrII	1	1	2	1	1	2	1+
6	836/08	IDC-NOS GrII	1	0	1	2	1	3	3+
7	840/08	IDC-NOS GrII	1	0	1	3	3	6	3+
8	841/08	IDC-NOS GrII	0	1	1	2	2	4	3+
9	1068/08	IDC-NOS GrII	2	1	3	1	1	2	3+
10	1114/08	IDC-NOS GrII	4	2	6	1	3	4	1+
11	1148/08	IDC-NOS GrII	1	0	1	2	1	3	1+
12	1183/08	Mucinous Ca	4	3	7	3	2	5	1+
13	1196/08	IDC-NOS GrII	1	1	2	3	2	5	1+
14	1215/08	Metaplastic Ca	0	1	1	0	1	1	1+
15	1227/08	IDC-NOS GrII	1	0	1	0	1	1	3+
16	1265/08	IDC-NOS GrII	1	0	1	3	2	5	2+
17	1292/08	IDC-NOS GrI	0	0	0	3	2	5	1+
18	1325/08	IDC-NOS GrII	2	1	3	3	3	6	2+
19	1332/08	IDC-NOS GrIII	1	1	2	1	1	2	3+
20	1429/08	IDC-NOS GrII	1	0	1	1	0	1	3+
21	1445/08	IDC-NOS GrII	5	2	7	1	1	2	2+
22	2672/08	IDC-NOS GrII	1	0	1	1	0	1	3+

23	2729/08	IDC-NOS GrII	**4**	**3**	**7**	**3**	**2**	**5**	**2+**
24	2834/08	IDC-NOS GrII	**4**	**2**	**6**	**4**	**2**	**6**	**3+**
25	33/09	IDC-NOS GrII	**2**	**2**	**4**	**1**	**1**	**2**	**3+**
26	34/09	Neuroendocrine	4	**3**	**7**	**4**	**3**	**7**	**2+**
27	82/09	IDC-NOS GrII	**1**	**1**	**2**	**1**	**1**	**2**	**3+**
28	105/09	IDC-NOS GrII	**1**	**0**	**2**	**0**	**0**	**0**	**3+**
29	134/09	IDC-NOS GrII	**5**	**3**	**8**	**2**	**1**	**3**	**3+**
30	157/09	Mucinous Ca	**4**	**3**	**7**	**4**	**2**	**6**	**1+**
31	834/09	Papillary Ca	**3**	**3**	**6**	**4**	**2**	**6**	**1+**
32	979/09	Lobular Ca	**1**	**0**	**1**	**1**	**0**	**1**	**1+**
33	1174/09	Medullary Ca	**2**	**1**	**3**	**2**	**0**	**2**	**2+**

PS - Proportion Score

IS - Intensity of Staining

TS - Total Score

TABLE 6. CORRELATION OF ESTROGEN RECEPTOR WITH PROGESTERONE RECEPTOR

S.No.	Group	No. of Cases	Percentage
1.	ER + / PR +	8	24.24%
2.	ER - / PR +	6	18.18%
3.	ER + / PR -	3	9.10%
4.	ER - / PR -	16	48.48%
	Total No of cases	**33**	**100%**

Table 6. shows correlation between Estrogen receptors and Progesterone receptors. Among the 33 cases, 8 cases were positive for both estrogen and progesterone receptors, 3 were positive for Estrogen receptor, 6 were positive for progesterone receptor, while 16 were negative for both receptors.

Estrogen receptor, progesterone receptor or both were positive in (17cases) 51.52 % where as Estrogen receptor, progesterone receptor or both were negative in (16cases) 48.48%

TABLE 7. EXPRESSION OF HER-2/neu IN BREAST CARCINOMA

S.no	HER-2/neu expression		Total No. of cases	Percentage %
1.	Positive	14	33	42.42%
2.	Negative	19		57.58%

Table 7. shows HER-2/neu overexpression in 33 breast carcinoma patients, among them 14 cases (42.42%) were found to be positive, while 19 cases (57.57%) were found to be negative.

TABLE 8. CORRELATION OF TUMOUR SIZE WITH HORMONE RECEPTORS.

S.No.	Tumor Size	Total	ER / PR Positive	Percentage %
1.	T_1 < 2 cm	3	2	66%
2.	T_2 2- 5 cm	12	9	75%
3.	T_3 >5 cm	18	6	33%
	Total No. of Cases	**33**	**17**	

Table 8. shows correlation of hormone receptors with tumor size. Estrogen receptor and Progesterone receptor positivity was noted in 66% of T_1 sized tumors, 75% of T_2 sized tumors, and 33% of T_3 sized tumors. The receptor status was found to be comparatively reduced in larger sized tumors as depicted.

The correlation of hormone receptors with tumor size was statistically analyzed and found to be significant (p=0.003).

TABLE 9.CORRELATION OF HER-2/neu WITH TUMOR SIZE

S.NO	Tumor size	HER-2/neu positive	Total cases	PERCENTAGE %
1.	<2cm	2	2	100%
2.	2-5cm	8	12	66.66%
3.	>5cm	9	19	47.3%

Table 9. shows correlation of HER-2 /neu with tumor size. HER-2/neu overexpression was noted in 100% of T_1 sized tumors, 66.66% in T_2 sized tumors and 47.3% in T_3 sized tumors. There was no significant correlation between the tumor size and oncoprotein expression. This was expressed consistently in tumors of all size.

TABLE 10. CORRELATION OF RECEPTOR STATUS WITH HISTOLOGICAL GRADING

S.NO	Histological grade	Total No. of cases	ER/PR positive	Percentage
1.	Grade I	1	1	100%
2	Grade II	23	14	60.8%
3.	Grade III	2	0	0%

Table12. shows correlation of hormone receptors with tumor grade, Estrogen, Progesterone receptor positivity was seen 100% in grade I tumors, 60% in grade II tumors and none in grade III tumors. High histological grade tumors showed low expression of receptors.

TABLE 11.CORRELATION OF HER-2/neu WITH HISTOLOGICAL GRADING

S.No	Histological grade	HER-2/neu positive	Percentage
1.	Grade I(1case)	0	0
2.	Grade II(23 cases)	13	56.5%
	Grade III(2cases)	2	100%

3.			

Table 13. shows correlation of HER-2/neu with histological grade. It was found that HER-2/neu overexpression was not seen in grade I tumors, where as it was expressed in 56.5% of grade II tumors and 100% of grade III tumors.

Statistical analysis was done and found to be significant (p=0.001).

TABLE 12.CORRELATION OF RECEPTOR STATUS WITH NODAL STATUS

S.NO	Nodal metastasis	ER/PR Positive	Percentage
1.	Present (15 cases)	5 Cases	33.3%
2.	Negative (18 cases)	10 Cases	55.5%

Table10. shows correlation of hormone receptors with nodal status. Out of 33 patients 15 had nodal metastasis, among whom 5 showed receptor positivity. Out of 18 nodal negative patients, the receptor status was positive in 10 patients. This explains higher receptor expression in nodal negative patients.

Statistical analysis was done and found to be significant (p=0.001)

TABLE 13.CORRELATION OF HER-2/neu WITH NODAL STATUS

S.NO	Nodal metastasis	HER-2/neu Positive	Percentage
1.	Present (15 cases)	10 cases	66.6%

2.	Negative (18 cases)	2 cases	11.1%

Table11. shows correlation of HER-2/neu with nodal status. Out of 33 patients, HER-2/neu overexpression was seen in 66.66% of nodal positive as opposed to 11.1% of nodal negative patients. Statistical analysis was done and found to be significant (p=0.001)

TABLE 14.CORRELATION OF RECEPTORS WITH ONCO PROTEIN EXPRESSION

ER/PR Status	**HER-2 / neu**		**Total No. of Cases**
	Positive	**Negative**	
Positive	2	15	17
Negative	12	4	16
Total No. of Cases	14	19	33

Table14. shows an inverse relationship of Estrogen , Progesterone receptor status with HER-2 / neu status.

Statistical analysis was performed with the SPSS version 11. and found to be significant (p=0.001).

DISCUSSION

Incidence and Age of Occurrence:

In the Indian scenario, breast carcinoma and cervical carcinoma account for about 60% of malignancies in women, the incidence of breast cancer alone being 10.4% [4].A study conducted by WHO [7] indicates that Chennai recorded the highest number of cases in India, the incidence being 26/1,00,000 women. The age of occurrence is between 41-50years. [7]

In concurrance with the above mentioned studies, the current study also revealed that the distribution of disease was about 9.4%. The age of peak occurrence of malignancy was 41–50 years group.

Table 15. Comparative analysis of Distribution of Histological variants of Breast carcinoma.

Histological types	Dixon JM etal	Omar Hameed	Current study
I DC – NOS type	79%	>70%	79%
Lobular carcinoma	10%	5 – 15%	3%
Mucinous carcinoma	2%	1-5%	6%
Medullary carcinoma	2%	1-7%	3%
Papillary carcinoma	1%	2%	3%
Solid neuroendocrine ca	< 1%	rare	3%
Metaplastic carcinoma	_	2-5%	3%

Table15. shows the comparative analysis of the distribution of histological variants of breast carcinoma. The incidence encountered by Dixon et al[22] and Omar Hameed et al [66] were comparable

with present study. The most common histological type in accordance with the other results was Invasive ductal carcinoma – NOS type accounting for 79%.

The identifiable histological variants are as follows:

1**. Invasive ductal carcinoma –NOS type:**

This comprises the largest group of malignant tumors accounting to 65-80% of breast carcinoma [14,15.] Our study shows 79% distribution.

Grossly the tumor size varied from 2.7cm to 18.5cm. Cut section of most of the tumor showed gray white, ill-defined tumor mass, firm to hard in consistency. (Fig.1) Occasionally tumor showed 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. (Fig.2) About Fifty percent of the cases (15/33) showed nodal metastasis. Most of the tumors were found to be in histological grade II. Resected margin involvement was seen in two patients and was confirmed by microscopic examination. None of these patients had either skin involvement or Paget's disease.

2. **Invasive Lobular carcinoma**:

Foot and Stewart [20] recorded an incidence of 4.9 -12% in post menopausal age group. In the present study this variant represented 3% of occurrence. But in contrast to Foot and Stewart [20] the age of occurrence was 35 years. Grossly the tumor was gray white, firm measuring 6cms in diameter (Fig.3). Microscopically "Indian file" pattern was noted (Fig.4). The cells were small to medium sized with uniform, hyperchromatic nuclei, and mild nuclear pleomorphism. Microscopically, metastasis was found in 23 lymph nodes.

3**. Mucinous carcinoma**:

In Omar Hameed et al[66] study incidence of Mucinous carcinomas was 5-15% and the mean age of occurrence was 58-68 years. The present study data represents 6% of distribution and the age of occurrence was 63years. This is parallel with above mentioned study.

Grossly the tumor mass was about 6cm -10cm, well circumscribed with gelatinous appearance. (Fig.5) On cut section soft, gel like material was noted. Microscopically clusters of bland appearing epithelial cells with abundant extracellular mucin were noted (Fig.6).

4**. Medullary Carcinoma**:

Omar Hameed[66] reported the incidence of Medullary carcinoma as 1-7%, but Dixon et al[22] showed relatively low incidence of this type approximately 2%. Current data represents 6% of distribution which is in comparable with Omar Hameed.

Grossly the tumor was about 5.5cm, well circumscribed, gray white, soft in consistency with foci of necrosis and hemorrhage (Fig.7). The WHO book describes the classical microscopic finding as follows (Fig.8).[17]

1) Syncytial growth pattern in >75% of the tumor.
2) Absence of glandular structure
3) Diffuse lymphoplasmacytic infiltration.
4) Microscopic circumscription: in that edge of the tumor should have a smooth rounded contour.
5) Poorly differentiated nuclear grade with high mitotic index.

5. Papillary carcinoma:

This tumor type accounts for 2% of all invasive carcinomas. It is most frequently encountered in post menopausal women.[22,66] In the current study the age of occurrence was 68 years and represents 3% of distribution. This is in parallel with the study of Dixon et al.[22]

Grossly the tumor measured about 3cm, appears well circumscribed, pale white in color with granular appearance and was easily detached from the surrounding tissue (Fig.9).Microscopic pathology revealed predominantly papillary structure with fibro-vascular core and lined by multilayered epithelial cells. In high power (40X) examination the absence of myoepithelial cells was confirmed. Invasion of papillary structure into the surrounding stroma with stromal reaction was noted (Fig.10).

6**. Metaplastic carcinoma:**

A very rare entity was included in our study. This represents 3% of distribution. Grossly the tumor was an ill defined gray white mass, firm to hard in consistency and was measuring about 19cms (Fig.11). Microscopically chondroid differentiation was noted predominantly. There was an abrupt transition from intraductal carcinoma to metaplastic component. Focal areas show spindle cell morphology (fig.12).

7**. Neuroendocrine carcinoma:**

In Omar Hameed et al[66] study incidence of Neuroendocrine carcinoma was 2-5%. The present study data showed 3% distribution, which is comparable with the above study. Grossly the tumor showed an ill defined, gray-white friable mass measuring about 2 cm (Fig.13).Microscopically, nested appearance of cell groups surrounded by a collagenous stroma was noted. The cells were composed of uniform small round hyperchromatic nuclei with very minimal pleomorphism. Mitotic figures were increased.

Hormone Receptor status in breast carcinomas:

Estrogen, Progesterone receptor positive tumors tend to have a significantly longer disease free

survival than with receptor negative tumors.[39, 52, 53, 54]

Earlier in 1975 Rosen et al [18] attempted to correlate Estrogen, Progesterone receptors status along with various histological types of breast carcinoma. In his study Estrogen, progesterone receptors were positive in 70-80% of the tumors and HER-2/ neu expression was present in 15-20% of the tumors.

Wilbur D et al [67] (1993) studied about hormone receptor status in 30 patients by immunohistochemical method on paraffin embedded blocks. He described Estrogen receptors positivity in 73% (22/30), Progesterone receptors positivity in 63% (19/30), and HER-2/neu expression in 37% (11/30).

Lici et al (2003) [68] have documented the incidence of invasive carcinoma by hormone receptor status from 1992 to 1998 in a population based study. He found that hormone receptor positivity increased from 75.4% to 77.5% in United States with a rise in prevalence over the years.

The number of studies performed on this topic is much less in Asian communities when compared with western world. Desai et al [69] (2000) documented the Estrogen, Progesterone receptors status of breast carcinoma in India. The procedure was done by immunohistochemical method. Out of 798 tumors, 32.6% were Estrogen receptor positive and 46.1% were Progesterone receptor positive. He obtained a high incidence of steroid receptors non-reactivity in breast carcinoma patients in India.

Col.V. Dutta et al [70] (2008) conducted a study in Armed Forces Medical College, Pune. The author analyzed Hormone receptors and HER-2/neu expression in breast carcinoma. In total of 75 tumors, 33% (25/75) cases expressed Estrogen receptor, Progesterone receptor or both where as 67% (50/75) were found to be both the receptor negative. HER-2/neu overexpression was seen in 58 % (43/75) of tumors. This study reveals that receptor negativity is higher in this subset of tumors when compared with western communities.

Lakmini K.B Mudduwa [71] (2009) studied Hormone receptor status of breast carcinoma by using Quick score method. She has reviewed 151 cases and documented prevalence of Estrogen receptor positivity in 45.7%, Progesterone receptor positivity in 48.3% and both receptors negativity in 54.3%. According to this study HER-2/neu overexpression was seen in 19.1% (26/136) cases.

Tanuja Shet et al [72] (2009) studied hormone receptors expression in the last 8 years from 1999 to 2006 in a cancer referral center in India. A total of 11,780 cases were reviewed. The percentage of hormone receptor expression varied from 52% to 57%.

Vikash Kumar et al [73] (2007) studied HER-2/neu oncogene overexpression which was much higher among Indian breast cancer patients 46.3% in comparison to 25-30% in Western literature.

In the present study Estrogen, Progesterone receptor or both were positive in 51.6% cases and both receptors were negative in 48.4% cases (Tab.6). HER-2/neu overexpression was in 42.7% cases (Tab.7). Hence this study is comparable with the studies conducted in the Asian countries. There appears to be a minimal variation in receptor expression; technically this could be explained by differences in technique of evaluation and inter laboratory variations.

Estrogen, Progesterone receptor and HER-2/neu status in Histological variants:

Rosen PP et al 1975 [18] found that Mucinous carcinomas, Papillary carcinomas are Estrogen, Progesterone receptors positive whereas Metaplastic carcinoma and Medullary carcinoma tend to be both receptor negative. The author has found that a small set of Invasive ductal carcinoma-NOS type and Lobular carcinomas showed receptor negativity.

Desai et al [69] (2000) described that Invasive lobular carcinoma, Mucinous carcinoma, mixed

tumors were Estrogen, Progesterone receptor positive, where as high grade infiltrating ductal carcinomas, Medullary carcinomas, in situ comedo-ductal carcinomas tended to be receptor negative.

Diab SG et al [26] has studied the tumor characteristics and clinical outcome of Tubular and Mucinous breast carcinomas, found Estrogen receptor positivity in 92% cases, Progesterone receptor positivity in 68% of cases and HER-2/neu expression in less than 5% of tumors. Similar results were also noted in studies conducted by Shousha S, et al [83] (1989).

Reiner et al (1988) [74] found that Papillary carcinomas are 100% Estrogen receptor positive, 80% Progesterone receptor positive and they tended to have a HER-2/neu negativity .

Rosen et al [38] (1995) studied HER-2/neu expression in nodal negative patients. He has documented low incidence of oncogene expression in Papillary carcinomas.

In 1991 Soomro S et al [75] studied about oncogene expression in different histological types of invasive breast carcinomas and quoted low expression in Neuroendocrine carcinomas.

Immunohistochemical studies by LeeAK[31] showed Neuroendocrine marker positivity in 82% of the cases. However 67% showed Estrogen receptor positivity and 56% exhibited Progesterone receptor positivity. [30, 32] HER-2/neu over expression was found to be negative. [32]

The present study shows Estrogen, Progesterone receptor positivity and HER-2/neu negativity in Mucinous carcinomas (Fig.21), Papillary carcinoma (Fig.23) and Neuroendocrine carcinoma (Fig.22). This is in correlation with above mentioned studies.

A study by Oberman HA et al [36] (1987) showed that Metaplastic carcinomas were Hormone receptor and oncoprotein negative. (Triple negative) [14, 17]

Horsfall et al [29] (1986) conducted study on relationship between ploidy and steroid receptors.

The author found that Medullary carcinoma is a high nuclear grade tumor with receptor negativity.

A study by Rosen PP et al [38] (1995) and Soomro S.et al [76] (1991) showed a low incidence of HER-2/neu receptor overexpression in Medullary and Metaplastic carcinoma.

The present study also denoted Triple negativity in both Medullary and Metaplastic carcinomas (Fig.24).

Kuenen–Boumeester V et al (1992)[76] studied immunohistochemistry of Androgen receptor in relation to Estrogen and Progesterone receptors. He has reported that a small set of lobular carcinomas tend to express both receptors negativity and Androgen receptor positivity.

A study by Rosen PP et al [38] (1995) showed Estrogen receptor positivity in 87.55% and, Progesterone receptor positivity in 75% of the tumours in Invasive lobular carcinomas. A small subset expressed both receptor negativity and invariably Androgen receptor positivity.

Riva et al [77] (2005) studied immunhistochemical analysis of Androgen receptor in breast carcinoma and showed frequent expression in Lobular carcinomas along with Estrogen, Progesterone receptors negativity.

In correlation with the above reference the Invasive lobular carcinoma in the present study also expressed both receptors and HER-2/neu negativity. Clinicopathologically this can be explained by aggressive nature of the tumor with higher nodal metastases.

Table.16 Correlation of Age and Receptors expression.

Age group	Total no of cases	ER/PR positive
31-40	6	3
41-50	11	3

51-60	9	7
61-70	5	4
> 70 yrs	2	-

Col.V. Dutta et al [70] (2008) studied about Estrogen, Progesterone receptor expression with the age. Out of 75 cases 35% of cases were in 51-60 years age group. The results showed that receptor positivity increases with age. Young patients tend to have a high level of circulating estrogen and correspondingly low expression of receptors.

Nidal M Almasri et al [61] (2005) has reviewed 91 specimens of breast tumors during the period of 1995-1999. The author has found significant receptor expression in 58% patients older than 50 years.

The present study shows receptor status positivity of 77.77% in patients older than 50years age group. The Increased immunoreactivity with advancing age is parallel to above mentioned studies.

Estrogen, Progesterone receptor, HER-2/neu with other variables:

Rosen PP et al [38] (1995) in his study correlated Estrogen, Progesterone receptor positivity with tumor size and histological grade. He concluded Estrogen, Progesterone receptor expressed more in low grade tumors and tumors of lesser diameter. HER-2/ neu overexpression was more among the nodal positive patients and tumors more than 2cm in diameter. [15]

J.Buon et al [78] found HER-2/neu receptor overexpression in higher grade tumors. Hormone receptor positivity was seen 100% in grade I tumors, 76.30% in grade II tumors, and 41.18% in grade

III tumors. Their positivity tends to have an inverse relationship with tumor grade.

S.Goyle et al [79] (2008) conducted a retrospective study in India. He has reviewed 131 patients and found that receptor and oncoprotein expression does not necessarily correlate with advanced tumors in our population.

In contrast with S.Goyle et al [79] (2008) the present study showed (Tab.12) Estrogen, Progesterone receptor positivity of 100% in grade I, 60.8% in grade II, none in grade III tumors. HER-2 /neu overexpression showed 100% in grade III, 56.5% in grade II, none in grade I tumors. This explains the overexpression of oncoprotein with higher histological grade tumors. Thus, it reflected a direct relationship with higher nuclear grade, which was comparable with J.Buon et al [78] & Rosen PP et al.[38]

Current study shows correlation of hormone receptors and HER-2/neu with tumor size. For practical purpose the tumors are categorized into three, T_1- < 2cm, T_2=2-5cm, T_3 = > 5 cm. As depicted in (Tab.8) 66% of T_1, 75% of T_2 and 33% of T_3 tumor showed receptor positivity. This explains that receptor positivity has an inverse relationship with tumor size.

HER-2/neu overexpression showed no expression in T_1, 66.6% in T2 and 48% in T_3 tumors. (Tab.9). This explains the higher expression among the tumors of more than 2 cm size.

Correlation with nodal status:

Col.V. Dutta et al [70] (2008) studied a strong correlation between HER-2/neu and nodal metastases. He has reported that 70% of nodal positive tumors overexpressed HER-2/neu protein.

H.J. Huang et al [80] conducted a study in 1362 women with primary breast tumor. He found that Estrogen receptor positivity was less in nodal positive tumors.

In present study out of 33 patients 15 cases showed metastasis while 18 cases did not. Receptor

positivity was found to be higher among the nodal metastasis negative patients 55.55% (10/18). HER-2/neu overexpression was seen in 66.66% of nodal positive cases than the nodal negative patients which was 11.1 %.

Correlation of Estrogen, Progesterone receptor with HER-2/neu:

H.J. Huang et al [80] conducted a study in 1362 women with primary breast tumor. The author has found an inverse relationship with receptor and oncoprotein expression. This can be explained by cross-linkage between the two pathways of tumor growth.

The present study (Tab.14) showed an inverse relationship between these hormone receptors and oncoprotein expression and therefore is comparable with above mentioned studies.

Francis G et al [81](2006) in a study of 591 tumors concluded that more than 20% of HER-2/neu positive tumors showed moderate or strong staining for Estrogen receptors.

Bhargava R et al [82] (2009) has reviewed 205 cases and concluded that 15% (32/205) were triple negative, 4% (8/205) were positive for Estrogen receptor and HER-2/neu hybrid expression.

In correlation with these studies the present study (Tab.14) showed two cases of Estrogen receptor- HER-2/neu positivity. Apart from Metaplastic carcinoma and Medullary carcinoma, one case was triple negative. There is no plausible explanation for this kind of receptor expression.

SUMMARY AND CONCLUSION

- The incidence of breast carcinoma in patients attending Coimbatore Medical College hospital for the year 2007-2009 was 9.4%.
- The distribution of benign breast lesions was 36.65% and that of malignant breast tumor was 63.35%.
- The benign lesions had a peak occurrence in the age group 21 to 30 years, whereas malignant tumors had a peak in the age group 41 to 50 years.
- Among the various histological variants in breast carcinoma, Invasive Ductal carcinoma – NOS type constituted 79% of cases.
- Estrogen, Progesterone receptor positivity and HER-2/neu negativity in Mucinous carcinomas, Papillary carcinoma and Neuroendocrine carcinoma.
- Triple negativity in Medullary, Metaplastic carcinomas and Invasive lobular carcinoma.
- Regarding the histological grade of breast carcinoma, Grade II tumors were common accounting for 88.5 %.
- Estrogen, Progesterone receptor or both was found in 51.52% while 48.48% were receptor negative.
- HER-2 /neu overexpression was found in 43.43% of tumors and it was negative in 57.57% tumors.
- Out of the total 33cases, 18 cases were T_3 tumors of more than 5cms in diameter.
- Larger the tumor size lesser the expression of hormone receptor, whereas smaller one

expressed more receptor positivity. This inverse correlation was statistically significant (P=0.003)

- HER-2/neu overexpression was found in tumors of all size. However, there was no significant correlation between the tumor size and HER-2/neu.
- Among 33 cases, nodal metastasis were found in 15 cases and negative in 18 cases.
- Among the 18 nodal negative patients Estrogen, Progesterone receptor were positive in 10 cases. Thus, there is higher receptor expression in nodal negative patients. Which was found to a have significant correlation (P=0.001).
- HER-2/neu overexpression was observed in 66.66% of nodal positive patients. Which statistically significant (P=0.001).
- Higher the histological grade of breast carcinoma, lower the receptor positivity. None of the grade III tumors expressed receptor positivity.
- Higher the histological grade of breast carcinoma, greater the HER-2/neu overexpression. Which was found to have a significant correlation .(P=0.001).
- Higher the Estrogen, Progesterone receptor positivity, lower was the HER-2/neu overexpression. Thus, there was an inverse relation between the receptor and HER-2/neu. which was found to be statistically significant (P=0.001).
- Receptor negativity is higher in this subset of tumors when compared with western communities.

CONCLUSION

Estrogen, Progesterone receptor positive tumors are common in post menopausal women, tumors of more than 2cm size, Histological grade I and in nodal negative patients. Oncoprotein overexpression is common among the tumors of more than 2cm size, grade III tumors and in nodal positive patients. When compared with western world Estrogen, Progesterone receptor positive tumors are found to be low, while HER-2/neu overexpression is high in this study group. Hormone receptor and oncoprotein expression has an inverse correlation with each other.

BIBLIOGRAPHAY

1. Dowsett M, Hanna W M, Kockx M et al. Standarisation of HER-2/neu testing: Result of an international proficiency –testing study. Mod. Pathol 2007: 20: 584-591.

2. Gown A M: Current issues in Estrogen receptor and HER-2/neu testing by immunohistochemical method in breast cancer. Mod. Pathol 2008; 21: S8-S15.

3. Schwartz's Principle of Surgery; F Charles Brunicardi 8th ed. 2005.Mc Graw –Hill Companies .Inc.The Breast. P 453-500.

4. Park's Textbook of Preventive and Social Medicine19th ed ,2007.P 378-380.

5. Bancroft JD, Marilyn Gamble (Ed) Theory and practice of histological techniques.
Churchill Livingstone 2002. P

6. Sondik.E.J.Breast cancer trends, Incidence, Mortality and Survival.
Cancer: 1994; 74; 995-999.

7. WHO (1999) Health Situation in South East Asia Region during 1994 -1997

8 .Government of India Ministry and Social Welfare, New Delhi .2001-2002.

9. Moore DH, Breast carcinoma Etiological Factors, Adv. Cancer Res; 1983:40;
189-253.

10. Pathak DR et al. Breast carcinoma Etiological Factors, Reproductive and Hormonal Factors.Cancer 2000; 88: 1230-1238.

11. Priti Lal MD, Lee K. Tan MD, et al .Correlation of HER-2/neu status with Estrogen, Progestrone receptor and histological features in 3,655 Invasive breast carcinoma. Am J Clin. Pathol 2005.

12.Susan c. Lester, The Breast . Robbins and Cotran Pathological basis of Disease , 7th Ed(2004) P.1120-1153.

13.M willams Audeg, The new era of cancer risk assessment in Surgical Oncology
P.261-265.

14.Gavin Harris, Sarah E Pinder , et al Invasive carcinoma special types. In Foundation in diagnostic pathology (2006). P. 201-220.

15. Paul Peter Rosen in Rosen 's Breast Pathology 3rd Ed(2009). Lippincott Williams &Wilkins.P.352-519.

16. Rosai Ackerman's Surgical Pathology .Juan Rosai,9th edition, 2005; Elsevier. Breast P. 1763-1876.

17. WHO Classification of tumors. Pathology and Genetics of Breast and Female genital organs. Lyon: IARC press 2003; 13-59.

18. Rosen PP, Menendez Botet C J, et al Pathological review of Breast lesions analyzed for Estrogen receptor protein. Cancer Res 1975; 35; 3187-3194.

19. Fisher ER ,Andersons, Dean , et al .Solving the dilemma of IHC and other methods. Cancer: 103: 164-173.

20.Foote FW Jr, Stewart FW et al. A Histological Classification Of Carcinoma of Breast. Surger 1946;19: 74-79.

21.Tavassoli FA, Pathology of Breast. Infiltrating and Familiar sub- types. Norwalk, Appleton and Lange.1992; 293-294.

22.Dixon JM et al Long term Survivors after Breast cancer. Br. J. Surg. 1985; 72:445.

23. Paterson D.A et al Assessment of ER by IHC on Fixed and Frozen Tissue by Ligand Assay are comparable by 75 % -80 % J.Clin Pathol 1990; 43 ; 46-51

24. Depotter CR, Schelfhout AM,et al The neu-protein and breast cancer
Virchow Archive: 1995; 426; 107-115

25.Suo Z, Risberg B, et al The expression of EGFR family Ligands in breast cancer I Int J .Surg pathol 2002 ; 10: 91-99.

26. Diab SG, Clark GM, Osborne C K, et al . Tumor characteristics and clinical out come of Tubular and Mucinous breast carcinoma. J Clin .Oncol 1999; 17:1442-1448.

27.Jensen ML, Kiaer A, Melsan F, et al . Medullary Carcinoma Vs poorly differentiated carcinoma on IHC study with keratin 19, and ER staining Histopathology

1996;29:241-245.

28. Ponsky JL, Gliga L, Reynolds S, et al. Medullary carcinoma of breast as association with negative hormone receptors J Surg Oncol 1984;25:76-78

29.Horsfall DJ. Tilley WD, Orell SR, Marshall VR, Cant EL et al.Relationship between ploidy and Steroid Hormone Receptor in Primary Invasive Breast Cancer.

30. Cubilla AL , Wooddruff JM et al . Primary Carcinoid tumor of Breast. A report of eight patients. Am J Surg. Pathol 1977;1 :283-292.

31. LeeAK, .Rosen PP, De Lellis RA et al. Tumor marker expression in carcinoma of breast and the relationship to prognosis an IHC study. Am J Clin Pathol 1985; 84:687-692

32.Niremudi A,Pinder SE, Lee AHS et al. Neuroendocrine differentiation and prognosis in Breast carcinoma . Histopathology 2000 ;40: 215-222.

33. Papootti M, Gugliottu P, Eusebi V et al. IHC analysis of benign and malignant papillary lesions of breast. . Am J Surg. Pathol 1983;7 :451-463.

34.ZekiogluO,Erhan Y,Ciris M et al. Invasive micropapillary carcinoma of breast. IHC profile compared with Invasive Ductal Carcinoma. Histopathology 2004; 44:18-23.

35.Kahfmann MW ,Marti JR, Gallenger HS et al . Carcinoma of Breast with Pseudosarcomatous Metaplasia. Cancer 1984; 53:1908-1917.

36.Oberman HA, Metaplastic carcinoma of breast. A Clinicopathological study of 29 patients. Am J Surg. Pathol 1987 ;11:918-929.

37.Wargotz ES, Norris HJ et al. Metaplastic carcinoma of breast .Cancer 1989;64: 1490-1499.

38.Rosen PP,Lesser ML, Arroya CD et al. IHC detection of HER-2 in patients with axillary lymphnode negative breast cancer. A study of epidemiological risk factors and prognosis.Cancer 1995; 75:01320-1326.

39. Brader B, Graf D, Padovan R etal ER, PR as prognostic marker Tumori 1988; 74 ; 45-52.

40. Mary US born, Wenaejusz, Domagula (Eds) in Comprehensive cytopathology by Bibbo 2nd ed

1997.

41. Jay H.B Histochemistry in Ivan D James L, (Eds) Anderson pathology,
Mosby St louis , 1996.

42. Pluzek, K.Y Sweeney , E,Miller, K.D Isaacson .P. A major advance for IHC
E pos J. Pathol 169 (Suppl) abstract 220

43. Stuart J Schnitt, Rose mary R Millis,Andrew M ,Hanby oberman H.A The breast Sternberg's Diagnostic Surgical Pathology (Eds) Lippincott Williams & wilkins 2004

44.Huang .S Minnassian H More J D et al. Application of immunofluorosent staining in paraffin sections improved by trypsin digestion Laboratory Investigation 35: 383-391

45Miller K,Auld . J, Jessup. E, Rhodes A et al. Antigen unmarking by pressure cooker method . A comparison with microwave oven heating and traditional methodsAdvances of anatomical pathology, 2 : 60-64

46. Langols, N.E.T , King G., Herricot R et al .Non-enzymatic retrieval of antigen staining of PGP 9.5 J.Pathol 173 ; 249-253

47.Jensen EV, Jacobson HI, Basic guides to mechanism of action of hormone receptors. Recent Prog Horm Res 1962; 18 ; 387-414.

48.Suzanne A.W Fuqua Rachel Schiff Disease of breast ed : The biology of ERs – 585 -590.

49.Shoker et al ER expression in normal and precancerous breast J Pathol 1999; 188: 237-244

50.Peterson OW, et al. Frequency and distribution of ER positive cells in normal non lacating human breast tissue .Cancer 1987; 47 : 5748 -5781

51.Li X, Huang J, Yip, Bambara R.A et al. Single chain estrogen receptor (ER s) reveal
that the ER alpha / Beta hetero dimer emulates functions of ER alpha dimer in genomic
Estrogen signaling path rays.Mol. Cell .Biol 2004 ; 24 (17) ; 7681-94
52. Brader B,Graf D , Padovan.R et al. ER, PR as Prognostic marker.Tumori 1988;47;364-367.

53. Kinne DW Ashikari R, Bulter A Rosen P.P ER Protein in breast cancer as predictor of recurrence. Cancer 1981; 47: 364 -367

54.Syed K . Mohsin. Molecular markers in invasive breast cancer. Foundation in diagnostic pathology(2006)P . 266-269.

55.Harvey JM, Clark GM et al. ER Status by IHC is superior to the LBA for predicting response to adjuvant endocrine therapy in breast cancer.J Clin Oncol 1999:17 :1474-1481.

56.Lesser ML ,Rosen PP,Kinne DW.Multicentricity and bilaterality in invasive breast carcinoma. PR is estrogen regulated. Most PR positive carcinoma is ER positive also.Surgery1982: 1:234-240.

57.Clark et al. Steroid receptors and other prognostic factors in breast cancer semin Oncol 1988;15:20-25

58.Goulding H, Pinder S, et al.A new method for assessment of ER on routine formalin fixed tissue. Sup L Hum Pathol 1995; 26 : 291 -294

59. Barnes DM , Hanby AM.et al. Estrogen and progesterone receptor in breast cancer Past, Present, Future. Scoring system for ER Histopathology 2001; 38: 271-274

60.Allred DC,Harvey JM,Berardo M, Clark GM . Prognostic and predictive factors in breast cancer by immunohistochemistry. Mod. Pathol 1998; 155-165

61.Nidal M. Almasri and Mohammad Al Hamad .Immunohistochemical evaluation of human epidermal growth factor receptor and estrogen and progesterone receptors in breast carcinoma in Jordan. Breast cancer research 2005; 7; R 598-604

62. Leake R Barness D Pinders . , et al . IHC detection of steroid receptors in breast cancer ; A working group protocol. J Clin .Pathol 2000; 53: 634-635.

63.Suo Z, Risberg B, et al The expression of EGFR family Ligands in breast cancer I Int J .Surg pathol 2002 ; 10: 91-99.

64. Rhodes A, Jasni B, et al .Evaluation of HER -2 / neu IHC assay sensitivity scoring in formalin fixed paraffin embedded cell in breast tumors.Am J Clin Pathol 200 2 ; 118 : 408 -407.

65.Ellis I O et al. Breast practice No 176, updated recommendation for HER -2 neu testing in U.K. . J Clin .Pathol 2004; 57(3);233-237.

66.Omar Hammed Washington manual of surgical pathology 2008.Lippincott Williams&Wilkins. P.256-258.

67. Wibur D.Barrows GH.Estrogen,Progestrone receptor and HER-2/neu analysis in pure in situ carcinoma –an IHC analysis. Mod. Pathol 1993 Mar; 6(2):114-120.

68. Lici, Darling JR, Malone KE Incidence of invasive breast cancer by HR status from 1992-1998. J Clin Oncol 2003:21;28-34.

69. Desai SB, Moonim MT, Gill AK, Punia RS, Naresh KN et al HR status of breast cancer in India A study of 798 tumours Breast 2000;9:267-270.

70. Col V. Dutta , Brig GS Chopra SM et al. HRs, HER-2/neu and chromosomal aberrations in breast cancer MJAFI 2008; 64: 11-15.

71.Lakhmini K.B. Mudduwa dept. of pathology, Sri Lanka .Quick score of hormone receptor status of breast carcinoma:Correlation with the other clinicopathological prognostic parameters Indian journal of Pathology & Microbiology 2009; vol 52:2: 159-163.

72. Tanuja shet, Atin Agarwal et al.Hormone receptors over the last 8 years in a cancer referral center in India.What was and what is Indian journal of Pathology & Microbiology 2009;52:2:171-174.

73. Vikash Kumar, Mallika Tewari, Usha singh et al.Significance of HER-2/neu protein overexpression in Indian breast cancer patients. Ind. J. Surg 2007 vol 69; No: 4:122-128.

74. Reiner A, Reiner G, Spona J, Schemper M, Holzner JH. Histopathologic characterization of human breast cancer in correlation with ER status. A comparison of IHC and biochemical analysis. Cancer 1988; 61:1149-1154.

75. Soomro S, Shousha S, Taylor P, Shephard HM, Feldmann M. c-erb-2 in different histological sub types.J Clin Pathol 1991;44:211-214

76.Kunen-Boumeester V,Vanderkwast TH ,Van putten WLJ et al. Immunohistochemistry of Androgen receptor in relation of ER,PR. Int.J.Cancer 1992; 52:581-584.

77. Riva C,Danese E, Caprara C et al. Immunohistochemical study of AR in breast carcinoma. Evidence of their frequent expression in Lobular carcinoma. Virchows Arch 2005;447:695-700.

78. Jovcic-Milentijeric M,Ilic R,Katic V, Zivkovic V. et al. Correlation of Hormone receptor status with histological and nuclear grading in breast carcinoma. Breast Cancer Res J. Buon 2004. Apr-June 9(2) 173-7

79. S,Goyle, S.Karmarker I, Ambulkar .Presentation trends of breast cancer patients at a single cancer institute in India..J Clin Oncol 2008;26:158.

80.Huang HJ,Neven P,Drijkoningen Met al. Association between HER-2/neu and the progesterone receptor in estrogen –dependent breast cancer is age related.Breast cancer Res Treat 2005,

81.Francis G, Beadle G, Thomas S, et al. Evaluation of Estrogen, Progesterone receptor status in HER-2/neu positive breast carcinoma and correlation with outcome. Pathology 2006 oct;38(5):391-398.

82.Bhargava R,Strieebel J, Beriwal S,et al. Prevalence, morphological features and proliferation indices of breast carcinoma molecular classes using IHc surrogate markers. Int J Clin PAthol.2009;2(5);444-55.

83.Shousha S, Coady AT, StampT, James KR , et al. HRs in Mucinous carcinoma of breast, immunohistochemical study on paraffin wax section J Clin Pathol 1989;42:902-905

PROFORMA

Serial No. : **Name** :

HPE No. : **Age** :

IP No. : **Sex** :

Clinical diagnosis :

Breast : **Right / Left**

Specimen : **Excisional (Lumpectomy / Mastectomy)**

Macroscopic Examination :

Specimen Size :

Skin and Nipple :

Tumor Size :

Tumor margins : **Circumscribed / Infiltrative**

Posterior margin :

Lymphnode :

Number :

Size :

C/S :

Histological type of lesion :

Skin : **Free / Involved**

Nipple : **Free / Involved**

Muscle : **Free / Involved**

LYMPHNODES

Total Number :

No.of nodes involved :

Other Findings :

Receptor Study :

ER Status:

Proportion Score :

Intensity Score :

Total Score :

PR Status:

Proportion Score :

Intensity Score :

Total Score :

HER – 2/ neu Status :

Weak :

Moderate :

Strong Positive :