CLINICO-PATHOLOGICAL STUDY OF BREAST CARCINOMAS WITH ER, PR STUDIES

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CERTIFICATE

This is to certify that **Dr.R.NARMADHA,** post graduate student (2008-2011) in the Department of Pathology**, Kilpauk Medical College,** has done dissertation on **'CLINICO-PATHOLOGICAL STUDY OF BREAST CARCINOMAS WITH ER, PR STUDIES'** under my guidance and supervision in partial fulfillment of the regulation laid down by the **'THE TAMILNADU DR MGR MEDICAL UNIVERSITY, CHENNAI - 32'** for M.D., Pathology degree examination to be held in April 2011.

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DECLARATION

I , **Dr.R.NARMADHA**, solemnly declare that the dissertation titled **'CLINICO-PATHOLOGICAL STUDY OF BREAST CARCINOMAS WITH ER, PR STUDIES'** is a Bonafide work done by me at Kilpauk Medical College between 2008 and 2010, under the guidance and supervision of our Head of the Department, **Prof. Dr. V. Rajalakshmi., M.D. , D.C.P.**

This dissertation is submitted to "**THE TAMILNADU DR MGR MEDICAL UNIVERSITY"**, towards partial fulfillment of regulation for the award of M.D.DEGREE BRANCH III in Pathology.

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INTRODUCTION

Breast carcinoma has a major impact on the health of women. Cancer of the breast is the most common cancer among women in many regions in India and has overtaken cancer cervix (1). Presently 75,000 new cases occur in India every year.

Breast cancer survival is linked to early detection and timely appropriate treatment . Prognosis is related to a variety of clinical, pathological and molecular features which includes stage of the carcinoma, histologic type, grade and lymph node metastasis . Estrogen and progesterone receptors have, with increasing importance, influenced the management of this malignancy(2).

With an established positive correlation of ER and PR with the degree of tumour differentiation, determination of ER and PR status on breast biopsy specimens, prior to therapeutic intervention is advocated as a standard practice(3). Survival and response to hormone therapy are most favourable among women who are receptor positive.

With these prognostic implications, the need for accurate and precise assessment of ER and PR in breast carcinomas is essential to

predict the response to hormone therapy. Immunohistochemistry is the most commonly used and the best method of testing ER and PR status(4,16).

This study is aimed at assessing the hormone receptor status in breast carcinomas and to correlate this reactivity pattern with histologic grade, tumor stage and lymph node metastasis.

AIMS AND OBJECTIVES

1. To assess the clinical stage using American Joint Committee Cancer staging system.

2. To grade the the breast tumors based on Nottinghams modification of Bloom & Richardson grading system.

3. To assess ER, PR status of breast carcinomas by immunohistochemistry using Quick score.

4. To study the correlation between ER, PR status and other prognostic indicators of breast cancer.

REVIEW OF LITERATURE

Breast is a modified sweat gland resting on pectoral muscle. It extends from 2nd to 6th rib and from sternal edge to near the mid axillary line.

Breast carcinoma is becoming the most common malignant tumor in women. It causes 3,76,000 deaths in women in a year worldwide and every year 9,00,000 new cases are diagnosed.

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

HISTOLOGY

The functional unit of breast is lobule. There are numerous lobules within each breast. Lobules consist of variable number of blind ended terminal ductules alternatively called acini which is lined by double layered epithelium- outer flattened myoepithelial cell, inner cuboidal epithelial cells with secretory function.

The acini drain into terminal duct. Each terminal duct and its acini are together referred to as Terminal Duct Lobular Unit(TDLU). The terminal duct drains into sub segmental, segmental ducts and finally into lactiferous duct. There are 15-20 lactiferous ducts which open into the nipple. Immediately below the nipple, the lactiferous duct dilates to form lactiferous sinus.

RISK FACTORS

The frequency of the disease has prompted an intensive study of risk factors. The common denominator for these factors is strong and prolonged estrogen stimulation operating on a genetically susceptible background.

1. **AGE:** Breast cancer is rarely found before the age of 25 years. 70% occur in women over 50 years. Incidence rises throughout a woman's life.

2. **REPRODUCTIVE & MENSTRUAL HISTORY:** Early menarche, late menopause, nulliparity, women over age 35 at first pregnancy have increased risk because of high estrogen stimulation(9).

3. **FAMILY HISTORY:** Women who have first degree relatives with breast carcinoma have increased risk (5), probably because of mutation in BRCA1 and BRCA2.

4. **PRENEOPLASTIC CONDITIONS:** Atypical hyperplasias and florid epitheliosis are associated with increased risk (8).

5. **RACE & SOCIOECONOMIC GROUP:** Incidence of breast carcinoma is high among high socio-economic group.

Additional risk factors are also recognised, but there is a lack of definitive correlation. The additional risk factors includes the following,

1. **ESTROGEN EXPOSURE:** Hormone replacement therapy and use of oral contraceptive pills are associated with increased risk of breast cancer. Oophorectomy reduces the risk of breast cancer by 75%(6,7).

2. **RADIATION EXPOSURE:** Therapeutic radiation and atom bomb survivors have increased risk.

3. **BREAST FEEDING:** The longer the women breast feed, the greater is the reduction in the risk of breast cancer.

4. **DIET:** High fat diet and obesity carry increased risk.

HISTOLOGICAL CLASSIFICATION OF TUMORS OF BREAST BY WHO

Epithelial tumors

- 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 tumors with abundant mucin
  - Mucinous carcinoma
  - Cystadenocarcinoma and columnar cell mucinous carcinoma
  - Signet ring cell carcinoma

- Neuroendocrine tumors
    - Solid neuroendocrine carcinoma
    - Atypical carcinoid tumor
    - 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 tumors

- Hemangioma
- Angiomatosis
- Hemangiopericytoma
- Pseudoangimatous stromal hyperplasia
- Myofibroblastoma
- Fibromatosis(aggressive)
- Inflammatory myofibroblastic tumor
- Lipoma
    - Angiolipoma
- Granular cell tumor
- Neurofibroma

- Schwannoma
- Angisarcoma
- Liposarcoma
- Rhabdomyosarcoma
- Osteosarcoma
- Leiomyoma
- Leiomyosarcoma
- Fibroepithelial tumors
- Fibroadenoma
- Phyllodes tumor
    - Benign
    - Borderline
    - Malignant
- Periductal stromal sarcoma, low grade
- Mammary hamartoma

Tumors of the nipple

- Nipple adenoma

- Syringomatous adenoma
- Paget's disease of the nipple

Malignant lymphoma

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

Metastatic tumors

Tumors of the male breast

- Gynaecomastia
- Carcinoma
  - Invasive
  - In situ

Almost all breast malignancies are adenocarcinomas, all other types making up fewer than 5% of the total.

Carcinomas are divided into in situ and invasive. Invasive carcinoma has invaded beyond the basement membrane into the stroma. All carcinomas are thought to arise from terminal duct lobular unit.

WHO classification is based on the growth pattern and cytologic features and does not imply histogenesis or site of origin within mammary duct system.

The most common histologic type of invasive breast cancer by far is invasive ductal carcinoma- not otherwise specified(11).

INVASIVE DUCTAL CARCINOMA- NOS TYPE:

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

Microscopically, architectural arrangement may be in cords, clusters and trabeculae while some are characterised by predominantly solid or syncytial infiltrative pattern(13).

In a study conducted by Paul Peter Rosen et al, these carcinomas show 70-80% ER, PR positivity(12). According to Lakhmini K.B. Mudduwa the prevalence of hormone receptor positive breast cancer in

Asian countries has found to be lower than western world where more than 50% tumors express hormone receptors(64).

Pleomorphic carcinoma is a rare variant of high grade ductal carcinoma -NOS characterised by pleomorphic and bizarre giant cells in more than 50% of tumor cells in a background of adenocarcinoma(13,14).

In a study conducted by Ellis I O et al, 10 year survival rate of this tumor ranges from 33-48%(28).

INVASIVE LOBULAR CARCINOMA

The classical form of infiltrating lobular carcinoma was first described by Foote & Stewart(15).

In a study conducted by Grazio Arpino et al, this type represents 4.9-15% of all invasive breast carcinomas(31). They are frequently bilateral and multicentric when compared with other subtypes(14).

Microscopically, cells are round or oval with eccentrically placed nuclei with small nucleoli and small amount of cytoplasm. They have characteristic Indian file or targetoid pattern.

Dixan.J.M. conducted receptor assay that reveals ER, PR positvity in 67-92% of cases(12).

It has a clinical outcome similar to IDC-NOS type. 10-year survival rate is 54%(27,28).

TUBULAR CARCINOMA:

Tubular carcinoma is usually smaller than 2cm and has two morphological types, ‘pure type’ with stellate nature and sclerosing type with more diffuse ill-defined nature.

Microscopically, it has irregularly arranged tubules lined by single layer of epithelial cells with little pleomorphism and low mitotic rate. The tubules are characteristically angulated and have open glandular lumina.

Pure tubular carcinomas have an excellent prognosis(17). 10-year survival rate is 90%(28).

MUCINOUS CARCINOMA:

WHO defines it as “large amount of extracellular mucin sufficient to be visible both grossly and microscopically surrounding the tumor cells”(10).

Microscopically, the tumor consists of small islands or clusters of epithelial cells floating in lakes of extracellular mucin divided by delicate

fibrous septae. The lakes of mucin are positive for PAS and mucicarmine stain.

ER, PR positivity ranges from 73-95%(12).These tumors carry a very good prognosis with 10-year survival data varying between 68 and 90%(17,28).

MEDULLARY CARCINOMA:

Grossly the tumor appears as well-circumscribed, soft and fleshy. WHO defines it as “well circumscribed carcinoma composed of poorly differentiated cells with scant stroma and prominent lymphocytic infiltration”. They carry a good prognosis with 10-year survival rate of 84%(12,30). Immunohistochemical studies of hormone expression conducted by Ponsky et al were negative(18,19).

PAPILLARY CARCINOMA:

Diagnosed predominantly in postmenopausal patients. Microscopically, circumscribed, show delicate or blunt papillae with focal solid areas of tumor growth. DCIS is present in >75% of cases and usually has papillary pattern(29). They have an excellent prognosis(27,28).

In a study conducted by Zekioglu et al hormone receptor positivity is seen in 89% of cases (24).

METAPLASTIC CARCINOMA:

WHO defines it as “a heterogenous group of neoplasms with spindle cells, squamous cells or with mesenchymal differentiation. Extensive sampling of metaplastic tumors should be done to identify carcinomatous foci and distinguish them from true sarcomas because of differences in biologic behaviour and response to therapy.

It behaves as a highly malignant tumor with early recurrence and poor survival(23,28).

According to Tzu-Chieh Chao et al, hormone receptors were negative in majority of the cases (22).

NEUROENDOCRINE CARCINOMA:

WHO defines this type as a carcinoma with neuroendocrine marker positivity noted in more than 50% of the cell population(10,20). This type has an infiltrative morphology with component cells arranged in nests, sheets or trabecular formation and peripheral palisading of cell groups((13,14,60).

In a study conducted by Niremudi et al, 55-65% showed ER, PR positivity.

PROGNOSTIC AND PREDICTIVE FACTORS

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. Major prognostic and predictive factors are:

1. TUMOR SIZE:

The diameter of the primary tumor shows a good correlation with the incidence of nodal metastases and with the survival rate. This easier, quicker and cheaply determined parameter has been found to be one of the strongest predictors of dissemination and rate of relapse in node negative breast carcinoma. Women with node negative carcinomas, which are less than 1cm in diameter, have a prognosis approaching that of women without treatment approximately 90% (14,33).

According to Michaelson et al, for correlation with prognosis, the size of tumor should be assessed only on pathological specimens, as clinical measurements may be inaccurate (36).

2. EXCISION MARGINS:

Microscopic examination of the excision margins is usually undertaken to assess the adequacy of surgical excision and hence the probability of recurrence. According to Swanson et al and Frazier et al ,it has been found that when the tumor reaches the excision margins, there is a significantly increased risk of local recurrence and distant metastasis.(37,38).

3. HISTOLOGIC SUBTYPE:

30-year survival rate of women with special type of carcinomas (tubular, mucinous, medullary, papillary)is greater than 60% compared with less than 20% for women with carcinomas of no specific type(27).

4. VASCULAR INVASION:

Tumors stimulate the growth of host blood vessels (angiogenesis). Tumor emboli are mainly seen within thin walled channels. Since it is almost impossible to determine whether such spaces are lymphatics or

venules, the broad term vascular invasion is used(39). Immunohistochemistry for endothelial markers can differentiate blood vessel and lymph vessel invasion(41).

There is significant relationship between the presence of vascular invasion and prognosis as judged by local recurrence and survival(40).

5. LYMPH NODE STAGE:

Axillary lymph node is the most important prognostic factor for invasive carcinoma in the absence of distant metastasis(43). The clinical assessment of nodal involvement is very inaccurate with both false positive (as in palpable reactive nodes) and false negative findings (as with small metastatic deposits). Hence biopsy is required for accurate assessment.

Numerous studies have shown that patients who have histologically confirmed loco-regional lymph node involvement have a poorer prognosis than those without nodal involvement(43). According to Veronesi et al, 10-year survival rate is reduced from 75% for patients with no lymph node involvement to 25-30% for those with lymph node metastasis(44).

Prognosis is more likely related to the number of nodes involved rather than size of the deposit.

For prognostic purpose, the best grouping seems to be the following

1- negative nodes

2- one to three positive nodes

3- four or more positive nodes

With no nodal involvement, the 10-year disease-free survival rate is close to 70 to 80%, the rate falls to 35-40% with one to three positive nodes and 10-15% in the presence of more than ten positive nodes(33).

The level of nodal involvement also provides useful prognostic information(44). Metastasis is not only a marker of diagnosis at a latter point in the history of breast cancer, but also a marker of aggressive phenotype(42).

6. HISTOLOGICAL GRADE:

Most commonly used grading system to assess the degree of differentiation is Nottingham modification of the Bloom-Richardson system.

The grading criteria for this system are:

a. Tubule formation:

Score1 - tubular formation in >75% of the tumor

Score2 - tubular formation in 10-75% of the tumor

Score3 - tubular formation in <10% of the tumor

b. Nuclear pleomorphism:

Score1 - nuclei with minimal variation in size and shape

Score2 - nuclei with moderate variation in size and shape

Score3 - nuclei with marked variation in size and shape

c. Mitotic count:

Mitotic figures are to be counted only at the periphery of the tumor. Counting should begin in the most mitotically active area; 10 high power fields are to be counted in the same area.

Score1 - 0-9 mitoses/10 hpf

Score2 - 10-19 mitoses/10hpf

Score3 - 20/> mitoses/10 hpf

Allocation of grade:

Scores are added together and allocated as

Score3-5	grade1
Score6-7	grade2
Score8-9	grade3

According to Enad.A.Rakha et al, histologic grade as assessed by Nottingham grading system, provides a strong predictor of outcome in patients with invasive breast cancer (34).

7. NOTTINGHAM PROGNOSTIC INDEX:

A study conducted by Galea et al showed a significant relationship of prognosis with size, grade and lymph node metastasis (45).

Using the coefficients of significance for these factors, an index predicting survival – Nottingham prognostic index is calculated (NPI).

NPI=0.2x tumor size (in cm)+ lymph node stage(1-3)+ histological grade (1-3)

NPI SCORE	PROGNOSIS
<3.4	good prognosis
3.4 - 5.4	moderate prognosis
>5.4	poor prognosis

It is a powerful and reproducible method of assessing prognosis and is the only integrated index which has been confirmed in prospective studies (46).

8. TNM STAGING:

The revised TNM staging for breast cancer, as approved by the AJCC is

PRIMARY TUMOUR (T) :

Tx : Primary tumour cannot be assessed

T0 : No evidence of primary tumour

Tis : DCIS, LCIS, Paget's disease of nipple with no tumour.

T1 :

T1mic : Microinvasion < 0.1 cm in greatest dimension.

T1a : Tumour more than 0.1 cm but < 0.5cm.

T1b : Tumour > 0.5cm but < 1 cm

T1c : Tumour 1-2 cms.

T2 : Tumour > 2cm but < 5 cms in greatest dimension

T3 : Tumour > 5 cms in greatest dimension

T4 : Tumour of any size

T4a : Extension to chest wall

T4b : Skin involvement (Peau d'orange, Ulcer, Satellite nodules)

T4c : Both T4a and T4b.

T4d : Inflammatory carcinoma.

REGIONAL LYMPHNODE (N)

Nx : Regional LN cannot be assessed (Eg. Previously removed)

N0 : No regional LN

N1 : Metastasis in mobile ipsilateral axillary LN (s).

N2a : Ipsilateral matted or fixed LNs.

N2b : Clinically apparent ipsilateral internal mammary nodes and in the absence of clinically evident axillary LNs.

N3a : Metastasis in ipsilateral axillary nodes and ipsilateral infraclavicular lymphnode.

N3b : Axillary LNs + Ipsilateral internal mammary LN(s)

N3c : Metastasis in ipsilateral supraclavicular LN (s).

Pathologic classification

pNX : Regional lymph nodes cannot be assessed (eg., not removed for pathologic study or removed previously)

pN0 : No regional lymph node metastasis

pN1 : Metastasis to movable ipsilateral axillary lymph node(s)

pN1a : Only micrometastasis (none >0.2 cm)

pN1b : Metastasis to lymph node(s), any larger than 0.2cm

pN1bi : Metastasis in 1-3 lymph nodes, any larger than 0.2 cm and all smaller than 2 cm in greatest dimension

pN1bii : Metastasis to 4 or more lymph nodes, any larger than 0.2 cm and all smaller than 2 cm in greatest dimension

pN1biii : Extension of tumor beyond the capsule of a lymph node metastasis, smaller than 2 cm in greatest dimension

pN1biv : Metastasis to a lymph node 2 cm or larger in greatest dimension

pN2 : Metastasis to ipsilateral axillary lymph node(s) fixed to each other or to other structures

pN3 : Metastasis to ipsilateral internal mammary lymph node(s)

METASTASIS

M0 : No distant metastasis

M1 : Distant Metastasis

TNM STAGE GROUPING

Stage I :	T1 N0 M0
Stage IIa :	T1 N1 M0
	T2 N0 M0
Stage II b :	T2 N1 M0
	T3 N0 M0
Stage IIIa :	T0 N2 M0
	T1 N2 M0
	T2 N2 M0
	T3 N1 M0
	T3 N2 M0
Stage IIIb :	T4 N0 M0
	T4 N1 M0
	T4 N2 M0

Stage IIIc : Any T N3 M0

Stage IV : Any T Any N M1

9. HORMONE RECEPTOR:

Women with estrogen and progesterone receptor positive cancer have better prognosis than do women with hormone receptor negative carcinomas. The evaluation of hormone receptors is very valuable to predict response to hormone therapy(47,48,49).

10. Her-2/neu:

It is a transmembrane glycoprotein involved in cell growth control. Over expression of Her-2 neu is associated with poor prognosis(47,48,49).

11. PROLIFERATION RATE:

Proliferation can be measured by flow cytometry, by thymidine labelling index, by mitotic counts or by immunohistochemical detection of cellular proteins produced during cell cycle. Tumors with high proliferation rate have the worst prognosis(48,49).

IMMUNOHISTOCHEMISTRY

Immunohistochemistry is a method based on the selective binding of specific immunologic reagents to specific antigenic determinants on a cell.

ANTIGEN: Any foreign material that may enter the body and trigger the mechanism of immune response, that results in the production of antibodies.

ANTIBODY: Substances produced in response to an antigenic stimulus.

Immunohistochemistry is used to determine expression of particular antigen and its microanatomic location in the tissue. IHC uses antibodies to distinguish the antigenic differences between the cells. These differences identify the lineage of cell population and define biologically distinct population of cells within the same lineage.

Immunohistochemistry was started in 1940 by Coons for frozen sections.

In 1966, Pierce modified it and used for paraffin sections. Antigen retrieval technique was introduced by Shi in 1991. Antigen retrieval

technique is a simple method that involves heating paraffin processed sections at high temperatures before IHC staining.

The use of antibody in immunohistochemistry depends on the sensitivity and specificity of antigen-antibody reaction and the Hybridoma technique provides limitless source of highly specific antibodies.

BLOCKING NON-SPECIFIC BACKGROUND STAINING:

Background staining is due to either non specific binding or presence of endogenous enzymes. Non-specific binding with polyclonal primary anrtibody is minimised by pre-incubating sections with serum from same species on optimal working dilution.

Endogenous enzymes such as peroxidase seen in normal and neoplastic tissues is abolished by peroxidase blocking or by using alternate systems such as immunogold technique.

Methods suggested to overcome endogenous activity include incubation in methanol containing 0.5% hydrogen peroxide for 10 minutes at room temperature.

DETECTION SYSTEMS:

Antibodies are labelled or flagged by some method to permit visualisation – these include fluorescent substances, heavy metals or enzymes.

Enzymes are the most widely used labels in immunohistochemistry and incubation with a chromogen using a standard histochemical method produces a stable coloured end product suitable for light microscopy.

METHODS:

DIRECT LABELLING METHOD:

Antibody is attached with a label by chemical means and directly applied to tissue sections. The advantage of this method is that they are simple to use. The main disadvantage is that the sensitivity is low.

INDIRECT LABELLING METHOD:

Enzymes are labelled with secondary antibody, which is produced against primary antibody. This technique is more sensitive.

AVIDIN BIOTIN CONJUGATE METHOD:

In this technique primary antibody is added followed by biotinylated secondary antibody and next by preformed complexes of Avidin and Biotin horse radish peroxidase conjugate. This is also more specific.

BIOTIN STREPTAVIDIN METHOD:

Modification of Avidin biotin with streptavidin being used instead of Avidin. Advantage is less non specific background staining.

IMMUNOGOLD WITH SILVER ENHANCEMENT :

It can be used in both direct and indirect methods and has found wide image in ultrastructural immuno location. Gold particles enhanced by addition of several layers of metallic sliver. This technique may represent the most sensitive and effective light microscopy immunohistochemical method currently available.

Tissue for IHC undergo fixation, dehydration and paraffin embedding.

FIXATION

This is a critical step as preservation of morphology is essential for interpretation of IHC. 10% buffered neutral formalin is commonly used. The disadvantage of masking antigens during fixation can be over come by antigen retrieval technique.

According to Elizabeth et al , biopsies fixed for intervals shorter than 6 hours or longer than 72 hours, sample where fixation delayed for more than one hour may not give proper results (63).

ANTIGEN RETRIEVAL

This procedure involves unmasking of the antigens. The following technique can be used.

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

Care should be taken not to allow the section to dry after heating, as this destroys antigenicity. Damage of nuclear details is seen in poorly

fixed tissues. Fibres and fatty tissues tend to detach from the slides while heating.

CONTROLS

Use of control tissue is essential in hormone receptor assays. Ideally, the test block should include normal breast lobules and ducts to provide an internal control population of cells, since a proportion of these should show positive reactivity. Use of internal control cells in this fashion protects against the effects of poor fixation.

HORMONE RECEPTOR

ER and PR are dimeric, gene - regulatory proteins. Estrogen and progesterone are well established endocrine steroid regulators that modulate multiple aspects of mammary gland pathology. These two hormones work together to direct mammary epithelial growth, differentiation and survival. Although both steroids are commonly thought to be of primary importance for tumours arising in the reproductively competent years, between puberty and menopause, local aromatization of adrenal androgens provides additional estrogens in the postmenopausal years. ER and PR belong to super family proteins

whose function is to control the transcription of the receptor of the cellular genes.

Estrogen and Progesterone receptor act through their nuclear receptors to modulate transcription of target genes (54).

ESTROGEN RECEPTORS

ER may exist either in homodimeric or hetero dimeric species, composed of alpha and beta receptors acting as hormone dependent transcriptional regulators (55). ER alpha is of key importance in mammary ductal elongation of puberty. PR and ER beta appears to be more involved with lactational differentiation of the lobules (56).

Over expression of ER alpha is a well established prognostic factor in breast cancer patients. Generally ER alpha positive cancers are associated with slow tumour growth , lower histology grade, DNA diploidy and thus a better over all prognosis.

Estrogen receptors are regarded as cytoplasmic receptors 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 hormone response elements.

PROGESTERONE RECEPTORS

PR is a heterodimeric protein with A and B subunits. Over expression of PR indicates that the ER pathway is intact, even if the tumour is reported as ER negative .

Hormone receptors are well established bio markers in breast carcinoma and their assessment helps in predicting the response to endocrine therapy.

SCORING SYSTEM

Estrogen and progesterone receptors express nuclear positivity. Different scoring systems are available and includes measurements of intensity of staining or percentage of positive cells or a combination of the two.

1. Quick Score

Assigns values to both intensity and proportion of staining (50).

Score for proportion staining

0 - No nuclear staining

1 - < 1% nuclear staining

2 - 1 - 10% nuclear staining

3 - 11 - 33% nuclear staining

4 - 34 - 66% nuclear staining

5 - 67 - 100% nuclear staining

Score for staining intensity

0 - No staining

1 - Weak staining

2 - Moderate staining

3 - Strong staining

This comes to a maximum score of 8.

There are many scoring system but Quick score, which considers both proportion of cells and intensity of staining is used by many laboratories. According to Leake R. Barnes et al., (50) , the results obtained from Quick score correlates well with the biochemical assays and provides significant predictive and prognostic information.

In a study conducted by Thusharie Liyanage, the Quick score appears as reliable scoring system at the therapeutic decision making level and a substantial to almost perfect inter observer agreement was seen in assigning an over all scoring (51).

2. H Score :

This score is based on the percentage of nuclei that stain and the intensity of the staining reaction i.e. based on the summation of proportion of tumour cells showing different degrees of reactivity.

Score :

0 - No reactivity

1 - Weak reaction

2 - Moderate reaction

3 - Strong reaction

This would give a maximum score of 300, if 100 percent of tumour cells shows strong reactivity.

3. Fractionated score (F score)

Six point score by estimating percentage of positive staining tumour cells (52).

Scoring

0 - None

1 - 1 - 10%

2 - 11 - 30%

3 - 31 - 50%

4 - 51 - 70%

5 - 71 - 100%

A percentage of 10% (i.e F score = 1) is chosen as cut-off value to dichotomise the results into positive versus negative.

4. J - Score

Evaluates only positive cell rate without taking the staining intensity into account (53).

Scoring Criteria

0 - No stained cells

1 - Stained cells < 1%

2 - Stained cells 1 - 10%

3 - Stained cells > 10%

Final decision on hormone receptor status

Score 0 - Negative

Score 1 & 2 - Uncertain (Equivocal)

Score 3 - Positive

5. Allred score

Hormone receptor expression was scored by assigning proportion score and intensity scores(57).

Proportion score

0 - None

1 - < 1/100

2 - 1/100 to 1/10

3 - 1/10 to 1/3

4 - 1/3 to 2/3

5 - > 2/3

Intensity score

0 - None

1 - Weak

2 - Intermediate

3 - Strong

The proportion and intensity scores were then added to obtain a total score, which ranges from 0 - 8.

Total Score

0 - 2 Negative

3 - 8 Positive

SIGNIFICANCE OF ER, PR STATUS ASSESSMENT IN BREAST CARCINOMAS

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 more valuable to predict response to therapy.

Patients with hormone receptor positive tumours benefit from adjuvant tamoxifen treatment, regardless of nodal status, menopausal status and age. Both recurrence free survival and breast cancer survival are improved (62).

According to Osborne et al., patient with ER + and PR + tumours have 78% response, those with ER + PR - have 34% response, those with ER -ve PR + have 45% response and ER - PR - tumours have 10% response to hormone therapy (65).

MATERIALS AND METHODS

A total of 73 mastectomy specimens were received in the Department of Pathology, Kilpauk Medical College, from the Department of Surgery between July 2008 and September 2010.

A detailed history regarding age, parity, socio economic status, family history and menstrual history were reviewed in all cases.

Inclusion criteria :

All female patients who underwent mastectomy irrespective of age and proved to be malignant histologically were included for study.

Exclusion criteria :

Excision and incision biopsies , proven to be malignant histologically, were not included in the study.

Of the 73 cases, ER, PR study was done for 55 cases. All the mastectomy specimens received were properly sliced and fixed in 10% formalin for 18 - 24 hours. Detailed gross examination pertaining to over all size of the specimen, nipple and areola, margin status and nodal status were carefully studied.

Histological grading was done by modified Bloom and Richardson scoring system.

Representative samples are taken from tumour, margins, nipple and areola and lymph nodes. The tissues were processed in various grades of alcohol and xylol using automated histokinette. Paraffin blocks were prepared and sections of 5micron thickness were cut in microtome using disposable blades and stained with hematoxylin and eosin. Suitable blocks were chosen for IHC.

Immunohistochemistry

Sections for Immunohistochemistry were also cut in microtome using disposable blades. Slides coated with chrome alum were used. Sections were subjected to antigen retrieval using pressure cooker technique using citrate retrieval solution (pH 6) and then treated by Horse Radish Peroxidase (HRP) polymer techniques.

Methodology

Coated slides after antigen retrieval were taken through following stages.

1. Treatment with peroxidase block for inhibiting endogenous peroxidases in the tissue for 5 minutes.

2. Washed twice in TRIS buffer for 5 minutes .

3. Application of power block for blocking non-specific antigen- antibody reaction for 5 minutes.

4. Washed twice in TRIS buffer for 5 minutes.

5. Application of primary antibody for 60 minutes.

6. Washed twice in TRIS buffer for 5 minutes.

7. Application of secondary antibody with the tagged Horse Radish Peroxidase enzyme for 30 minutes.

8. Washed twice in TRIS buffer for 5 minutes.

9. Application of super enhancer for 30 minutes which enhances the final reaction product by increasing the sensitivity of antigen - antibody reaction.

10. Washed twice in TRIS buffer for 5 minutes.

11. Application of DAB (Diamino benzidine) chromogen for 5 minutes - this is cleaved by enzyme to give coloured product at the antigen sides.

12. Washed in distilled water for 5 minutes.

13. Slides are counter stained with hematoxylin.

14. Air dried and mounted with DPX .

Scoring system

Scoring done by Quick Score System

Score for proportion staining

0 - No nuclear staining

1 - < 1% nuclear staining

2 - 1 - 10% nuclear staining

3 - 11 - 33% nuclear staining

4 - 34 - 66% nuclear staining

5 - 67 - 100% nuclear staining

Score for staining intensity

0 - No staining

1 - Weak staining

2 - Moderate staining

3 - Strong staining

Scores are summed to give a maximum score of 8.

OBSERVATION AND RESULTS

TABLE - 1

AGE DISTRIBUTION OF BREAST CARCINOMA

AGE (years)	CASES	
	NUMBER	%
21-30	2	2.7
31-40	12	16.4
41-50	27	37
51-60	21	28.8
61-70	7	9.6
71-80	4	5.5
TOTAL	73	100
MEAN	**50.18**	

Table 1 shows the incidence of breast carcinoma in different age groups in our study. The youngest patient was 28 years old and the oldest patient was 80 years old. Maximum number of cases were seen in 41-50 years age group. Mean age was 50.18 years. 80% of the cases were more than 40 years.

CHART - 1

AGE DISTRIBUTION OF BREAST CARCINOMA

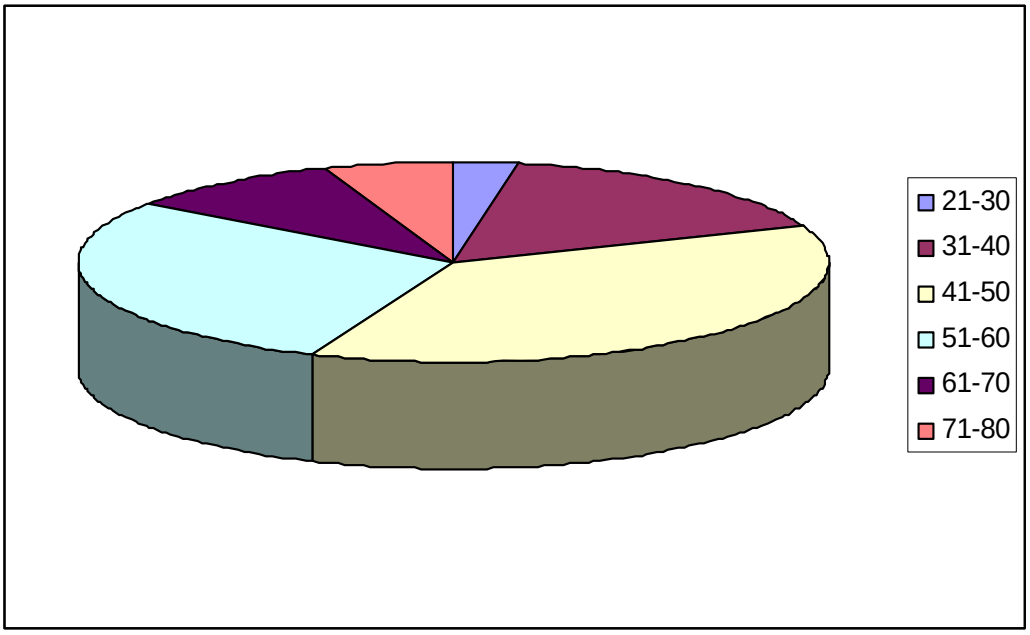

TABLE - 2

MENSTRUAL STATUS IN BREAST CARCINOMAS

MENSTRUAL STATUS	NO. OF CASES	%
PREMENOPAUSAL	32	43.8
POSTMENOPAUSAL	41	56.2

Table 6 shows number of cases in premenopausal and postmenopausal age groups. Majority of cases were postmenopausal.

CHART - 2

MENSTRUAL STATUS IN BREAST CARCINOMAS

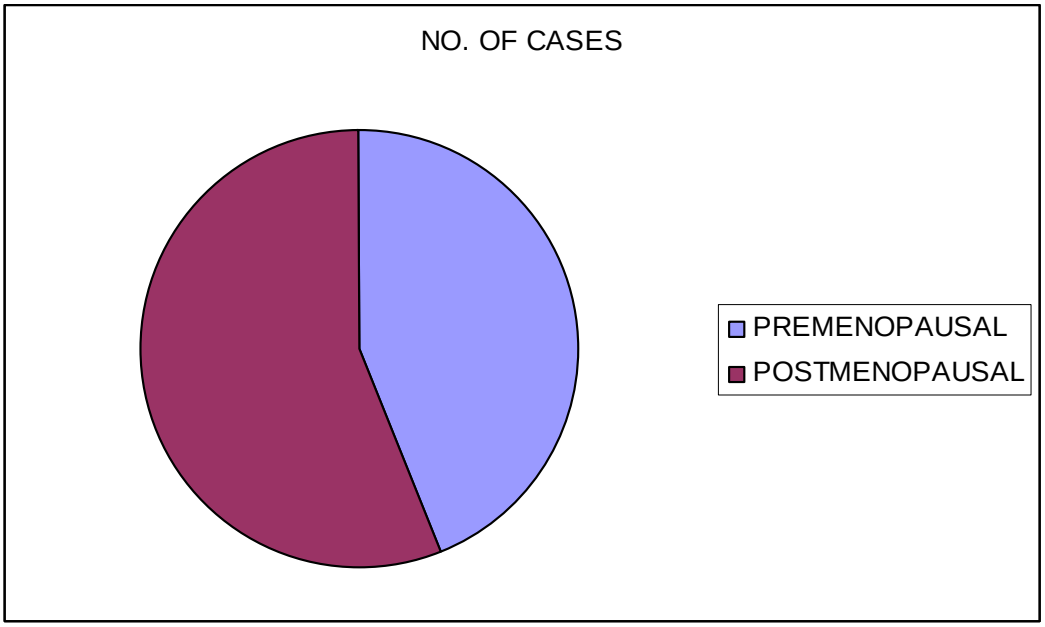

TABLE - 3

CLINICAL STAGEWISE DISTRIBUTION IN BREAST CARCINOMAS

S.NO	STAGE	CASES	
		NUMBER	%
1	1	6	8.2
2	2	48	65.8
3	3	19	26
4	4	NIL	NIL

Table 2 shows percentage of cases in each stage in our study. Maximum number of cases were stage 2. None of our cases were of stage 4.

CHART - 3

CLINICAL STAGEWISE DISTRIBUTION IN BREAST CARCINOMAS

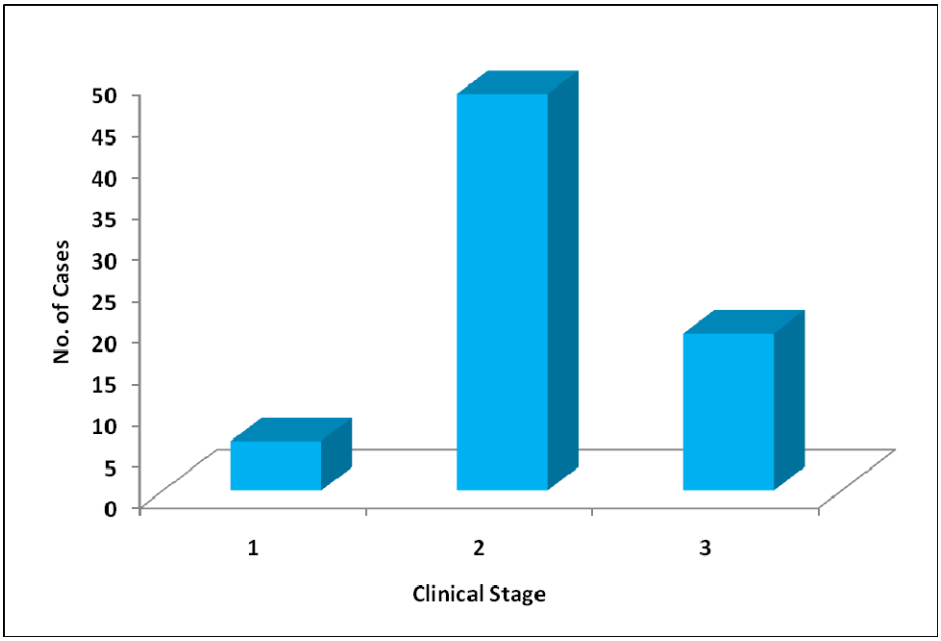

TABLE - 4

HISTOLOGICAL GRADEWISE DISTRIBUTION OF BREAST CARCINOMAS

S.NO.	GRADE	CASES	
		NUMBER	%
1	1	7	9.6
2	2	53	72.6
3	3	13	17.8

Table 3 shows percentage of cases in each grade. Maximum number of cases are grade 2.

CHART - 4

HISTOLOGICAL GRADEWISE DISTRIBUTION OF BREAST CARCINOMAS

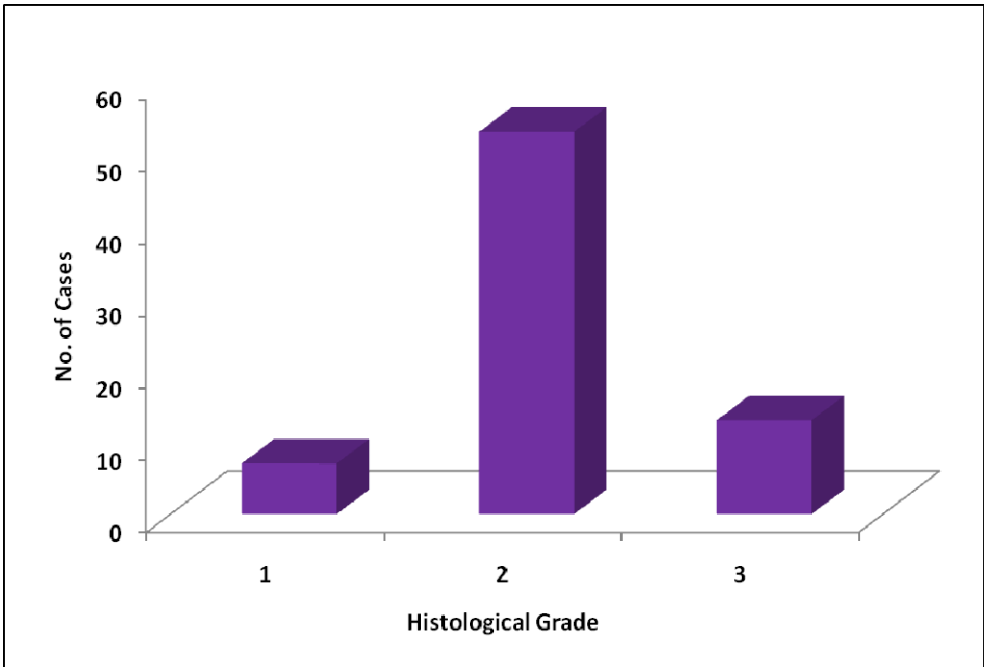

TABLE - 5

LYMPH NODE STAGEWISE DISTRIBUTION OF BREAST CARCINOMAS

S.NO.	LYMPH NODE STAGE	CASES	
		NUMBER	%
1	1	30	41.1
2	2	25	34.2
3	3	18	24.7

Table 4 shows percentage of cases in each lymph node stage. Maximum number of cases were in stage 1.

CHART - 5

LYMPH NODE STAGEWISE DISTRIBUTION OF BREAST CARCINOMAS

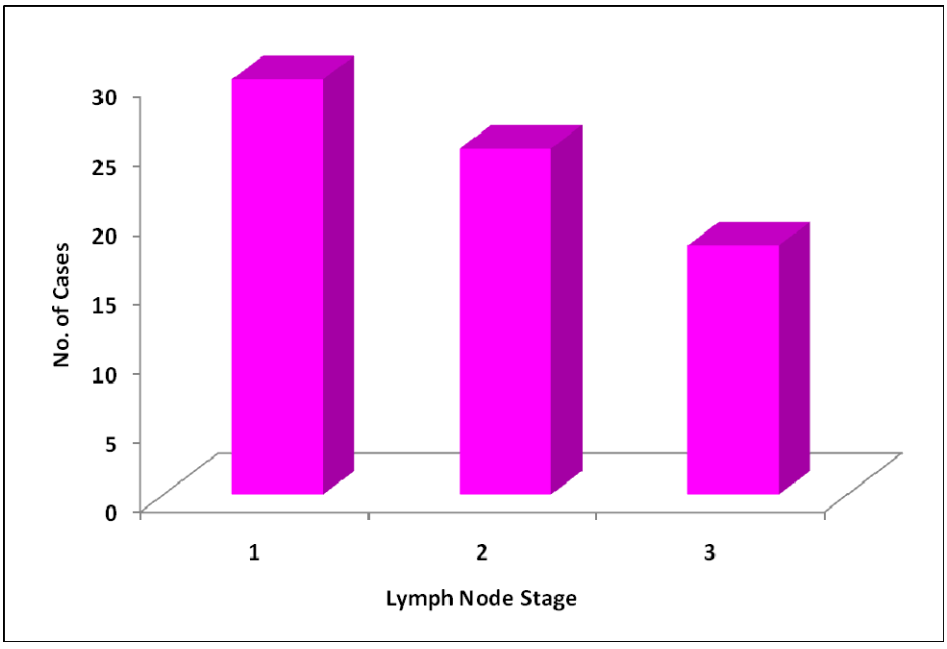

TABLE - 6

NPI DISTRIBUTION IN BREAST CARCINOMAS

S.NO.	NPI	CASES	
		NUMBER	%
1	GOOD PROGNOSIS	5	6.9
2	MODERATE PROGNOSIS	46	63
3	POOR PROGNOSIS	22	30.1

Table 5 shows percentage of cases belonging to each group of NPI score. Majority of cases were having moderate prognosis.

CHART - 6

NPI DISTRIBUTION IN BREAST CARCINOMAS

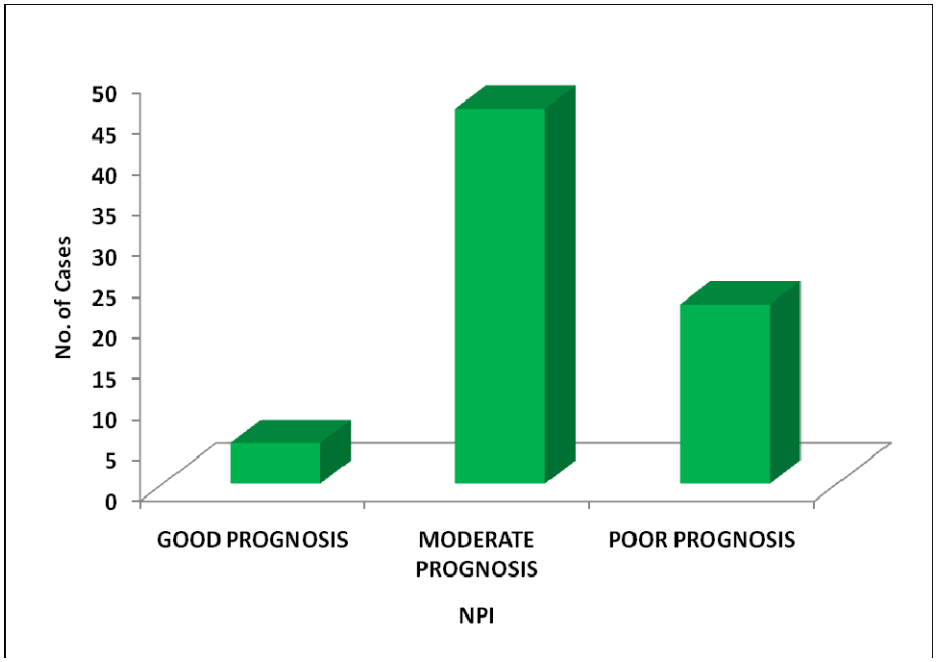

TABLE - 7

CORRELATION OF CLINICAL STAGING WITH NPI SCORE

STAGE	GOOD PROGNOSIS	MODERATE PROGNOSIS	POOR PROGNOSIS
1	2	4	NIL
2	2	38	9
3	1	4	13
4	NIL	NIL	NIL

Table 8 shows correlation between clinical staging and NPI score. There is statistically significant correlation between the two variables with a **p value of 0.001.** Majority of poor prognosis cases are of grade 3.

TABLE - 8

ER, PR STATUS IN BREAST CARCINOMAS

S.NO.	ER,PR STATUS	NO.OF CASES	%
1	ER+,PR+	9	16.4
2	ER+,PR-	8	14.5
3	ER-,PR+	5	9.1
4	ER-,PR-	33	60

Table 7 shows ER,PR status in breast carcinomas.16.4% were positive for both. 14.5% were ER positive but PR negative. 9.1% cases were ER negative but PR positive. 60% of the cases were negative for both the receptors. ER positivity is seen in 30.9% of cases and PR positivity is seen in 25.5% of the cases.

CHART - 8

ER, PR STATUS IN BREAST CARCINOMAS

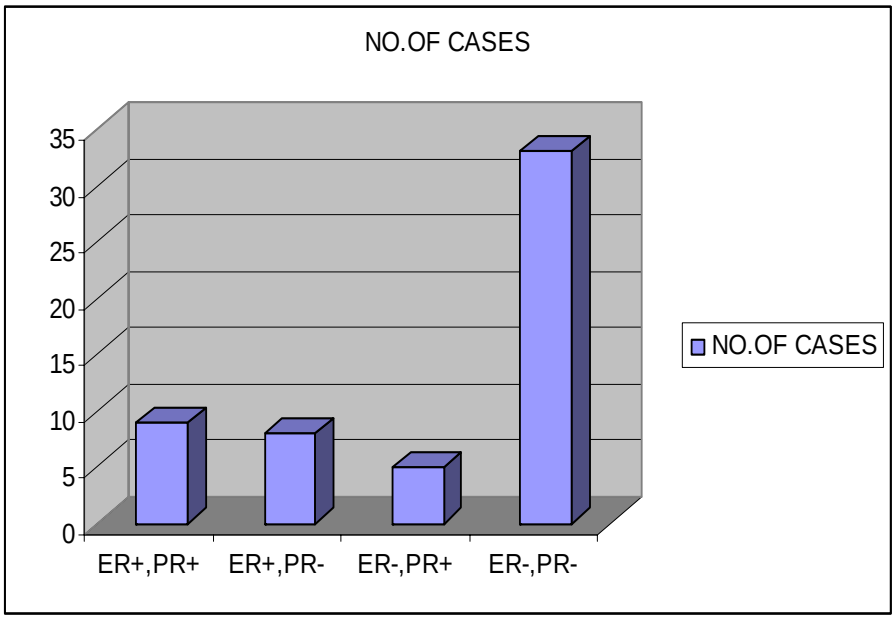

TABLE - 9

CORRELATION OF HISTOLOGICAL GRADING WITH ER, PR STATUS

S.NO	GRADE	NO.OF CASES	ER/PR + CASES
1	1	7	6
2	2	40	16
3	3	8	2

Table 9 shows correlation between histological grading and ER, PR status. There is a statistically significant correlation between the two variables with a **p value of 0.01.**

CHART - 9

CORRELATION OF HISTOLOGICAL GRADING WITH ER, PR STATUS

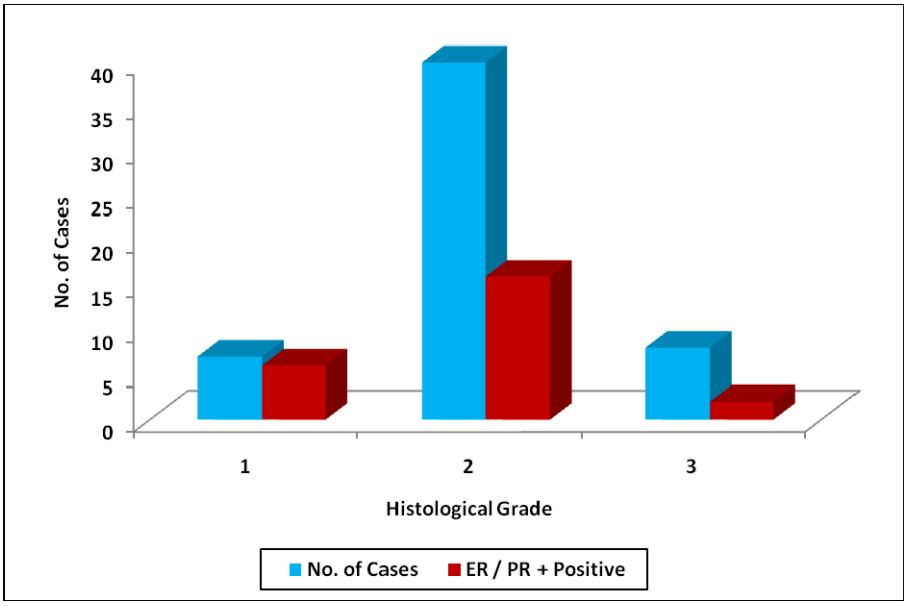

TABLE - 10

CORRELATION OF TUMOR SIZE WITH ER, PR STATUS

S.NO	TUMOR SIZE (in cm)	NO.OF CASES	ER/PR+ CASES	%
1	1-2	10	5	50
2	>2-5	39	16	41
3	>5	3	1	33.3

Table 10 shows correlation between tumor size and ER, PR status. Percentage of ER/PR positivity decreases with increase in tumor size.

TABLE - 11

CORRELATION OF LYMPH NODE STAGE WITH ER, PR STATUS

S.NO	LYMPH NODE STAGE	NO.OF CASES	ER/PR + CASES
1	1	24	7
2	2	16	8
3	3	15	7

Table 11 shows correlation between ER, PR status and lymph node stage. There is no statistically significant correlation between the two variables.

TABLE - 12

CORRELATION OF CLINICAL STAGING WITH ER, PR STATUS

S.NO	STAGE	NO.OF CASES	ER/PR+ CASES
1	1	5	2
2	2	38	14
3	3	12	6
4	4	NIL	NIL

Table 12 shows correlation between ER, PR status and clinical stage. There is no statistically significant correlation between the two variables.

TABLE - 13

CORRELATION OF NPI SCORE WITH ER, PR STATUS

S.NO.	NPI	NO.OF CASES	ER/PR+
1	GOOD PROGNOSIS	3	2
2	MODERATE PROGNOSIS	37	14
3	POOR PROGNOSIS	15	6

Table 13 shows correlation between ER, PR status and NPI score. There is no statistically significant correlation between the two variables.

DISCUSSION

Incidence of breast carcinoma is increasing in India. Prognosis is related to a variety of clinical, pathological and molecular features which include stage of the carcinoma, histologic type, grade and lymph node metastasis. Estrogen and progesterone receptors, have with increasing importance, influenced the management of this malignancy.

AGE DISTRIBUTION:

As seen in Table 1, mean age of patients included in our study was 50.18years. 80.9% of the cases were more than 40years of age. Maximum number of cases were in the age group of 41-50years.

This is less than the observation made by RhodesDT et al, who found more than 75% of the cases were above 50years and the mean age was 64 years(67).

But usually in Asian countries breast carcinoma occurs a decade earlier. Our results are in concordance with the study conducted by Lakmini.K.B.Mudduwa in which mean age was 52.5 years and 85.7% of the patients were more than 40years.

MENSTRUAL STATUS:

As shown in Table 6, 56.2% of the patients were postmenopausal women. This is in concordance with the study conducted by Louis.W.C.Chow et al, in which 52% of the women were postmenopausal(68) and Col V Dutta et al, in which 59% of the cases were postmenopausal women(69).

HORMONE RECEPTOR STATUS IN BREAST CARCINOMAS:

The hormone receptor status of breast carcinoma can predict the response to adjuvant endocrine therapy.

In a study conducted by Priti Lal et al at NewYork with 3655 breast carcinomas, ER was positive in 71.6% and PR in 47.4%(66).

Mehedad Nadji et al found in Miami with 5993 breast cancers ,that ER was positive in 75% of the cases and PR in 55% of the cases(74).

Li CI et al from Seattle conducted a study between 1992 to 1998 and found ER positivity in 77.5% and PR positivity in 67.7%(58).

These are some of the studies conducted in western population.

According to Lakhmini K.B.Mudduwa the prevelance of hormone receptor positive breast cancer in Asian countries has found to be lower

than western world where more than 50% tumors express hormone receptors(64). However the number of studies performed on this topic is much less in the Asian communities compared with the western world.

Ljiljana Hulpic et al conducted a study in Croatia with 242 cases and found ER positivity in 37.5%, PR positivity in 40.6% of the cases(47).

Azizun Nisa et al studied 150 cases in Karachi and found that ER and PR was positive in 32.7% and 25.3% of the cases respectively(73).

In a study conducted by Desai SB et al in India of 798 cases ER was positive in 32.6% of the tumors and PR was positive in 46.1% of the cases(59).

Col V Dutta et al conducted a study in Army Hospital and Research centre in New Delhi and found that out of 75 cases, 24% were ER positive and 30% were PR positive(69).

In this study 40% of the cases were either ER or PR positive and 60% of the cases are negative for both the receptors. ER is positive in 30.9% of the cases and PR is positive in 25.5% of the cases.

These results are not in concordance with the studies conducted in western population.

But the results of our study are in concordance with studies conducted in Asian population and one study of western population . The overall positivity rate for ER and PR is lower possibly because of the difference in techniques of evaluation (70), high tumour grades and majority being menopausal women in our study.

Nulliparity, late age at first birth, early age at menarche, higher body mass index and the use of hormone replacement therapy have all been associated with increased risk of developing an ER + tumour but with a decreased risk of developing an ER- tumour. Young patients have high levels of circulating oestrogens and a correspondingly low expression of steroid receptors, which is reflected in their tumours. There appears to be a variation in steroid receptor positivity in the Asian population (69).

CORRELATION OF HORMONE RECEPTOR POSITIVITY WITH OTHER PROGNOSTIC VARIABLES:

In this study there is a statistically significant association between ER, PR status and histological grade. Hormone receptor expression decreases with increasing tumor size but no statistically significant association between the two variables. No significant of ER, PR status with clinical staging, lymph node metastasis and NPI score noted.

Lakmini.K.B.Mudduwa has found a significant inverse relation ship with the grade and ER, PR expression in his study. His study also shows no significant association of hormone receptor status with tumor size and lymph node metastases(64).

Ana Lucia Amaral Eisenberg et al in Brazil also has established a significant correlation between ER, PR status and histological grade(61).

Col.V.Dutta in India observed that the reactivity for steroid receptor decreases with increasing grade but no significant association with other variables like lymph node metastases, tumor size(69).

Ljiljana Hupic has found no statistically significant association between ER, PR status and NPI score in concordance with our study but in contrast to this study there is a significant association with lymph node metastases(47).

Kenneth McCarty and Rosemary.R.Millis et al have also obtained similar results of association between ER, PR status and histological grade but no association with other prognostic variables (71,72).

This study shows results of association between ER, PR status and other prognostic variables comparable to most of the studies conducted especially in Asian population.

SUMMARY AND CONCLUSION

73 cases of mastectomy specimens were received and clinical staging, histological grading and NPI score were analysed for these cases. 55 cases were selected at random and ER, PR status was analysed using Quick score.

Greater than 80% of the cases were 40 years and above and majority were postmenopausal. Maximum number of cases were stage 2 and grade 2 with majority having no lymph node metastases.

ER was positive in 30.9% and PR in 25.5%, as the prevalence of hormone receptor positive breast cancers is less in the study population of Asian women compared with western world. There was a statistically significant association between hormone receptor expression and histological grade but not with other prognostic factors.

Presence of hormone receptors correlates well with response to hormone therapy. There is a significant decrease in mortality and tumor recurrences with hormone therapy. So, determination of ER, PR status is essential in all cases irrespective of clinical staging and lymph node metastasis.

MASTER CHART

S. No.	Bx. No.	Age	Menstrual Status	Tumor size	Lymph node stage	Stage	Grade	NPI	ER	PR	TUMOR TYPE
1	1872/08	54	post menopausal	5	4	3A	2	6	negative	negative	IDC-NOS
2	2162/08	38	pre menopausal	3	6	2B	2	5.6	negative	negative	IDC-NOS
3	2206/08	50	pre menopausal	3	NIL	2A	2	3.6	negative	negative	IDC-NOS
4	2374/08	50	post menopausal	7	4	3A	3	7.4	negative	negative	IDC-NOS
5	2502/08	50	post menopausal	4	1	2B	2	4.8	negative	negative	IDC-NOS
6	2579/08	60	post menopausal	4	3	2B	2	4.8	negative	negative	Mucinous CA
7	2812/08	41	pre menopausal	4	NIL	2A	2	3.8	negative	negative	IDC-NOS
8	3032/08	45	pre menopausal	2	NIL	1	2	3.4	negative	negative	IDC-NOS
9	3069/08	55	post menopausal	3	NIL	2A	2	3.6	negative	negative	Mucinous CA
10	3085/08	31	pre menopausal	4	9	3A	2	5.8	negative	negative	IDC-NOS
11	3100/08	46	pre menopausal	4	NIL	2A	3	4.8	negative	negative	IDC-NOS
12	3240/08	50	post menopausal	4	NIL	2A	2	3.8	negative	negative	IDC-NOS
13	3594/08	53	post menopausal	5	NIL	2B	3	5	negative	negative	IDC-NOS
14	3748/08	55	post menopausal	3	4	2B	2	5.6	negative	negative	IDC-NOS
15	3926/08	50	pre menopausal	2.5	NIL	2A	2	3.5	negative	negative	IDC-NOS
16	0012/09	53	post menopausal	5	4	3A	2	6	negative	positive	IDC-NOS
17	56/09	28	pre menopausal	5	NIL	2B	2	4	negative	negative	IDC-NOS
18	95/09	53	post menopausal	3	1	2A	2	4.6	negative	negative	IDC-NOS
19	481/09	52	post menopausal	4	NIL	2A	2	3.8	negative	negative	IDC-NOS
20	485/09	50	pre menopausal	5	2	3A	2	5	negative	negative	IDC-NOS
21	562/09	43	pre menopausal	3	NIL	2B	2	3.6	positive	negative	IDC-NOS
22	645/09	60	post menopausal	3	NIL	2A	1	2.6	positive	negative	Papillary CA
23	659/09	34	pre menopausal	3	1	2B	1	3.6	negative	positive	IDC-NOS
24	782/09	56	post menopausal	3	1	2B	2	4.6	negative	negative	IDC-NOS

S. No.	Bx. No.	Age	Menstrual Status	Tumor size	Lymph node stage	Stage	Grade	NPI	ER	PR	TUMOR TYPE
25	1104/09	44	pre menopausal	5	2	3A	2	5	negative	negative	IDC-NOS
26	1283/09	53	post menopausal	4	7	3A	2	5.8	positive	negative	IDC-NOS
27	1301/09	45	pre menopausal	5	4	2B	2	6	positive	positive	IDC-NOS
28	1329/09	57	post menopausal	7	4	3A	1	5.4	negative	positive	IDC-NOS
29	1396/09	45	pre menopausal	3	3	2B	2	4.6	positive	negative	IDC-NOS
30	1645/09	72	post menopausal	3	9	2A	2	5.6	negative	negative	IDC-NOS
31	1731/09	46	pre menopausal	3	4	2B	2	5.6	negative	negative	IDC-NOS
32	1788/09	75	post menopausal	3	NIL	2A	2	3.6	negative	positive	IDC-NOS
33	1874/09	45	pre menopausal	2	3	2A	2	4.4	positive	positive	IDC-NOS
34	1879/09	43	pre menopausal	6	2	3A	3	6.2	positive	positive	IDC-NOS
35	1737/09	45	pre menopausal	2	3	2A	1	3.4	positive	negative	IDC-NOS
36	1948/09	50	pre menopausal	7	NIL	2B	2	4.4	positive	negative	IDC-NOS
37	2028/09	55	post menopausal	2	7	2A	2	5.4	negative	positive	IDC-NOS
38	2037/09	80	post menopausal	5	4	3A	2	6	positive	negative	IDC-NOS
39	2110/09	55	post menopausal	2	NIL	1	2	3.4	negative	negative	IDC-NOS
40	2130/09	65	post menopausal	1	NIL	1	1	2.2	positive	positive	IDC-NOS
41	2134/09	52	post menopausal	1	2	2A	1	3.2	negative	negative	IDC-NOS
42	2154/09	65	post menopausal	8	NIL	2B	2	4.6	negative	negative	IDC-NOS
43	2156/09	45	pre menopausal	2	3	2A	2	4.4	negative	negative	IDC-NOS
44	2255/09	47	pre menopausal	8	2	3A	2	5.6			IDC-NOS
45	2345/09	50	post menopausal	3	NIL	2A	3	4.6			IDC-NOS
46	2347/09	50	post menopausal	5	5	3A	3	7	negative	negative	IDC-NOS
47	2744/09	48	post menopausal	4	NIL	2A	2	3.8			IDC-NOS
48	2490/09	52	post menopausal	1	NIL	1	2	3.2			IDC-NOS
49	2558/09	45	post menopausal	4	1	2B	3	5.8			IDC-NOS
50	2563/09	50	post menopausal	3	NIL	2A	2	3.6	negative	negative	IDC-NOS

S. No.	Bx. No.	Age	Menstrual Status	Tumor size	Lymph node stage	Stage	Grade	NPI	ER	PR	TUMOR TYPE
51	2769/09	37	pre menopausal	4	2	2B	3	5.8			IDC-NOS
52	2655/09	55	post menopausal	7	NIL	2B	2	4.4	negative	negative	IDC-NOS
53	2791/09	55	post menopausal	3	NIL	3B	1	2.6			IDC-NOS
54	2845/09	36	pre menopausal	4	1	2A	2	4.8	positive	positive	IDC-NOS
55	0030/10	40	pre menopausal	4	1	2B	3	5.8	negative	negative	IDC-NOS
56	43/10	35	pre menopausal	3	7	3A	3	6.6	positive	positive	IDC-NOS
57	318/10	63	post menopausal	4	1	2B	2	4.8	positive	positive	IDC-NOS
58	334/10	65	post menopausal	1.5	NIL	1	3	4.3	positive	positive	IDC-NOS
59	487/10	30	pre menopausal	4	NIL	2A	2	3.8	positive	positive	IDC-NOS
60	576/10	34	pre menopausal	3	NIL	2A	2	3.6	negative	negative	IDC-NOS
61	896/10	34	pre menopausal	2	NIL	1	2	3.4	negative	negative	IDC-NOS
62	1059/10	40	pre menopausal	3	1	2B	2	3.6	positive	negative	IDC-NOS
63	1132/10	45	post menopausal	2	2	2A	2	4.4			IDC-NOS
64	1172/10	75	post menopausal	8	5	3A	2	6.6			IDC with Neuroendocrine Differentiation
65	1286/10	55	post menopausal	5	4	3A	2	6			IDC-NOS
66	1240/10	65	post menopausal	4	1	2B	2	4.8			IDC-NOS
67	1286/10	55	post menopausal	5	4	3A	2	6			IDC-NOS
68	1385/10	37	pre menopausal	7	NIL	3B	2	4.4			IDC-NOS
69	1436/10	52	post menopausal	2	1	2A	2	4.4			IDC-NOS
70	1449/10	36	pre menopausal	2	3	2A	3	5.4			IDC-NOS
71	1471/10	70	post menopausal	2.5	NIL	2A	2	3.5			IDC-NOS
72	1472/10	68	post menopausal	3	NIL	2A	2	3.6			IDC-NOS
73	1648/10	45	pre menopausal	2.5	3	2B	3	5.5			IDC-NOS

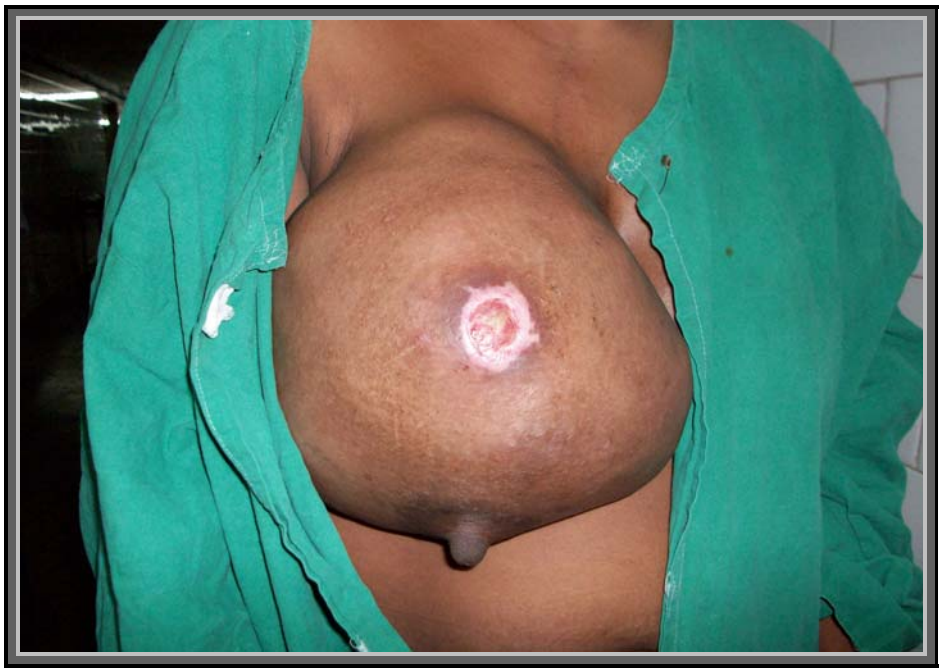

Fig.1 : 50/F, HARD PALPABLE MASS IN RIGHT BREAST INVOLVING ALL QUADRANTS AND SKIN INVOLVEMENT

Fig.2 : 46/F, HARD PALPABLE MASS IN LEFT BREAST INVOLVING UPPER QUADRANT

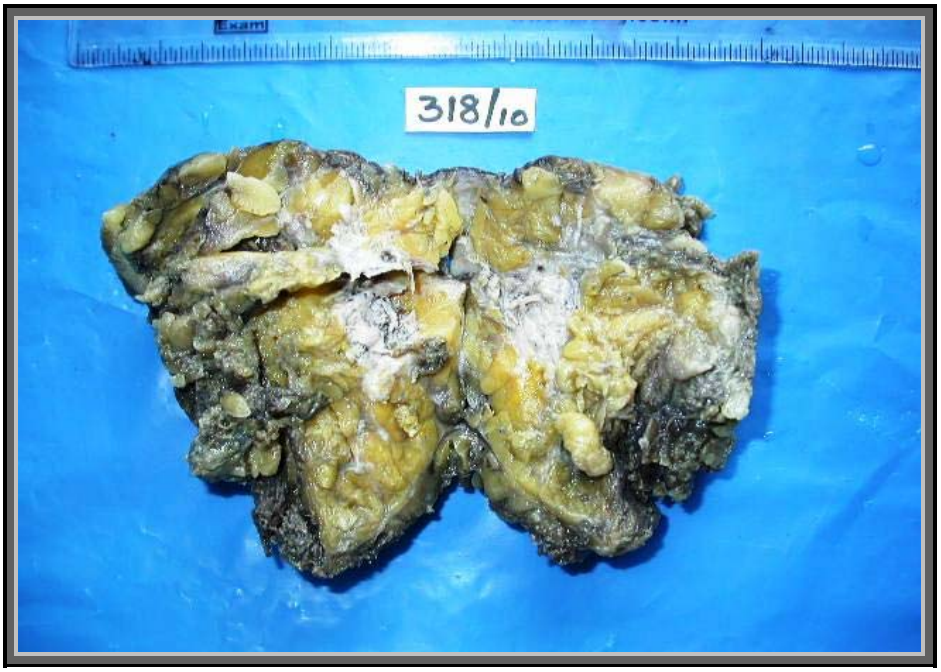

Fig. 3 : MRM SPECIMEN SHOWING A GROWTH MEASURING 4X3CM

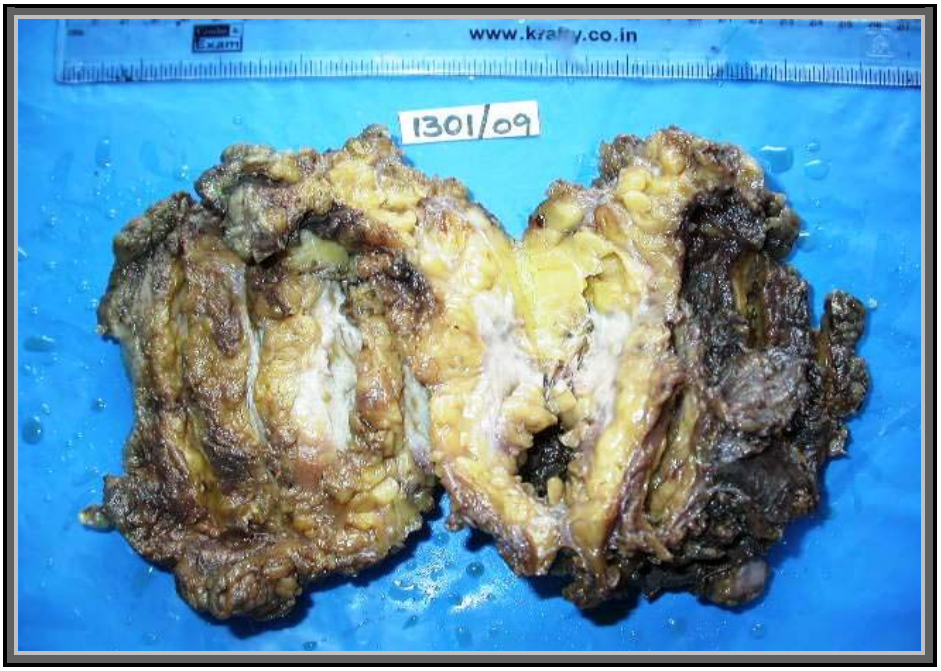

Fig. 4 : MRM SPECIMEN SHOWING A GROWTH MEASURING 5X4CM

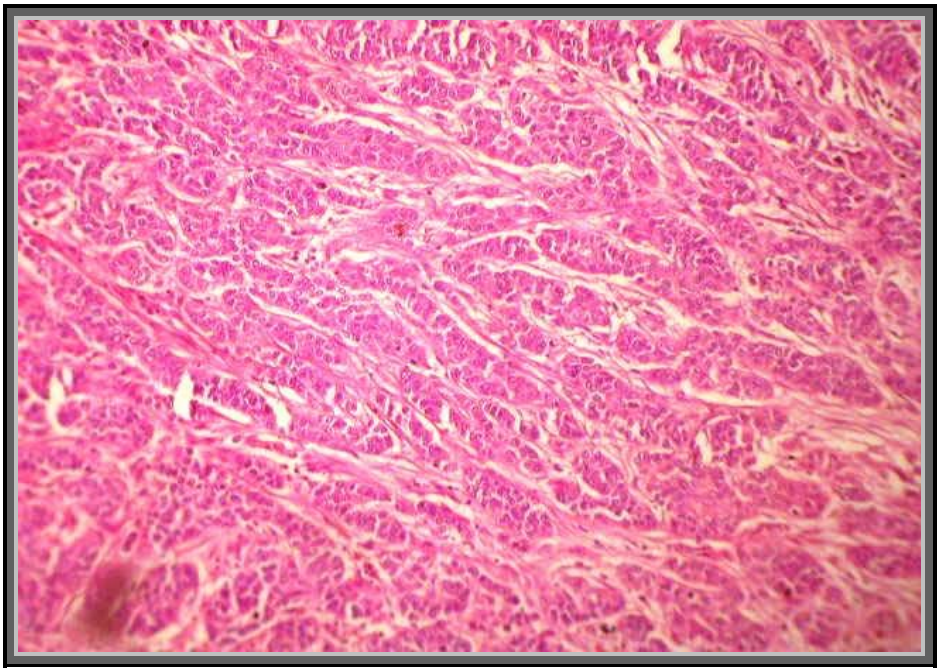

Fig. 5 : IDC-NOS, GRADE1, LOW POWER

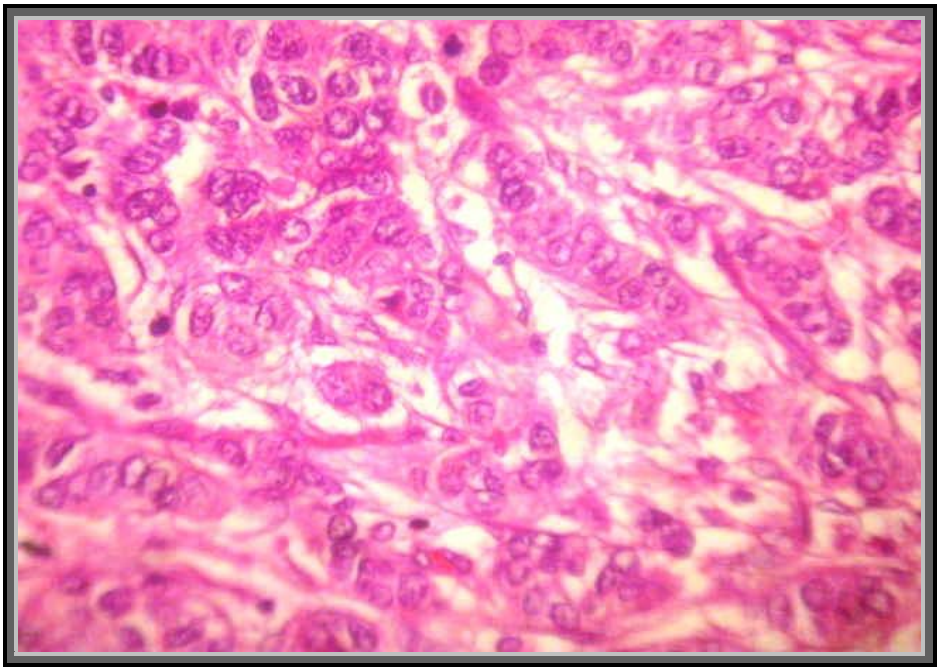

Fig. 6 : IDC-NOS, GRADE1, HIGH POWER

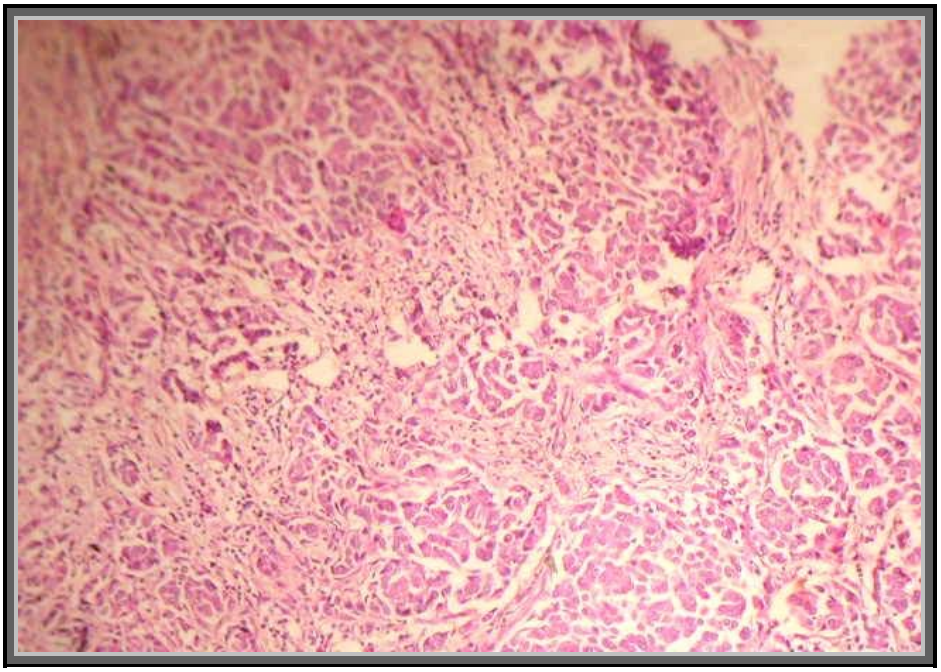

Fig. 7 : IDC-NOS, GRADE2, LOW POWER

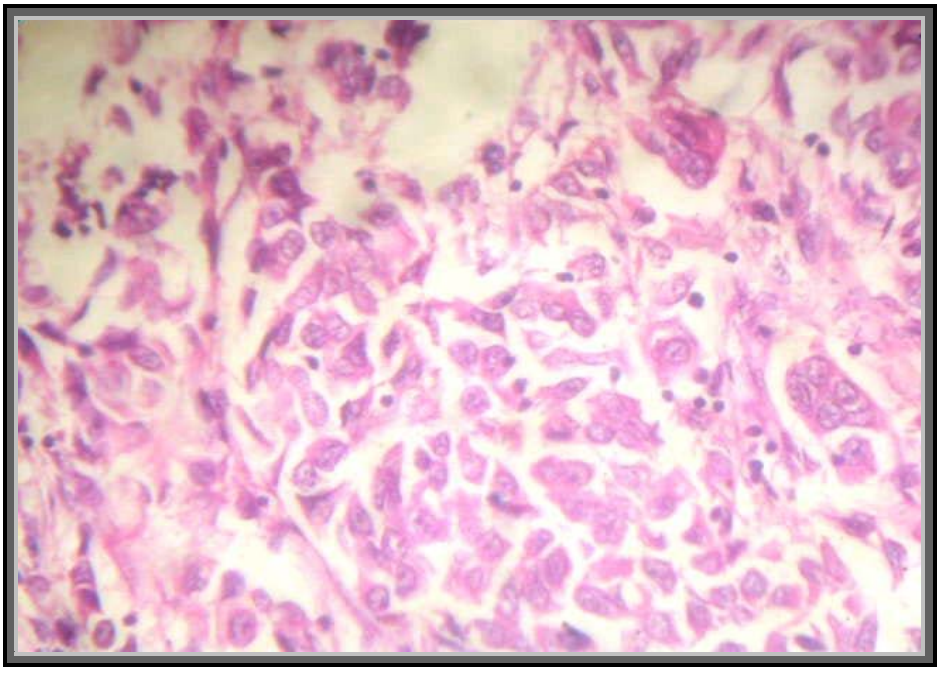

Fig. 8 : IDC-NOS, GRADE2, HIGH POWER

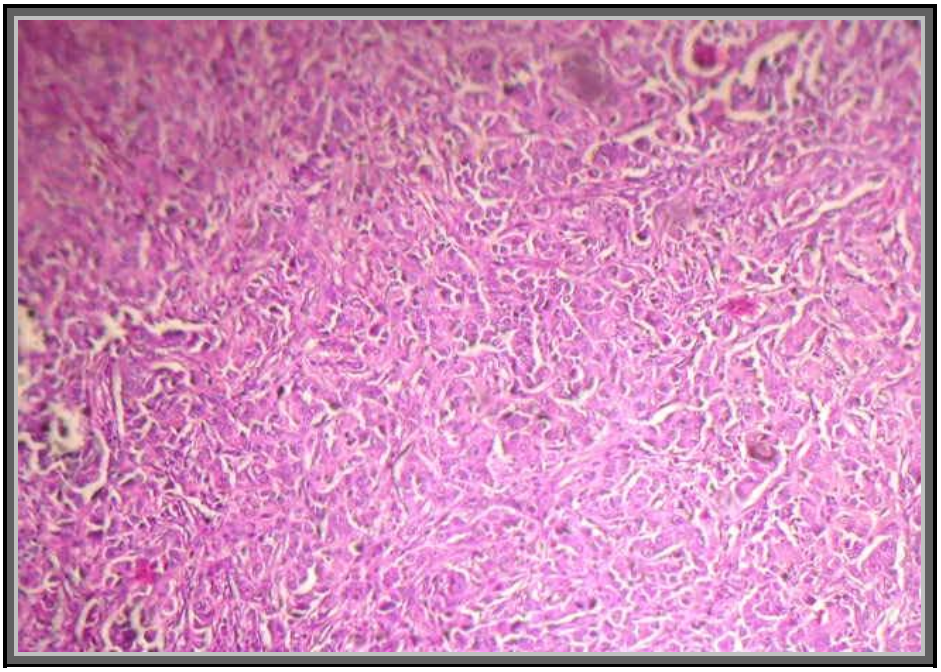

Fig. 9 : IDC-NOS, GRADE3, LOW POWER

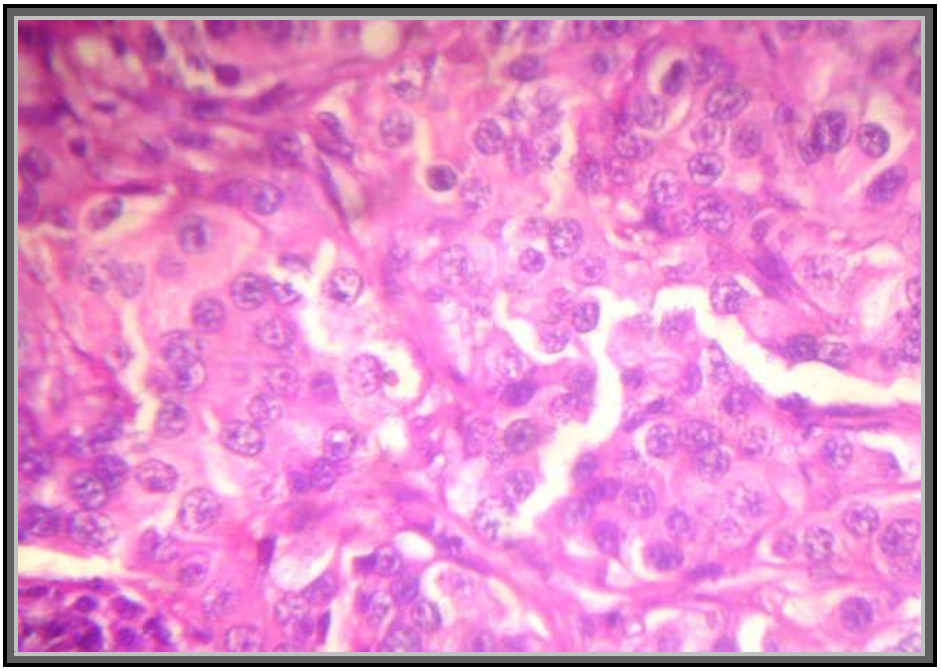

Fig. 10 : IDC-NOS, GRADE3, HIGH POWER

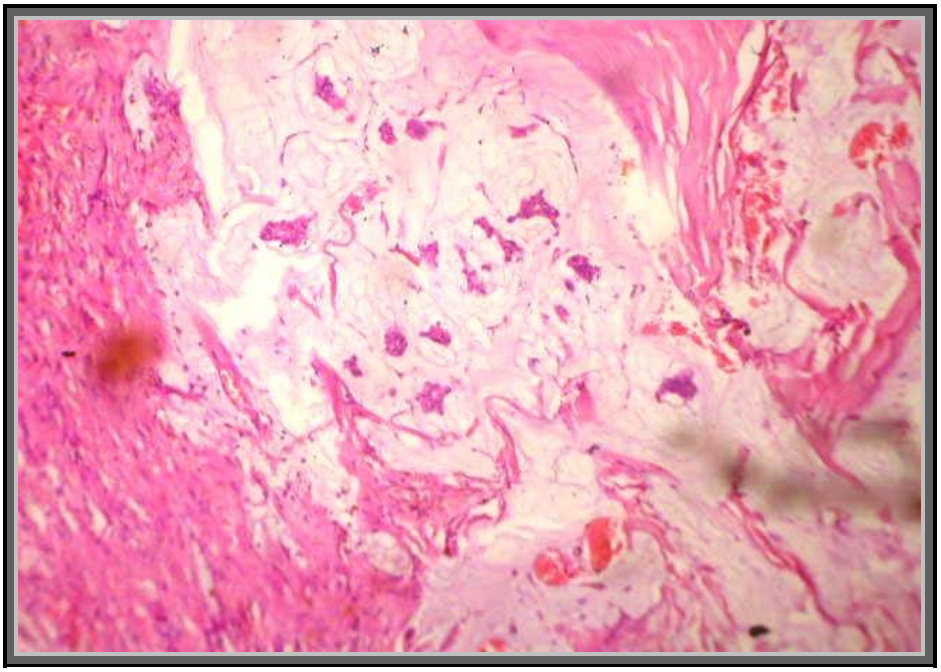

Fig. 11 : MUCINOUS CARCINOMA, LOW POWER

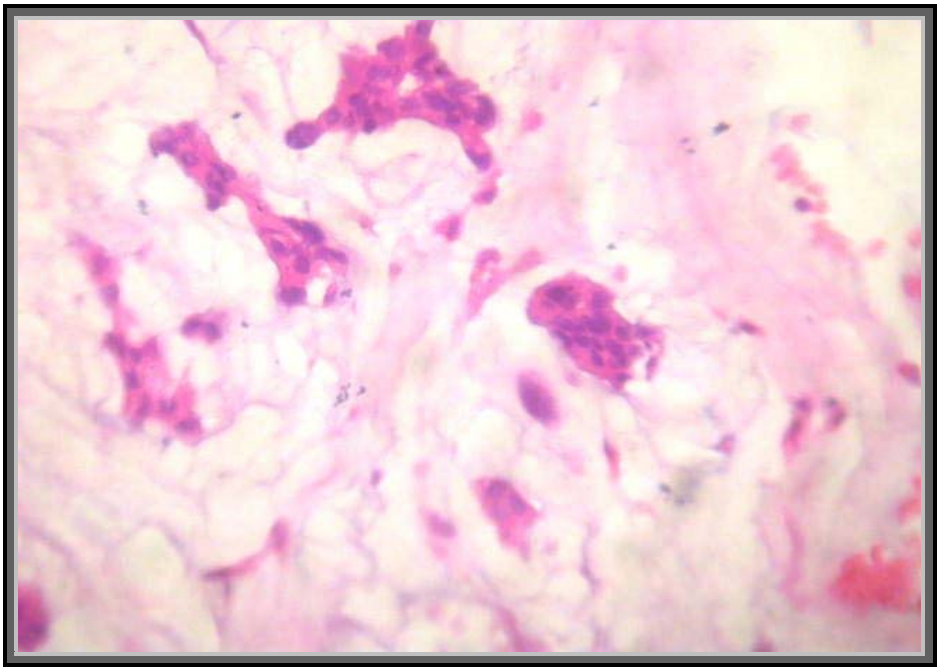

Fig. 12 : MUCINOUS CARCINOMA, HIGH POWER

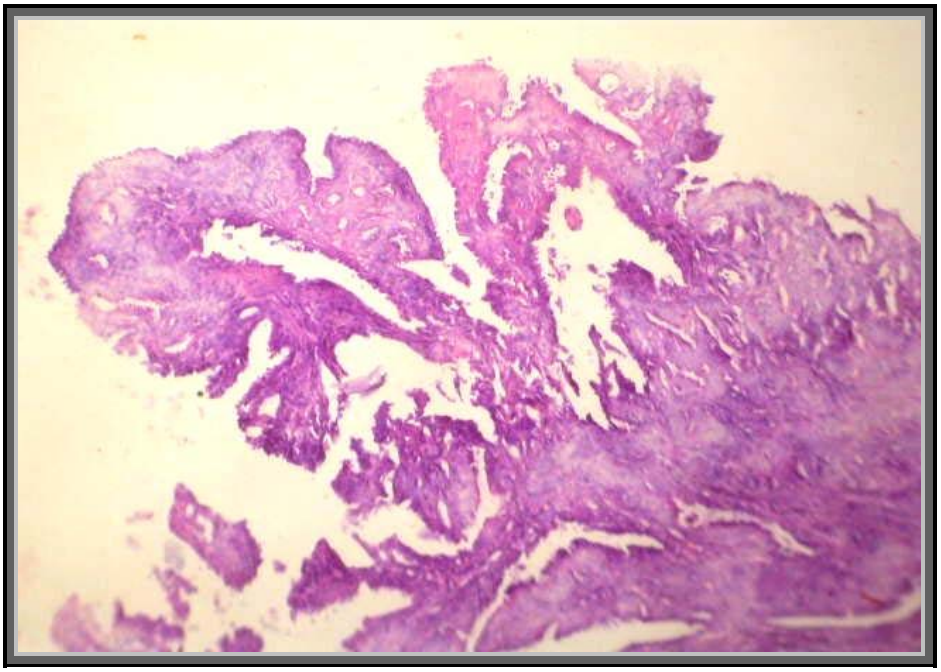

Fig. 13 : PAPILLARY CARCINOMA, LOW POWER

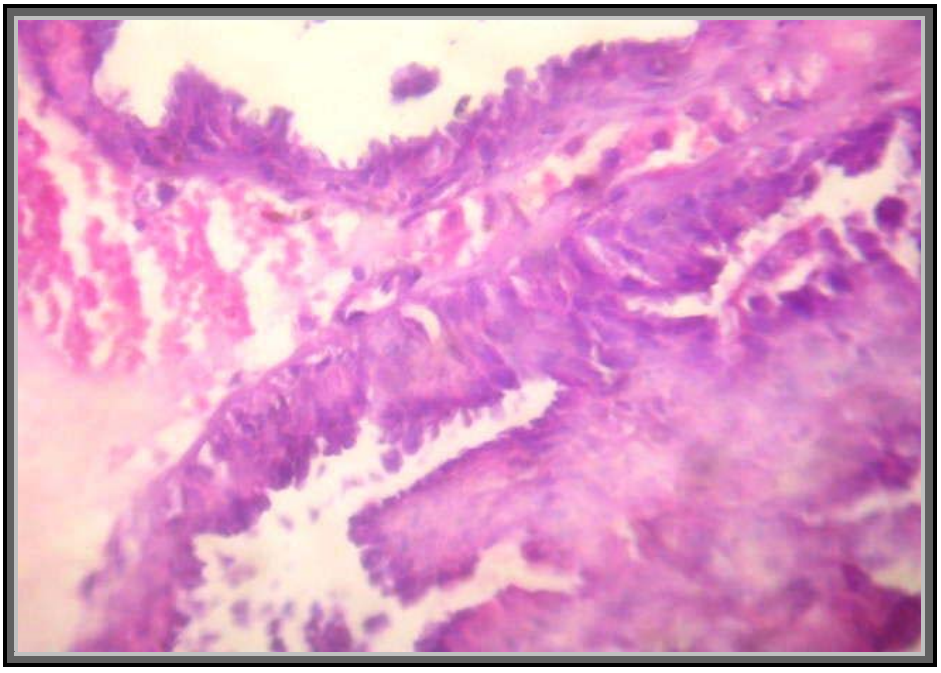

Fig. 14 : PAPILLARY CARCINOMA, HIGH POWER

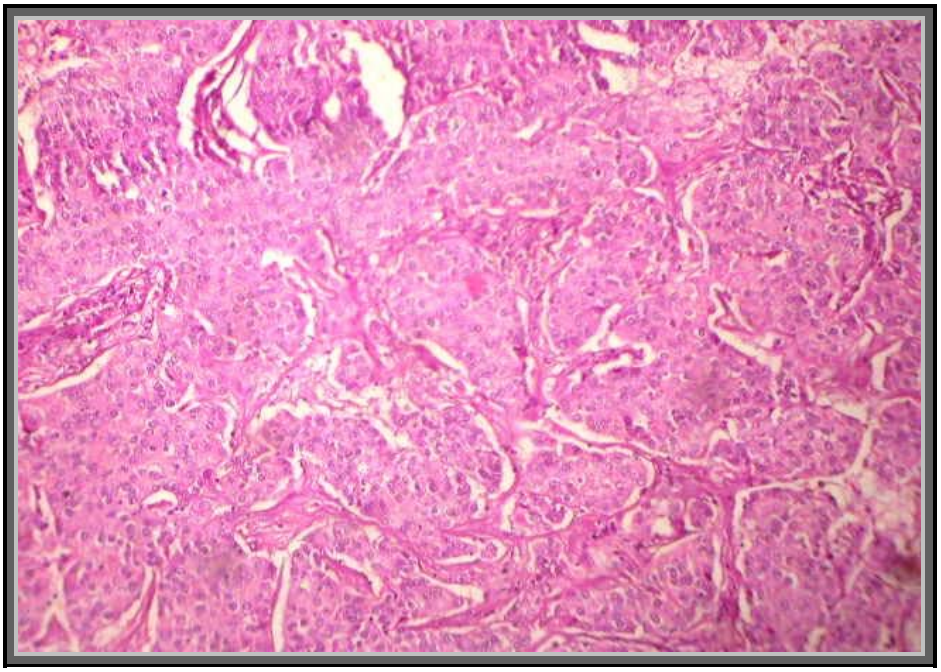

Fig. 15 : IDC WITH NEUROENDOCRINE DIFFERENTIATION, LOW POWER

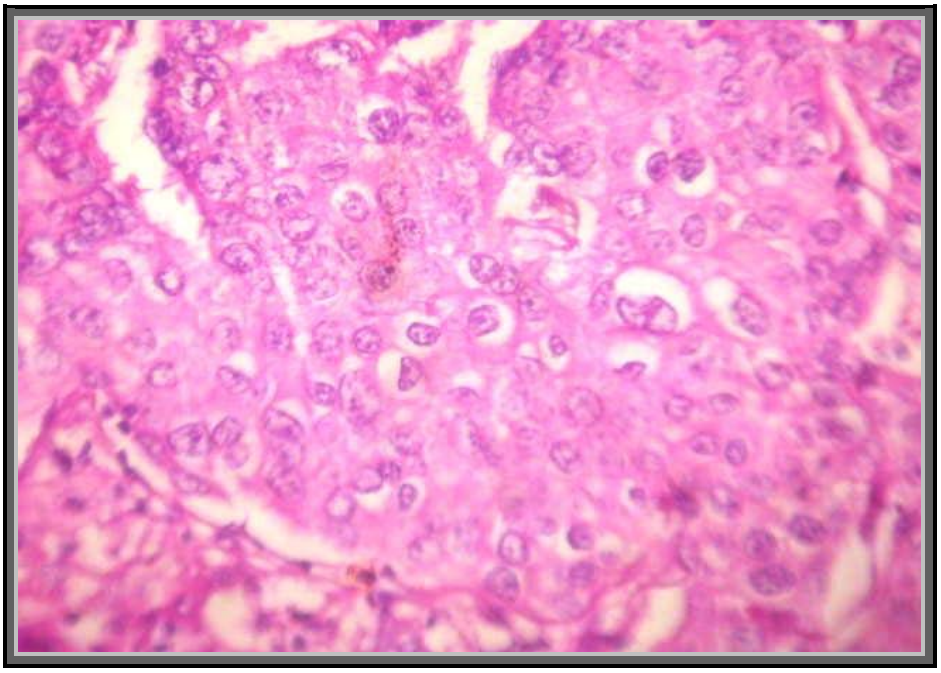

Fig. 16 : IDC WITH NEUROENDOCRINE DIFFERENTIATION, HIGH POWER

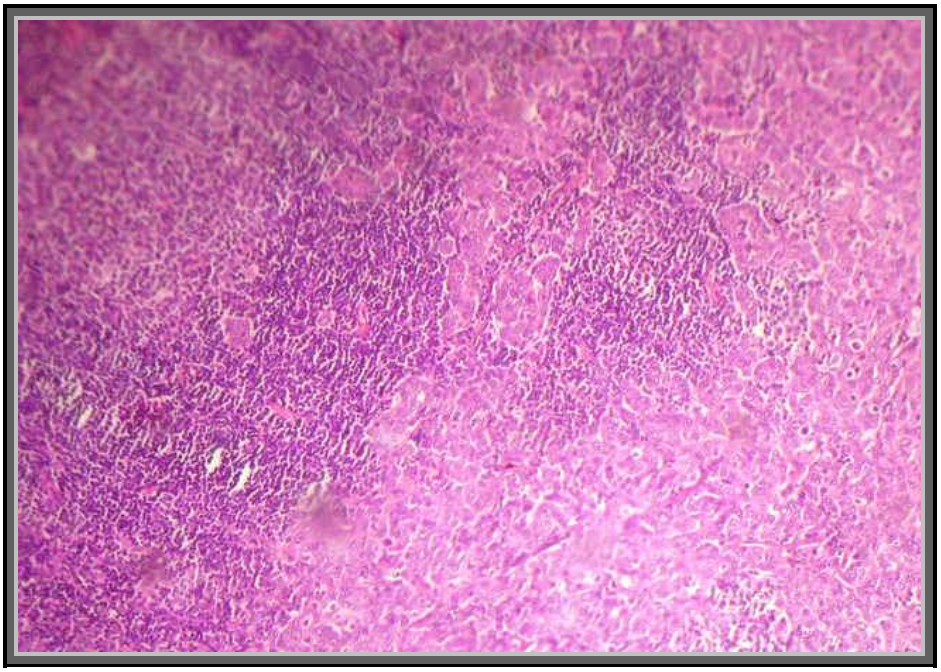

Fig. 17 : METASTATIC DEPOSIT IN LYMPH NODE, LOW POWER

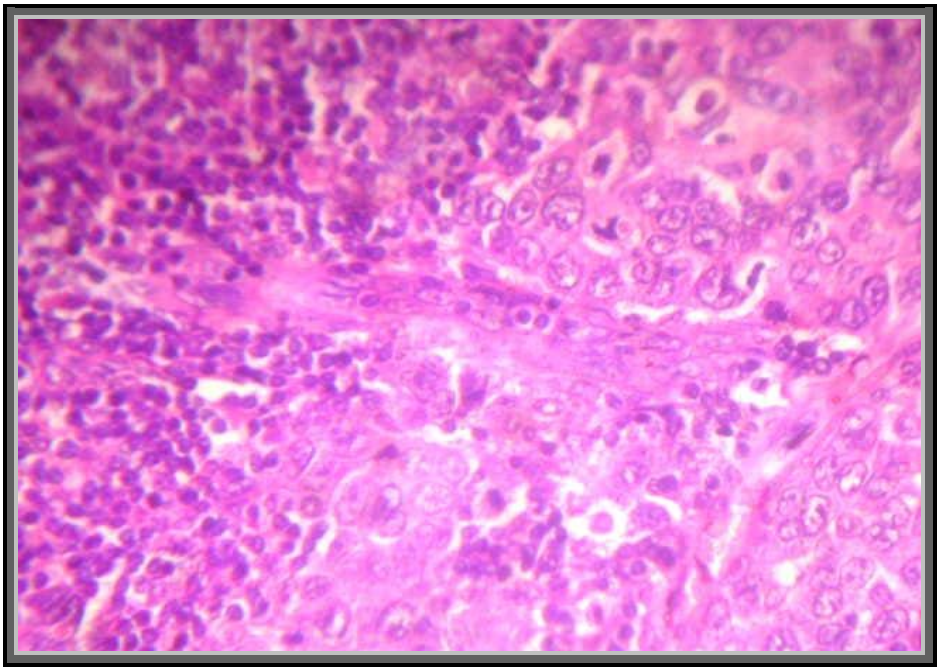

Fig. 18 : METASTATIC DEPOSIT IN LYMPH NODE, HIGH POWER

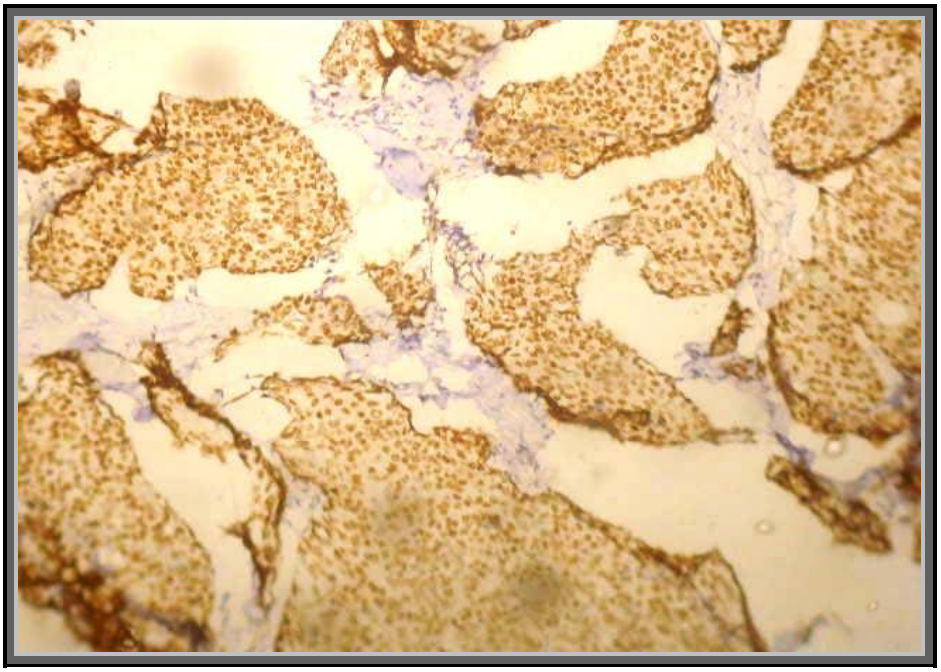

Fig. 19 : IHC, ER POSITIVITY, LOW POWER

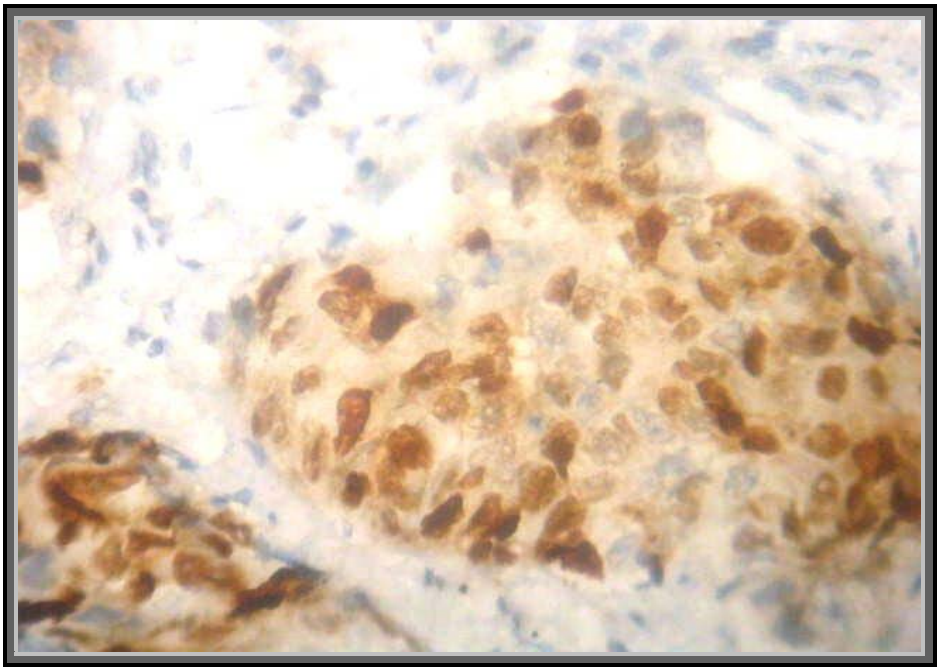

Fig. 20 : IHC, ER POSITIVITY, HIGH POWER

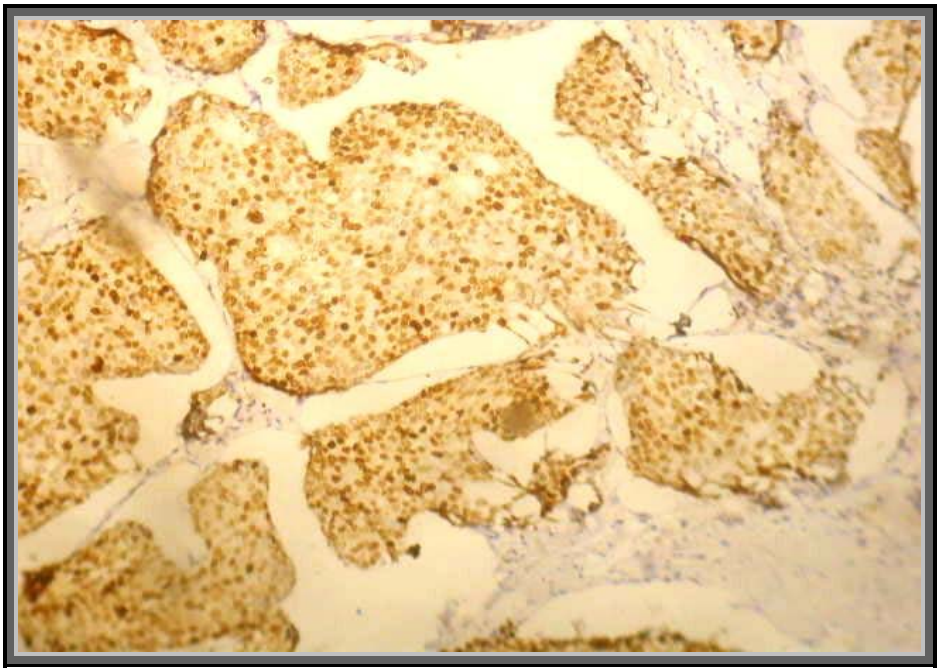

Fig. 21 : IHC, PR POSTIVITY, LOW POWER

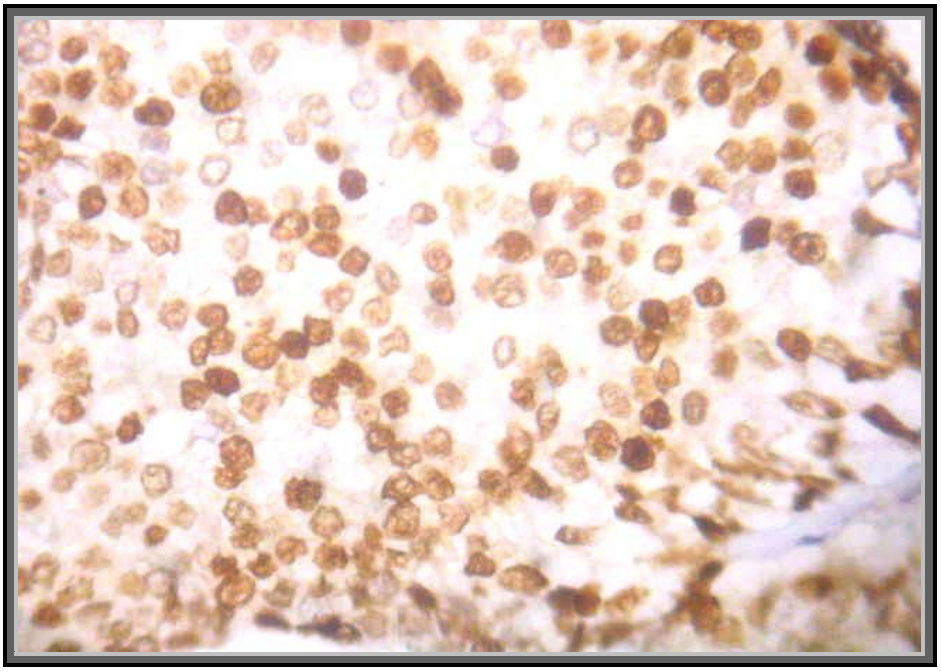

Fig. 22 : IHC, PR POSTIVITY, HIGH POWER

MASTER CHART

S. No.	Bx. No.	Age	Menstrual Status	Tumor size	Lymph node stage	Stage	Grade	NPI	ER	PR	TUMOR TYPE
1	1872/08	54	post menopausal	5	4	3A	2	6	negative	negative	IDC-NOS
2	2162/08	38	pre menopausal	3	6	2B	2	5.6	negative	negative	IDC-NOS
3	2206/08	50	pre menopausal	3	NIL	2A	2	3.6	negative	negative	IDC-NOS
4	2374/08	50	post menopausal	7	4	3A	3	7.4	negative	negative	IDC-NOS
5	2502/08	50	post menopausal	4	1	2B	2	4.8	negative	negative	IDC-NOS
6	2579/08	60	post menopausal	4	3	2B	2	4.8	negative	negative	Mucinous CA
7	2812/08	41	pre menopausal	4	NIL	2A	2	3.8	negative	negative	IDC-NOS
8	3032/08	45	pre menopausal	2	NIL	1	2	3.4	negative	negative	IDC-NOS
9	3069/08	55	post menopausal	3	NIL	2A	2	3.6	negative	negative	Mucinous CA
10	3085/08	31	pre menopausal	4	9	3A	2	5.8	negative	negative	IDC-NOS
11	3100/08	46	pre menopausal	4	NIL	2A	3	4.8	negative	negative	IDC-NOS
12	3240/08	50	post menopausal	4	NIL	2A	2	3.8	negative	negative	IDC-NOS
13	3594/08	53	post menopausal	5	NIL	2B	3	5	negative	negative	IDC-NOS
14	3748/08	55	post menopausal	3	4	2B	2	5.6	negative	negative	IDC-NOS
15	3926/08	50	pre menopausal	2.5	NIL	2A	2	3.5	negative	negative	IDC-NOS
16	0012/09	53	post menopausal	5	4	3A	2	6	negative	positive	IDC-NOS
17	56/09	28	pre menopausal	5	NIL	2B	2	4	negative	negative	IDC-NOS
18	95/09	53	post menopausal	3	1	2A	2	4.6	negative	negative	IDC-NOS
19	481/09	52	post menopausal	4	NIL	2A	2	3.8	negative	negative	IDC-NOS
20	485/09	50	pre menopausal	5	2	3A	2	5	negative	negative	IDC-NOS
21	562/09	43	pre menopausal	3	NIL	2B	2	3.6	positive	negative	IDC-NOS
22	645/09	60	post menopausal	3	NIL	2A	1	2.6	positive	negative	Papillary CA
23	659/09	34	pre menopausal	3	1	2B	1	3.6	negative	positive	IDC-NOS
24	782/09	56	post menopausal	3	1	2B	2	4.6	negative	negative	IDC-NOS

S. No.	Bx. No.	Age	Menstrual Status	Tumor size	Lymph node stage	Stage	Grade	NPI	ER	PR	TUMOR TYPE
25	1104/09	44	pre menopausal	5	2	3A	2	5	negative	negative	IDC-NOS
26	1283/09	53	post menopausal	4	7	3A	2	5.8	positive	negative	IDC-NOS
27	1301/09	45	pre menopausal	5	4	2B	2	6	positive	positive	IDC-NOS
28	1329/09	57	post menopausal	7	4	3A	1	5.4	negative	positive	IDC-NOS
29	1396/09	45	pre menopausal	3	3	2B	2	4.6	positive	negative	IDC-NOS
30	1645/09	72	post menopausal	3	9	2A	2	5.6	negative	negative	IDC-NOS
31	1731/09	46	pre menopausal	3	4	2B	2	5.6	negative	negative	IDC-NOS
32	1788/09	75	post menopausal	3	NIL	2A	2	3.6	negative	positive	IDC-NOS
33	1874/09	45	pre menopausal	2	3	2A	2	4.4	positive	positive	IDC-NOS
34	1879/09	43	pre menopausal	6	2	3A	3	6.2	positive	positive	IDC-NOS
35	1737/09	45	pre menopausal	2	3	2A	1	3.4	positive	negative	IDC-NOS
36	1948/09	50	pre menopausal	7	NIL	2B	2	4.4	positive	negative	IDC-NOS
37	2028/09	55	post menopausal	2	7	2A	2	5.4	negative	positive	IDC-NOS
38	2037/09	80	post menopausal	5	4	3A	2	6	positive	negative	IDC-NOS
39	2110/09	55	post menopausal	2	NIL	1	2	3.4	negative	negative	IDC-NOS
40	2130/09	65	post menopausal	1	NIL	1	1	2.2	positive	positive	IDC-NOS
41	2134/09	52	post menopausal	1	2	2A	1	3.2	negative	negative	IDC-NOS
42	2154/09	65	post menopausal	8	NIL	2B	2	4.6	negative	negative	IDC-NOS
43	2156/09	45	pre menopausal	2	3	2A	2	4.4	negative	negative	IDC-NOS
44	2255/09	47	pre menopausal	8	2	3A	2	5.6			IDC-NOS
45	2345/09	50	post menopausal	3	NIL	2A	3	4.6			IDC-NOS
46	2347/09	50	post menopausal	5	5	3A	3	7	negative	negative	IDC-NOS
47	2744/09	48	post menopausal	4	NIL	2A	2	3.8			IDC-NOS
48	2490/09	52	post menopausal	1	NIL	1	2	3.2			IDC-NOS
49	2558/09	45	post menopausal	4	1	2B	3	5.8			IDC-NOS
50	2563/09	50	post menopausal	3	NIL	2A	2	3.6	negative	negative	IDC-NOS

S. No.	Bx. No.	Age	Menstrual Status	Tumor size	Lymph node stage	Stage	Grade	NPI	ER	PR	TUMOR TYPE
51	2769/09	37	pre menopausal	4	2	2B	3	5.8			IDC-NOS
52	2655/09	55	post menopausal	7	NIL	2B	2	4.4	negative	negative	IDC-NOS
53	2791/09	55	post menopausal	3	NIL	3B	1	2.6			IDC-NOS
54	2845/09	36	pre menopausal	4	1	2A	2	4.8	positive	positive	IDC-NOS
55	0030/10	40	pre menopausal	4	1	2B	3	5.8	negative	negative	IDC-NOS
56	43/10	35	pre menopausal	3	7	3A	3	6.6	positive	positive	IDC-NOS
57	318/10	63	post menopausal	4	1	2B	2	4.8	positive	positive	IDC-NOS
58	334/10	65	post menopausal	1.5	NIL	1	3	4.3	positive	positive	IDC-NOS
59	487/10	30	pre menopausal	4	NIL	2A	2	3.8	positive	positive	IDC-NOS
60	576/10	34	pre menopausal	3	NIL	2A	2	3.6	negative	negative	IDC-NOS
61	896/10	34	pre menopausal	2	NIL	1	2	3.4	negative	negative	IDC-NOS
62	1059/10	40	pre menopausal	3	1	2B	2	3.6	positive	negative	IDC-NOS
63	1132/10	45	post menopausal	2	2	2A	2	4.4			IDC-NOS
64	1172/10	75	post menopausal	8	5	3A	2	6.6			IDC with Neuroendocrine Differentiation
65	1286/10	55	post menopausal	5	4	3A	2	6			IDC-NOS
66	1240/10	65	post menopausal	4	1	2B	2	4.8			IDC-NOS
67	1286/10	55	post menopausal	5	4	3A	2	6			IDC-NOS
68	1385/10	37	pre menopausal	7	NIL	3B	2	4.4			IDC-NOS
69	1436/10	52	post menopausal	2	1	2A	2	4.4			IDC-NOS
70	1449/10	36	pre menopausal	2	3	2A	3	5.4			IDC-NOS
71	1471/10	70	post menopausal	2.5	NIL	2A	2	3.5			IDC-NOS
72	1472/10	68	post menopausal	3	NIL	2A	2	3.6			IDC-NOS
73	1648/10	45	pre menopausal	2.5	3	2B	3	5.5			IDC-NOS

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ABBREVIATIONS

ER	-	Estrogen Receptor
PR	-	Progesterone Receptor
IDC-NOS	-	Invasive Ductal Carcinoma – Not Otherwise Specified
DCIS	-	Ductal Carcinoma In Situ
IHC	-	Immunohistochemistry
MRM	-	Modified Radical Mastectomy
CA	-	Carcinoma