TO ASSESS THE STROMAL EXPRESSION OF CD10 IN INVASIVE DUCTAL CARCINOMA OF BREAST AND ITS CORRELATION WITH HISTOLOGICAL GRADE, ER, PR AND HER2/ NEU EXPRESSION

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M.D. DEGREE

In

PATHOLOGY – BRANCH III

THE   TAMILNADU

DR. M.G.R. MEDICAL UNIVERSITY

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DECLARATION

I hereby declare that the dissertation entitled **"TO ASSESS THE STROMAL EXPRESSION OF CD10 IN INVASIVE DUCTAL CARCINOMA OF BREAST AND ITS CORRELATION WITH HISTOLOGICAL GRADE, ER, PR AND HER2/ NEU EXPRESSION"** is a bonafide research work done by me in the Department of Pathology, Coimbatore Medical College during the period from JULY 2014 TO JUNE 2015 under the guidance and supervision of Dr. G. S. Thiriveni Balajji, M.D, Associate Professor, Department of Pathology, Coimbatore Medical College.

This dissertation is submitted to The Tamilnadu Dr.MGR Medical University, Chennai towards the partial fulfillment of the requirement for the award of M.D., Degree(Branch III) in Pathology. I have not submitted this dissertation on any previous occasion to any University for the award of any Degree.

Place: Coimbatore

Dr. S. ULAGANATHAN

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CERTIFICATE

This is to certify that dissertation entitled **"To assess the stromal expression of cd10 in invasive ductal carcinoma of breast and its correlation with histological grade, ER, PR AND HER2/ neu expression"**is a bonafide work done by **Dr. S. ULAGANATHAN**, a postgraduate student in the Department of Pathology, Coimbatore Medical College, Coimbatore under guidance and supervision of **DR. G. S. THIRIVENI BALAJJI, M.D**, Associate Professor, Department of Pathology, Coimbatore Medical College, Coimbatore in partial fulfillment of the regulations of the Tamilnadu Dr. M. G. R. Medical University, Chennai towards the award of M. D. Degree   (Branch III) in Pathology.

Guide	**Head of the Depatrment**
Dr. G. S. Thiriveni Balajji, M.D,	**Dr.C.Lalitha,M.D,**
Associate professor,	Professor,
Department of Pathology,	Department of Pathology,
Coimbatore Medical College	Coimbatore Medical College
Coimbatore.	Coimbatore.

Dr.A.EDWIN JOE,M.D;B.L,

Dean

Coimbatore Medical College

Coimbatore

Coimbatore Medical College

COIMBATORE, TAMILNADU, INDIA - 641 014

(Affiliated to The Tamilnadu Dr. MGR Medical University, Chennai)

ETHICS COMMITTEE

CERTIFICATE

Name of the Candidate : Dr. S. Vlaganathan

Course : MD Pathology

Period of Study : 2013 - 2016

College : Coimbatore Medical College

Dissertation Topic : Stromal expression of CD 10 in Invasive ductal carcinoma of breast & its Correlation with histopathology grade and expression of ER, PR, HER 2.

The Ethics Committee, Coimbatore Medical College has decided to inform that your Dissertation Proposal is accepted / ~~Not accepted~~ and you are permitted / ~~Not permitted~~ to proceed with the above Study.

DEAN
Coimbatore Medical College & Hospital,
Coimbatore

15.7.2014.

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INTRODUCTION

Breast cancer is a heterogeneous disease with wide histological appearance. Breast cancer is the most frequent cancer in women worldwide. Incidence rate is differs worldwide from 27/100000 females in Eastern Africa to 96/100000 females in Europe.[1]

Breast cancer is the most common cancer among women in India according to National cancer registry programme 2011 report.[2] It is the most common non skin cancer in females worldwide. A female has one in eight chance of developing breast cancer during her lifetime if she lives up to the age of ninety years. By the year 2030 global burden of breast cancer will be more than two million every year.[1]

In India the incidence of carcinoma of breast is increasing[1] and the mortality rate for breast cancer in India is 11.1per 10,000.[3]

Lobule of the breast, part of terminal duct lobular unit, is composed of inner secretory epithelial cells and outer myoepithelial cells. Immunohistochemistry plays a pivotal role in therapeutic categorization. Estrogen receptor (ER) positive and ER negative breast cancers show obvious differences in patient characteristics,

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INTRODUCTION

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Lobule of the breast, part of terminal duct lobular unit, is composed of inner secretory epithelial cells and outer myoepithelial cells. Immunohistochemistry plays a pivotal role in therapeutic categorization. Estrogen receptor (ER) positive and ER negative breast cancers show obvious differences in patient characteristics, pathological features, response to treatment and prognosis.

Growth of the tumor is directly influenced by the communication between tumor cells and stromal cells via the chemical mediators. Loss of CD10 in myoepithelial cells and expression in stromal cells of invasive ductal carcinoma of breast is associated with poor prognosis.[4]

In addition to tumor cells, tumors also contain innate and adaptive immune cells, fibroblasts, etc. Recent studies suggest that genetic changes in stroma can promote carcinogenesis.[5] In some condition tumor behavior is predicted based on expression of certain genes in stroma. Studies highlights the role of stroma in tumor growth, progression and prognosis of breast cancer.[6]

CD10(common acute lymphoblastic leukemia antigen, CALLA) is a cell surface zinc dependant protease. CD10 act as a stem cell regulator in the breast and prevents uncontrolled proliferation of stem cells.[7] It is expressed in breast myoepithelial cells, lymphoid stem cells, neutrophils, and other epithelial cells.[8] CD10 also expressed in stroma of prostate, lung and colorectal cancers.[8] Stromal expression of

CD10 is associated with aggressive behavior of epithelial cancers. Few studies have indicated that CD10 expression of breast cancer is associated with aggressive behavior and poor prognosis.[4,7] Routine chemotherapeutic drugs target the epithelial cells while stromal cells are spared which could be responsible for recurrence. It indicates a novel form of therapy and stromal cells could be potential therapeutic targets.

AIM AND OBJECTIVES

AIM OF THE STUDY

To assess the stromal expression of CD10 in invasive ductal carcinoma of breast NOS type and its correlation with histological grade, ER, PR and HER2/ neu expression.

OBJECTIVES

To analyze the expression of CD10 in invasive ductal carcinoma of breast NOS type.

To analyze the correlation of stromal expression of CD10 with ER, PR and HER2 neu expression.

To analyze the correlation of histological grade of carcinoma of breast and stromal expression of CD10.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Breast cancer is the most common non skin cancer in women.[7] In India it is the most common cancer among women according to National cancer registry programme 2011 report.[2] 1.67 million new cases were diagnosed in 2012 worldwide.[1] In India 1,45,000 new cases were diagnosed in 2012 and about 70,000 deaths were occurred due to breast cancer.[1] Mortality rate for breast cancer in India was 11.1 per 100,000[.3]

Incidence and Epidemiology

Breast cancer incidence is increased partly due to mammographic screening programme. The major benefit of mammographic screening is the detection of in situ carcinoma and number of cases with advanced stage is diminished. In India the incidence of breast cancer is increasing and the average age of developing a breast cancer is decreasing.[2] It is more common in fourth and fifth decades of life than in the past.

Breast cancer mortality rate is decreased. It is due to the diagnosis of clinically significant cancer at an early stage because of screening and effective therapeutic methods.
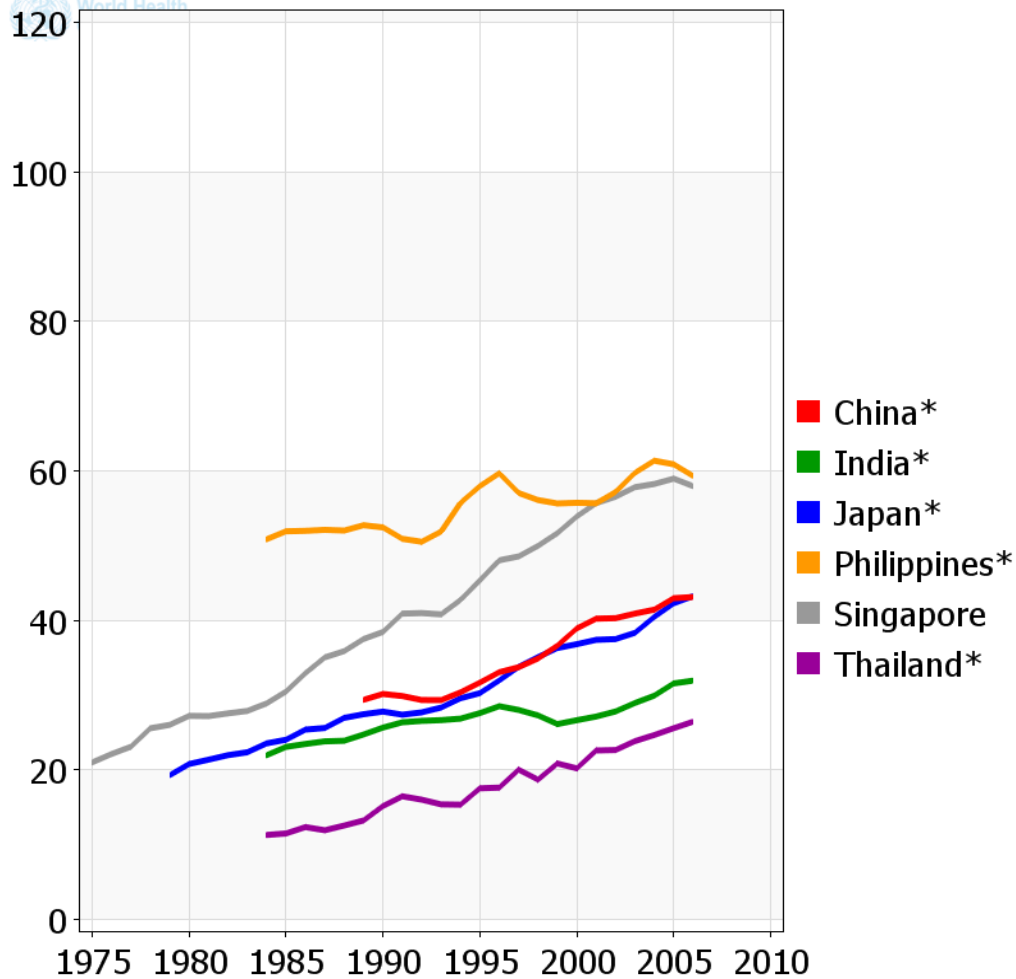
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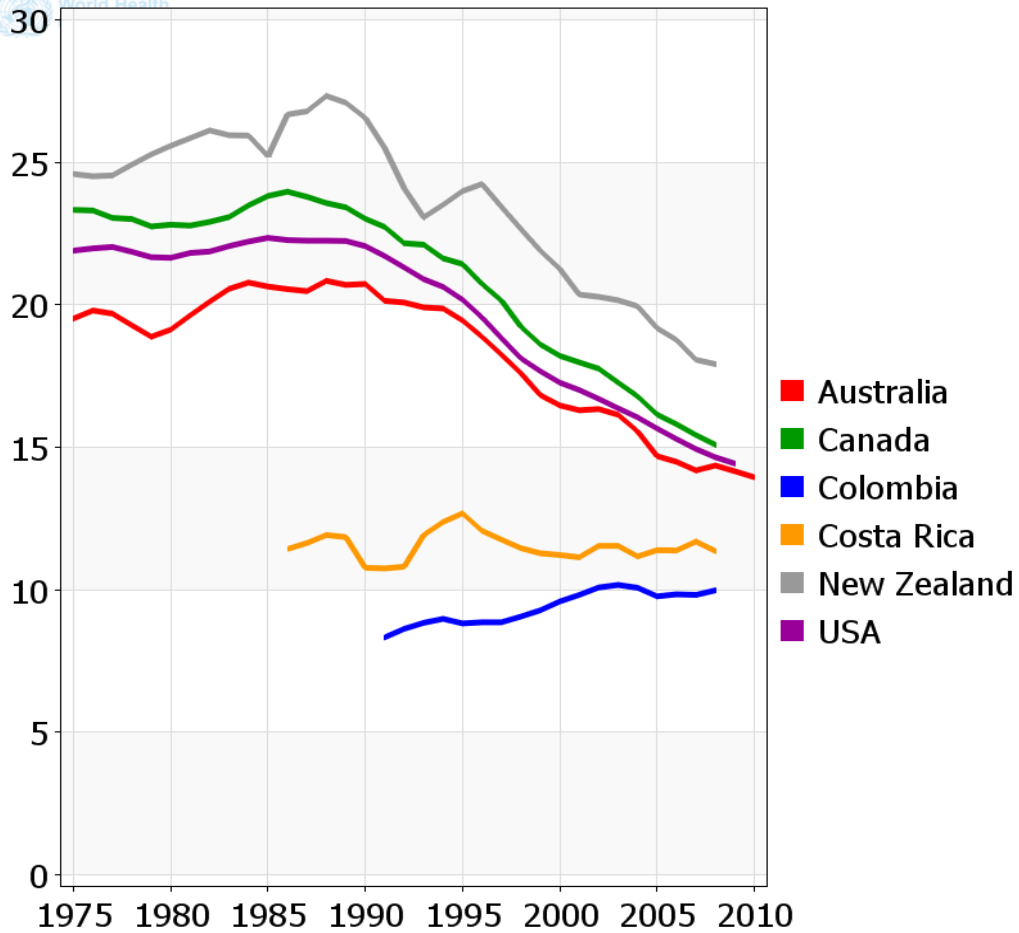
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Risk factors

Gender is the important risk factor, only one percent carcinoma of breast occurs in males.

Age

Breast cancer can occur in any age from childhood to old age. Most of the cases occur in the reproductive age groups. Breast cancer is rare below twenty five years of age. More than 50% of breast cancer in young women are either ER negative or HER2/neu positive. Early menarche and late menopause increases the risk of breast cancer. Females who has first term pregnancy at ages below twenty years have less risk than nulliparous women or female over the age of thirty five when they have first birth . It is believed that the pregnancy causes terminal differentiation of luminal cells and removes the cancer precursor cells. The changes in stroma that occurs in pregnancy help the development of invasive breast cancer from in situ component.[9]

Family history of breast cancer in the first degree relatives increase the risk. The risk is due to germline mutation in genes BRCA1, located on chromosome 17q21 and BRAC2 located on chromosome

13q12.3. But these genes are responsible only in 16% of familial breast cancer.[10,11] Affected individuals to be closely followed up or can have prophylactic mastectomy.[9]

Epithelial hyperplasia and fibrocystic disease

Atypical hyperplasia and fibrocystic disease increases the risk of breast cancer.

Estrogen exposure

There is 1.2 to 1.7 fold increased risk associated with hormonal replacement therapy with estrogen and progesterone.[12] Most of the cases are ER positive and invasive lobular carcinoma. Oral contraceptive use does not affect breast cancer risk.

Density

Increased radio density is a risk factor. It is correlated with young age and exposure to hormone. Detection of cancer in the screening programme is difficult. In some cases MRI may be useful.

Radiation

Exposure to radiation in any form is associated with increased risk for breast cancer.

Geography

Incidence in USA and Europe is 4 to 7 times higher when compared to other country. But number of cases in India is increasing.

Diet

Caffeine consumption may lower the risk and alcohol may increase the risk of breast cancer.

Obesity

In obese people the risk is lower below the age of 40 due to low progesterone level. In obese postmenopausal women risk is increased due to estrogen synthesis in fat.

Exercise

Risk for breast cancer is slightly lower for physically active women.

Breast feeding

Increased risk reduction for breast cancer is noted in women who breastfeed longer. Low level of incidence in developing countries may be explained by frequent and longer breast feeding.[13]

ETIOLOGY AND PATHOGENESIS

Hormones and genetics are the major risk factors for breast cancer.

Hereditary breast cancer

10 to 12% of all breast cancer is due to inheritance of a gene or genes.[14] Multiple affected first degree relatives, breast cancer in younger age group, multiple cancer in the family suggest a hereditary etiology. Mutation in highly penetrant breast cancer gene is associated with increased risk. BRAC1 and BRCA2 accounts for 3% of breast cancer. BRCA1 gene is located on chromosome 17q21 is also associated with increased risk for ovarian carcinoma. BRCA2 gene is located on chromosome 13q12.3.[15] BRAC2 gene is increases the breast cancer risk in men. Germline mutation in both the genes is associated with prostatic and pancreatic cancer.

BRCA1 and BRCA2 are large genes. BRCA1 encodes a protein with many function. Functions include recombination DNA repair, cell cycle control, chromatin remodeling.

Protein encoded by BRCA2 gene is also involved in DNA repair and cytokinesis.[12]

Breast cancer occurring with BRCA1 is usually poorly differentiated, have high percentage medullary features and triple negative phenotype or overexpression of HER2/neu. These tumors are similar to basal like breast cancer. There are some other susceptibility genes which less commonly associated with familial breast cancer. Li Fraumani syndrome (germline mutation in p53) and Li Fraumani variant syndrome (due to mutation in HEK2) together account for about 8% of carcinoma breast. Other syndromes with breast cancer include Cowden syndrome (PTEN gene), Peutz Jeghers syndrome, familial linitis plastica type c, Louis-Bar syndrome and Faconi anemia.

SPORADIC BREAST CANCER

The major risk factor in this group is related to hormone exposure, gender, age at menarche and menopause, reproductive history, breast feeding and hormone replacement. Most of the cases occur in postmenopausal females and are ER positive.

Hormonal exposure causes growth of target cells, proliferation increase the chances of DNA damage. Hormone also stimulate growth of

premalignant and malignant cells as well as stromal cells which helps in tumor development. Estrogen may cause mutation either directly or through free radicals in the cells.[16]

Genetic and epigenetic changes in some cells results in recognizable breast lesion. Earliest such lesions are proliferative changes. These changes include loss of growth inhibiting signals or reduced apoptosis. Hormone receptors are increasingly expressed in atypical hyperplasia. At some point these changes further acquire additional mutation which results in tumor development and neoangiogenesis. According to "cancer stem cell hypothesis" cancer occur from stem cell population. The most likely cells are ER positive luminal cells. Breast cancers which are ER negative may arise from myoepithelial cells which are ER negative. Alternatively an ER positive precursor lesion may loss its ER positivity during development of cancer.

Invasion from an in situ cancer occur probably due to an interplay between luminal cells, epithelial cells and stromal cells.

ROLE OF STROMA IN CANCER

Microenvironment in the stroma plays a crucial role in neoplastic cell proliferation and metastasis.[17,18] The interaction between epithelial cells and stromal cells is disrupted by many factors produced by neoplastic cells or by stromal cells.[19,20]

Matrix metalloproteinase, produced by stromal cells plays a crucial role in tumor invasion and metastasis. Studying the role of stromal components may help in developing new treatment for neoplastic conditions.[21]

CD10 is a zinc dependent metalloproteinase, present on cell surface of the stromal cells and are upregulated in the neoplastic cells.[22] Matrix metalloproteinases are involved in the cleavage of proteins of extracellular matrix thereby has a key role in remodeling of tissues.[23] Since CD10 cleaves the drug doxorubicin thereby causes chemoresistance. Thus inhibiting the activity of CD10 may have an increased response to chemotherapeutic agents.[24]

In the normal breast CD10 mostly acts as a stem cell regulator.[25] CD10 inhibit signaling proteins which cause differentiation of early common progenitor cells in to epithelial and myoepithelial cells.[26,27]

Maguer-Satta et al proposed that the transformation of stem cells in to neoplastic cells results in altered expression of CD10. A decreased activity of CD10 leads to lineage commitment and neoplastic proliferation of cells. Thus CD10 loss in myoepithelial cells leads to progression of DCIS in to invasive ductal carcinoma of breast.[28]

At the same time an increased level of CD10 activity from stromal cells leads to inhibition of epithelial cell differentiation. Thus cancer stem cells are maintained. Undifferentiated carcinoma have increased expression of CD10.[7]

CLASSIFICATION

More than 95% of breast cancers are adenocarcinomas. They are divided into in situ carcinomas and invasive carcinomas.[29] Neoplastic proliferation that is restricted to ducts and lobules is called as in situ carcinomas. When penetration occurs to stroma through basement membrane it is called as invasive carcinoma.

WHO HISTOLOGICAL CLASSIFICATION OF TUMOURS OF THE BREAST

A) EPITHELIAL TUMORS:

Invasive ductal carcinoma, Not otherwise specified

Invasive Lobular carcinoma

Tubular carcinoma

Invasive Cribriform carcinoma

Invasive Papillary carcinoma

Invasive Micropapillary carcinoma

Medullary carcinoma

Mucinous carcinoma

Apocrine carcinoma

Metaplastic carcinoma

Secretory carcinoma

Neuro Endocrine carcinoma

Oncocytic carcinoma

Adenoid cystic carcinoma

Acinic cell carcinoma

Glycogen rich clear cell carcinoma

Sebaceous carcinoma

Inflammatory carcinoma

Micro invasive carcinoma

Intraductal proliferative lesions

    Usual ductal hyperplasia

    Flat ductal hyperplasia

    Atypical ductal hyperplasia

Ductal carcinoma in situ

Intraductal papillary neoplasm

Central papilloma

peripheral papilloma

Intraductal papillary carcinoma

Benign epithelial proliferation

Adenosis and its variant

Radial scar or complex sclerosing lesion

Adenomas and its variants

B) MYOEPITHELIAL LESIONS:

Myoepitheliosis

Adenomyoepithelial adenosis

Adenomyoepithelioma

Malignant myoepithelioma

C) MESENCHYMAL TUMORS:

Hemangioma

Angiomatosis

Hemangiopericytoma

Myofibroblastoma

Fibromatosis

Inflammatory myofibroblastoma

Lipoma, Leiomyoma

Granular cell tumor

Neurofibroma

Schwannoma

Angiosarcoma,

Liposarcoma,

Rhabdomyosarcoma,

Osteosarcoma

Leiomyosarcoma

D) FIBROEPITHELIAL TUMORS:

Fibroadenoma

Phyllodes tumor

Periductal stromal sarcoma

Mammary hamartoma

E) TUMORS OF NIPPLE:

Nipple adenoma

Paget's disease of nipple

F) MALIGNANT LYMPHOMA:

Diffuse Large B cell Lymphoma

Burkitt Lymphoma

Extra nodal marginal zone B cell Lymphoma of MALT type

Follicular Lymphoma

G) METASTATIC TUMORS

H) TUMORS OF MALE BREAST:

Gynaecomastia ,

Carcinoma- invasive and in situ

DUCTAL CARCINOMA IN SITU(DCIS)

Detection of DCIS is increased due to mammography which detects calcification and less commonly periductal fibrosis.[30] It may spread throughout duct and lobule.

Morphological variants of DCIS[31]

Comedocarcinoma

Solid

Cribriform

Papillary

Micropapillary

Clinging

Cystic hypersecretory

Macroscopy

DCIS may have an ill defined area of fibrous tissue. Soft, pale cheese like necrotic debris can be seen on cut surface in comedocarcinoma.[32] Most of the small lesion are invisible.

Grading

Using nuclear grade DCIS is divided into three grades[33,34]

1. High

2. Intermediate

3. Low

High grade

This is consists of large pleomorphic cells and high nucleocytoplasmic ratio. Nucleus is enlarged, have coarse chromatin and large nucleoli. Frequent and atypical mitosis are seen. Comedocarcinoma, cribriform or micropapillary are the patterns associated with high grade DCIS.

Low grade

This grade will have uniform cells with small regular nuclei. Nucleoli is indistinct. Necrosis and mitoses are uncommon. Cribriform,

micropapillary and less commonly solid patterns are the patterns associated with low grade DCIS.

Intermediate grade

Nuclei in this grade show less pleomorphism than high grade and they lack uniformity. Necrosis and nucleoli may be seen but they are not large.The architectural patterns includes cribriform, solid and micropapillary.

Rare variants

Apocrine

Neuroendicrine

Signet ring cell

Cystic hypersecretory

Untreated cases of DCIS turn into invasive cancer at a rate of 1% per year. Surgical excision followed by radiation is the usual treatment. Recurrence rarely occurs due to residual DCIS or occult foci of invasion.

LCIS

Lobular carcinoma in situ(LCIS), otherwise called as lobular neoplasia, is usually diagnosed as an incidental finding. It has no distinguishing features grossly. Bilateral LCIS is more common than DCIS. Lobular pattern is attributed to the loss of E cadherin, a transmembrane adhesion molecule, that causes cell to cell cohesion in normal breast.

Microscopically LCIS consist of dyscohesive round cells with oval to round nuclei and small nucleoli. Signet ring cells are frequently seen.[35] Development of Invasion is more common than DCIS. Choices of treatment include bilateral prophylactic mastectomy, tamoxifen or clinical follow up with mammography.

INVASIVE CARCINOMA

When stromal invasion is seen it is called as invasive carcinoma, whether in situ cancer present or not. If the invasion is less than 0.1cm it is called as **microinvasion**.[36] Although the carcinoma of breast is develops from stem cells, depending upon pattern of differentiation it is divided in to two major categories- ductal type and lobular type.[29]

Clinical features

Most of the cancers present as a palpable mass, which are associated with axillary lymph node involvement in more than 50% cases. Fixation to the chest wall and skin dimpling can occur. Tumor involving the centre of breast causes nipple retraction. Blockage of lymphatics produces skin thickening and lymphedema. In such cases peau d' orange appearance occurs due to tethering of skin to the breast by cooper ligaments.[37]

With the introduction of mammographic screening small sized tumors are increasingly detected. They may present as radiodense mass and microcalcification

Rarely axillary nodal metastasis or distant metastasis may be the mode of presentation of an obscured or occult primary breast cancer.

At molecular level breast cancer is classified based on DNA, RNA, and proteins of cancer breast.[38]

DISTRIBUTION OF HISTOLOGICAL TYPE[39]

CARCINOMA IN SITU	**15-30%**
Ductal carcinoma in situ	80%
Lobular carcinoma in situ	20%
INVASIVE CARCINOMA	**70-85%**
No special type	79%
Lobular carcinoma	10%
Tubular carcinoma	6%
Mucinous carcinoma	2%
Medullary carcinoma	2%
Papillary carcinoma	1%
Metaplastic carcinoma	<1%

Invasive carcinoma no special type

70-80% breast cancers are invasive carcinoma of no special type.[40,41] It fails to exhibit histologic characters to classify other specific types such as lobular, mucinous or tubular carcinoma.

Macroscopic appearance

Most of the cancers are firm to hard in consistency with irregular border. Varied gross appearance can occur. Size of the tumor ranges from 0.5cm to above 10cm.[42] Presence of small foci of elastic stroma and occasional calcification gives a gritty feeling on cutting the tumor. High grade lesions are associated with massive necrosis or calcification.

Histological appearance

Histological appearance is variable. The tumor cells are arranged in sheets or cords. Some time they are diffusely infiltrative. Individual cells have abundant eosinophilic cytoplasm with pleomorphic nuclei. Glandular structure may be extensive or absent. Stromal component varies from desmoplastic proliferation to scanty connective tissue. Foci of elastosis and necrosis present which may be extensive. Features of special type of breast cancer is seen in variable proportion. Occasionally metaplastia, bizarre tumor giant cells are seen.[43] Up to 80% cases associated with DCIS component.

Molecular subtypes

There are five major pattern of gene expression in no special type group. These are identified with Gene expression profiling. They luminal A, luminal B, normal, basal like and HER2 positive.[44]

Luminal A

It is the largest group and 40-55% of no special type cancer are belong to this group. Luminal A type of cancers are ER positive and HER2/neu negative. Most of the tumors are well or moderately differentiated and mostly occur in post menopausal women. These tumors are slow growing and respond well to hormonal therapies.[38]

Luminal B

15-20% of no special type cancer are luminal B type. It is not only express ER but also often over express HER2/neu. These tumors are generally high grade. Most likely to have nodal spread. These tumors respond well to chemotherapy.[38]

Normal breast like

6-10% cancers are normal breast like type. These are usually ER positive, HER2/neu negative well differentiated cancers. Their gene expression pattern is similar to normal breast.[38]

Basal like

It accounts for 13-25% of no special type cases. These are characterized by ER, PR and HER2/neu negativity. They express markers of myoepithelial cells (P- cadherin, keratin, p63) or stem cells (cytokeratin 5 and 6). Basal like cancers are subgroup of triple negative carcinomas.[45,46] Most of the cancer occurring in women with BRCA1 mutation are basal like cancers. These are generally high grade and associated with poor prognosis. Only 15-20% respond to chemotherapy.

Her2 positive

It accounts for 7-12% of no special type cancers. This group over expresses Her2 protein. They are ER negative cancers. Amplification of a segment of DNA in chromosome 17q21 causes over expression of Her2 neu.[47] These tumors are poorly differentiated and frequently have brain metastasis.

INVASIVE LOBULAR CARCINOMA

This occurs commonly in older age group than ductal type. They frequently present with larger tumor size, low histological grade and hormone receptor positivity. It is the most common special type of breast cancer.[48] Higher rate of multicentricity, bilaterality, subsequent involvement of contralateral breast are frequently seen in invasive lobular carcinoma. Lobular morphology is due to loss of E-cadherin and loss of chromosome 16q.[49,50]

Macroscopy

Most of the invasive lobular carcinoma forms a mass which is indistinguishable from ductal type. Rarely ill defined lesion may occur which is difficult to detect clinically and with imaging techniques.[51]

Histopathology of classic type

This type accounts for 40% cases.[48] Small to moderately sized cells, arranged in dyscohesive cords, sheets, clusters or single file pattern are the hallmark features of this type. Concentric infiltration of tumor cells around normal ductular structures gives rise to targetoid appearance. Tumor cells are more or less uniform and have round to oval nuclei with inconspicuous nucleoli and a thin rim of cytoplasm. Nuclei often eccentrically placed.

31

Intracytoplasmic lumina is commonly seen.[52] Mitosis and desmoplasia are infrequent.

Other variants

Alveolar,

Solid,

Tubulolobular,

Pleomorphic

Mixed type.

Immunoprofile

They are commonly positive for ER, PR than in ductal type. HER2 positivity is lower than ductal type. They also express CEA. 10-16% cases express E-cadherin which is an uncommon finding.[53]

Metastatic pattern is different in lobular carcinoma and tend to involve the peritoneum, retroperioneum, leptomeninges, gastrointestinal tract and ovaries.[54]

Tubular carcinoma

It is an uncommon subtype of breast cancer. Accounts for 1-4% if invasive breast cancer. Other features include smaller tumor size, low lymph node metastasis and lower recurrence. They have favorable prognosis.[55]

Macroscopy

Grossly difficult to differentiate from ductal carcinoma no special type. Tumor size ranges from 2mm to 1.5cm. Stellate appearance seen in" pure" type. The appearance is diffuse and ill defined in sclerosing type.[56]

Histopathology

These tumors composed of predominantly well formed tubules lined by single layer of epithelial cells enclosing a lumen. Cells are small to

medium in size, cuboidal to columnar in shape and have low grade nuclear features. Elastosis is considered as hallmark of tubular carcinoma. Mitosis is rare. Stroma is desmoplastic in tubular carcinoma. 90% of the lesion should have tubular morphology to be classified as tubular carcinoma.[48] They are ER and PR positive.

Invasive cribriform carcinoma

This is an uncommon special type of breast cancer and they have excellent prognosis.[57]

Macroscopy

Tumors are firm and may have stellate configuration. Size ranges from 1 to 3cm.[58]

Histopathology

It shows a sieve like pattern. Tumors consist of round, angulated mass and islands of small cells in a desmoplastic stroma. Well defined punched out spaces filled with mucin secretion are seen. 90% of the lesion should show cribriform morphology.[58]

MUCINOUS CARCINOMA

Mucinous carcinomas are uncommon (1 to 4%) tumors. It is more common in women over 60 years of age.[48]

Macroscopy

They are well defined, soft tumor and have a glistening gelatinous surface on cut section. Tumor size ranges from 1 to 5cm.[59]

Histopathology

Mucinous carcinoma composed of small islands or clusters of uniformly round cells in an extensive extracellular mucin background. Tumor cells are small with minimal cytoplasm and have dark staining nuclei. 90% of the tumor mass shows mucinous morphology.[60] They have good prognosis.

They are ER, PR positive and HER2/neu negative. Mucinous carcinoma showing neuroendocrine differentiation are associated with good prognosis.

MEDULLARY CARCINOMA

Medullary carcinoma is usually occurs in the sixth decade of life. It has a better prognosis although they have high nuclear grade, aneuploidy and absence of hormone receptors expression. There is overexpression of adhesion molecules like E-cadherin which limits metastasis. 13% of cancers arising in BRCA1 carrier are medullary type.[61]

Macroscopy

They are well circumscribed mass, soft in consistency and are measuring 1 to 4 cm in diameter.[62]

Histopathology

Main criteria includes

1. Syncitium like sheets of large cells present. Cells have abundant cytoplasm with pleomorphic vesicular nuclei and prominent nucleoli. This pattern should be present at least 75% of tumor. Glands and tubules are not seen.

2. Scanty stroma with moderate to severe lymphoplasmacytic infiltrate

3. Pushing borders.[62]

Invasive papillary carcinoma

It is a rare breast cancer type and frequently occurs in elderly women.[63]

Macroscopy

Grossly they are well demarcated tumor, soft in consistency and the size varies from 1 to 3cm in diameter.[62]

Histopathology

Papillary structure with fibrovascular core is the characteristic feature of this tumor. Most of the carcinomas are surrounded by fibrosis and chronic inflammatory cells. Cells may have nuclear pleomorphism. Mitosis is increased.[64]

Invasive micropapillary carcinoma

It is an uncommon type, the epithelial cells form micropapillae with out fibrovascular core. It presents as solid tumor and has high lymph node metastasis. Tumor cells have moderate pleomorphism and low mitotic activity.[65]

MIXED TYPE

Mixed ductal and lobular carcinoma

In this tumor ductal component accounts for 10 to 90% of the tumor. Incidence varied from 2 to 6% of all breast tumors.[66]

Mixed ductal and special type of tumor

In this tumor, special type of tumor may be tubular, invasive cribriform or mucinous. Special type of tumor should form more than 10% of tumor mass.

Rare types

Rare types of breast tumor includes

       1. Secretory carcinoma

       2. Lipid rich, glycogen rich

       3. Apocrine carcinoma

       4. Neuroendocrine carcinoma

Secretory carcinoma

It occurs in all ages. They are well defined and firm neoplasm. They are less than 3cm in diameter. Microscopically they have well defined border. It is a low grade tumor with solid, microcystic and tubular pattern. The characteristic feature is intracellular and extracellular vacuoles which may contain mucinous material. Prognosis is excellent in children but in adults it is not so good.[67]

Apocrine carcinoma

Carcinomas showing cytological and immunological features of apocrine cells in more than 90% of tumor are called as apocrine carcinoma. They account for less than 1% of the breast tumors. Neoplastic cells have abundant, granular, eosinophilic cytoplasm with high grade nuclear features. They usually negative for ER, PR but frequently positive for androgen receptor and GCDFP-15.[68]

Neuroendocrine carcinoma

Primary neuroendocrine tumor of the breast will express neuroendocrine markers in more than 50% of the cells.[69] 1 to 4% of the tumors are neuroendocrine tumors. Histologically they include solid, small

cell and large cell neuroendocrine tumors. Solid type will have nest and trabeculae separated by thin fibrvascular stroma. They may have rosette like structure and peripheral palisadding. They tend to have poor prognosis than conventional invasive ductal carcinoma breast.[70]

Metaplastic carcinoma

These are heterogeneous group of tumors contain features of malignant epithelial elements and mesenchymal elements. Squamous and/or spindle cell components are present. Mesenchymal elements includes cartilage, bone and myxoid stroma. These tumors are uncommon.[71] Grossly they form large, firm, well defined tumor measuring up to 5cm in diameter. Microscopically two major subtypes include monophasic and biphasic. Conventional invasive carcinoma no special type present in biphasic form.[72] These tumors are triple negative and express cytokeratin 5/6, cytokeratin 14, cytokeratin 7 and CAM5.2.

Investigation

    1. Mammography

    2. cytology

    3. Needle core biopsy

    4. Open biopsy and frozen section

1. Mammography

Mammography helps in detecting small tumors of size 1mm to 2mm. Mammography detects calcified lesions. Cancer breast is associated with 50%to 60% of calcifications.

2. cytology

All palpable breast lesions are initially investigated with fine needle aspiration cytology. Although it is a cost effective, simple method but invasion can not be detected.

Role of FNAC in cancer breast

The confiramation of clinically suspected and inoperable cancer.

To investigate the suspected recurrence or metastasis in case of previously diagnosed cancer.

To obtain tumor cells for special analysis such as IHC and cytogenetic analysis.

3. Needle core biopsy

Needle core biopsy is useful in diagnosing cancer breast with invasive component. Hormonal status(ER,PR) and HER2 overexpression can be studied.

4. Open biopsy and frozen section

There are two types of biopsy one is excision biopsy and the other one is incisional biopsy.

Frozen sections are used to evaluate margin and to confirm the diagnosis of suspected invasive cancer detected by other methods.

Disadvantages of frozen section

Sampling error

Technical error

Histological misinterpretation

Staging

TNM system of staging is commonly used and it is adopted by American joint committee on cancer(AJCC).

Grading

Nottingham modification of the Bloom-Richardson system of grading is commonly used. It is based on microscopic assessment of tubule formation, nuclear pleomorphism and mitotic count.[73]

Prognostic and predictive factors

Prognosis of breast cancer varies widely. Prognosis mainly determined by pathological examination of primary cancer and axillary node. It is useful for patient counseling, appropriate treatment and clinical trials.[74]

Invasive versus in situ carcinoma

Most of the in situ carcinoma can be cured with adequate where as more than 50% patients with invasive cancer have metastasis.[74]

Distant metastasis

Distant metastasis is a poor prognostic factor. But it depends up on other factors like tumor type and location metastasis.[75]

Lymph node metastasis

In the absence of distant metastasis axillary nodal involvement is the important factor. Metastatic foci of less than 0.2cm are called as micrometastasis. Macrometastasis are more than 0.2cm in size and are important prognostic factor. Nearly 10% - 20% of women without axillary

node metastasis may have recurrence due to metastasis via blood or internal mammary node.[74]

Tumor size

Size of the tumor is second most important prognostic indicator. Number of axillary nodal metastasis is increases with the size of the tumor.[74]

Locally advanced disease

Cancer involving chest wall and skin are difficult to treat. They are associated with poor prognosis.

Inflammatory carcinoma

Carcinoma breast presenting with swelling and skin involvement have poor prognosis.

Histologic subtype

When compared to carcinoma of breast no special type women with special type of invasive cancer (tubular, medullary, mucinous, lobular) have increased survival rate.

Histologic grade

The Nottingham Histologic Score is the commonly used grading system. It combines nuclear grade, mitotic rate and tubule formation to classify carcinoma of breast as Grade I, II and III carcinoma.[76]

Estrogen and progesterone receptors

Patients with ER and PR positive breast cancer have increased disease free survival rate. ER and PR positivity is positively correlated with response to hormonal therapy.[77]

HER2/neu

Response to trastuzumab is predicted with overexpression of HER2/neu oncogene. Women with tumor which showing overexpression will have poor prognosis. Her2/neu overexpression is correlated with high grade tumor.[78]

Stromal CD10 expression

Stromal expression of CD10 is associated with hormonal receptor (ER) negativity, high grade tumor and low survival rate.[4,7]

Types of margin

Better prognosis has been observed in tumors with pushing type of margin. This applies to medullary carcinoma as well as other well circumscribed tumors.

Micro vessel density

Invasive breast carcinoma will behave in aggressive manner when they have increased vascularity in the surrounding stroma.

Elastosis

Tumors with no elastosis will have reduced response to hormonal therapy.

TREATMENT

Type and extent of the breast cancer determines the treatment .

    1) Surgery

    2) Radiation therapy

    3) Hormonal therapy

4) Chemotherapy

5) Target therapy

Simple mastectomy, radical mastectomy and modified radical mastectomy are the some of the surgical methods. Radiation therapy is used in postoperatively and to control the local recurrence of carcinoma. The chemotherapy is given as an neoadjuvant therapy and after local treatment and also given in breast cancers with axillary node metastasis. Chemotherapy can be used in combination with surgery and radiation in patients with large (>3 cm) tumors in order to avoid mastectomy. The hormonal therapy is used in hormone receptor-positive breast carcinomas.

MATERIALS & METHODS

MATERIALS AND METHODS

STUDY DESIGN:

The present study is a Prospective study conducted in the Department of Pathology during the period from July 2014 to June 2015. Ethical clearance for the study was obtained from the Ethics Committee of Coimbatore Medical College, Coimbatore.

A total sample of 30 cases of invasive ductal carcinoma breast NOS type were analyzed.

PLACE OF STUDY:

Department of Pathology, Coimbatore Medical College, Coimbatore

STUDY PERIOD:

July 2014 – June 2015

INCLUSION CRITERIA:

Invasive ductal carcinoma of breast not otherwise specified type.

EXCLUSION CRITERIA:

1. Patients on chemotherapy and radiotherapy

2. Male patients

3. Ill fixed specimen

The study was done in 30 invasive ductal carcinoma of breast cases sent from Department of Surgery and Department of Surgical Oncology, Coimbatore Medical College Hospital, Coimbatore for histopathological examination .

Hematoxylin and Eosin stained microscopic slides of the primary tumor were reviewed to confirm the diagnosis, to define tumor subtype and to standardize grading of invasive ductal carcinoma according to the Nottingham Modification of the Bloom and Richardson system.

GRADE	SCORE
I	3T0 5
II	6&7
III	8&9

Microscopic grading of carcinoma of breast: Nottingham Modification of the Bloom and Richardson system
Tubule formation
1 point: Tubular formations in >75% of the tumor
2 point: Tubular formations in 10-75% of the tumor
3 point: Tubular formations in <10% of the tumor
Nuclear pleomorphism
1 point: Nuclei with minimal variation in size and shape
2 point: Nuclei with moderate variation in size and shape
3 point: Nuclei with marked variation in size and shape
Mitotic count
1 point: upto 11/10 hpf, 2 point: 12 to 23/10 hpf, 3 point: 23 or more/ 10 hpf

Allred/ Quick Score system[82]

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

ER and PR markers were considered positive when the combined score for proportion and intensity is 3 or more.

HER 2-neu scoring was done as per following table[83]

Staining pattern	Score	HER2 neu Overexpression
No staining or membrane staining<10% tumor cells	0	Negative
Faint/perceptible membrane staining in >10% cells	1+	Negative
Weak to moderate complete membrane staining in >10% cells	2+	Weak
Strong complete membrane staining in >30% cells	3+	Strong

CD10 scoring was done as per the following table[79]. Pattern of staining for CD10 is cytoplasmic and membranous positivity in stromal cells. Both negative and weak expression were considered as

negative. Only strong CD10 expression was considered as positive for statistical purpose.

Score	Result	CD10 staining
0	Negative	<10% stromal positive cells (cytoplasmic and membrane positivity)
1	Weak	10%-30% stromal positive cells
2	Strong	>30% stromal positive cells

Formalin fixed and paraffin embedded tissue specimen of invasive breast carcinoma were examined. Four micron sections were cut and stained for immunohistochemistry with mouse monoclonal antibodies.[79] The staining was done as per the following protocol.

REAGENTS USED IN IMMUNOHISTOCHEMISTRY

1.Peroxide block

2. Power block

3. Chromogen - Diaminobenzidine

4. Liquid DAB substrate

5. Super enhancer

6. Poly HRP reagent

7. Hematoxylin- counter stain

8. Buffer solutions

BUFFERS USED

1.   TRIS EDTA : pH- 9.0

   TRIS buffer salt : 6.05 gm

   Disodium EDTA: 0.744 gm

   Distilled water :   1000ml

2. TRIS BUFFER pH - 8

    TRIS buffer salt : 6.05 gm

    Sodium chloride : 8 gm

    Distilled water : 1000ml

    1N Hydrochloric acid : 3 ml

3. CITRATE BUFFER pH-6

    Trisodium citrate : 2.94 gm

    Distilled water : 1000ml

    1N Hydrochloric acid : 5 ml

IMMUNOHISTOCHEMISTRY PROCEDURE

1. Overnight incupation of slides in incubator at $60^{0}C$

2. Deparaffinisation of the tissue sections in xylene for 30 minutes

3. Wash with absolute alcohol for five minutes with two changes

4. Tap water wash for ten minutes

5. Rinse in distilled water for five minutes

6.   Antigen retrieval was done by placing the slides in microwave with appropriate buffers for 20 minutes.

7.   Cool in room temperature, then rinse in distilled water.

8.   Wash in TBS buffer for five minutes with two changes.

9.   Apply peroxide block for ten minutes.

10.   Wash in TBS buffer for five minutes with two changes

11.   Apply power block on sections for ten minutes.

12.   Drain the slide, add primary antibody and incupation at Room temperature in moisture chamber for 1 hour.

13.   Wash in TBS buffer for five minutes with two changes.

14.   Cover the slides with superenhancer for thirty minutes

15.   Wash in TBS buffer for five minutes with two changes

16.   Then apply reagent of poly HRP for thirty minutes.

17.   Wash in TBS buffer for five minutes with two changes.

18.   Apply DAB chromogen for five to eight minutes.

19.   Wash in TBS buffer for five minutes with two changes.

20.   Tap water wash for five minutes.

21.   Counterstain with Mayers hematoxylin for one minute.

22.   Tap water wash for five minutes.

23.   Air dry and mount in DPX.

Statistical analysis:

The collected data was tabulated and analyzed. Continuous data was expressed as mean. Statistical correlation between stromal expression of CD10 and histopathological grade, ER, PR and HER2/neu expression were performed as per Chi square test. p values of less than 0.05 were considered as significant. Pearson coefficient of correlation was used to assess the relationship between the variables.

OBSERVATION & RESULTS

OBSERVATION AND RESULTS

Table 1: Distribution of IDC of breast according to different age group

AGE	NUMBER	%
21-30	1	3.3%
31-40	6	20%
41-50	11	36.7%
51-60	9	30%
>60	3	10%
TOTAL	**30**	**100%**

Total of 30 cases were studied and the following observations were obtained. The age of the patients ranges from 23 to 72 years with mean age of 48 years.

Majority of IDC of breast cases belong to 41- 50 age group(36.7%).

CHART 1: DISTRIBUTION OF IDC OF BREAST ACCORDING TO

DIFFERENT AGE GROUP

Age Distribution(N=30)

	<40	41 - 50	51 - 60	> 60
(%)	23%	37%	30%	10%
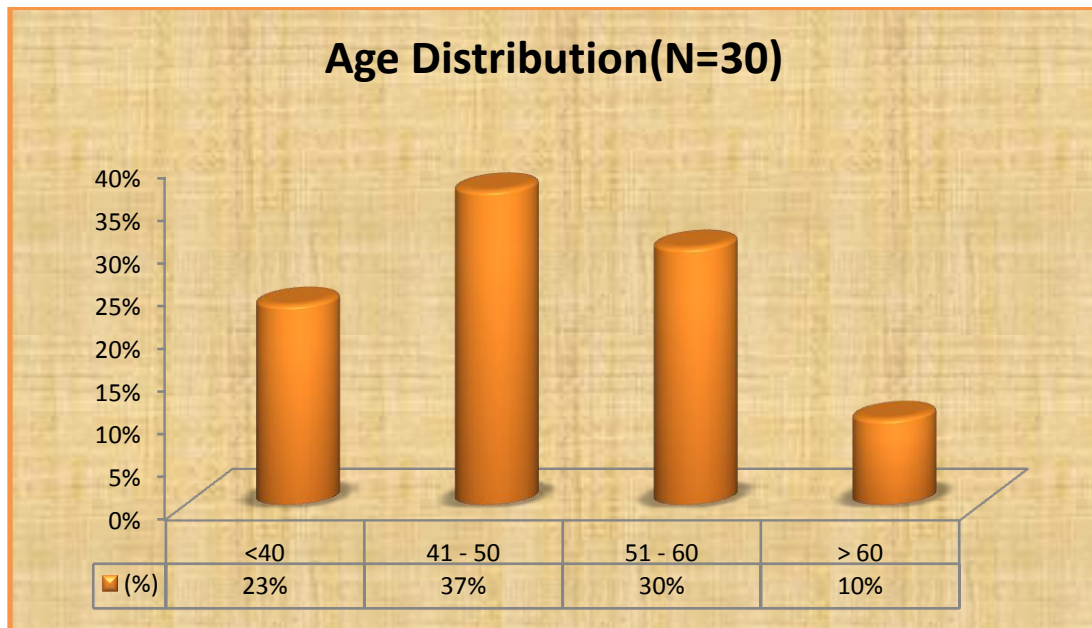
Majority of IDC of breast cases belong to 41- 50 age group(37%).

MEAN AGE OF GROUP

AGE	MEAN	SD	95% CI OF MEAN		Lowest age	Highest age
			LOWER	**UPPER**		
TOTAL	48.1 yrs	10.5 yr	44 yrs	52 yrs	23 yrs	72 yrs

Mean age of the study group is 48.1years

Lowest age in this study is 23 years.

Highest age in this study is 72 years.

TABLE 2: STROMAL EXPRESSION OF CD1O IN BREAST CARCINOMA

STROMAL CD10 EXPRESSION			
NEGATIVE	WEAK POSITIVE	STRONG POSITIVE	TOTAL
8(27%)	8(27%)	14(46%)	30

CHART 2: STROMAL EXPRESSION OF CD1O IN BREAST CARCINOMA
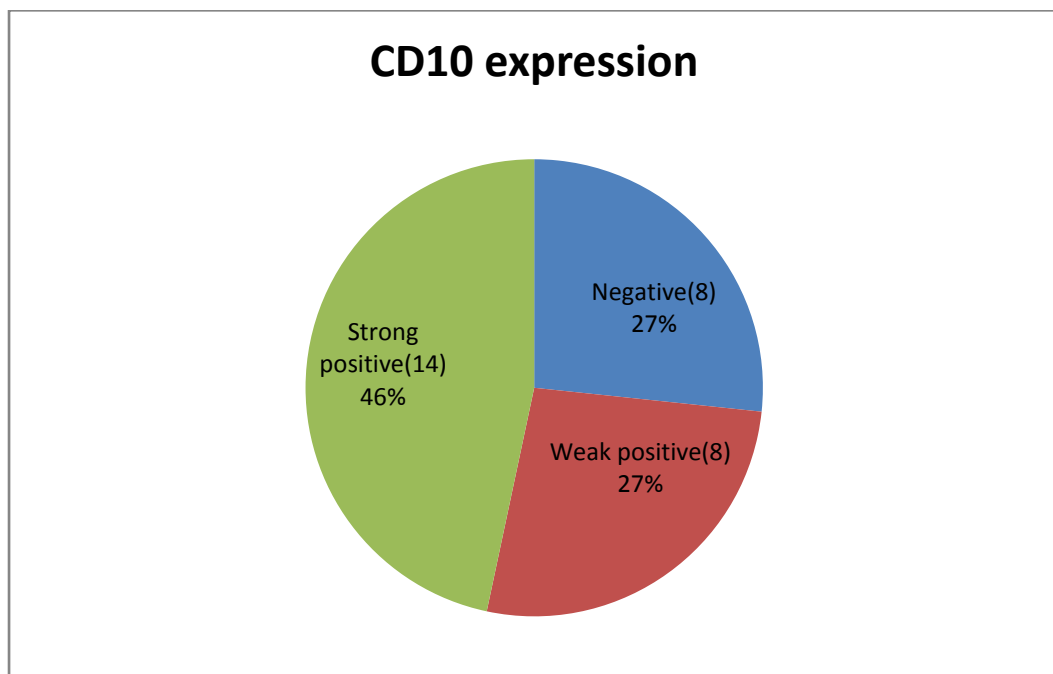
73% (22 out of 30) of the cases showed positivity for CD10 in the stroma, of which 46%(14) cases were strongly positive and 27%(8) were weakly positive.

TABLE 3: STROMAL CD10 EXPRESSION WITH

HISTOPATHOLOGICAL GRADE

	CD10			
GRADE	NEGATIVE	WEAK POSITIVE	STRONG POSITIVE	TOTAL
I	2	3	3	8
II	4	4	1	9
III	2	1	10	13
TOTAL	**8**	**8**	**14**	**30**

77% (10 out of 13) of the grade III Invasive ductal carcinoma of breast showed strong stromal CD10 expression. The association is   statistically significant,   p value is less than 0.05 (p value 0.04, Chi -square test).

CHART 3: ASSOCIATION OF STROMAL CD10 EXPRESSION
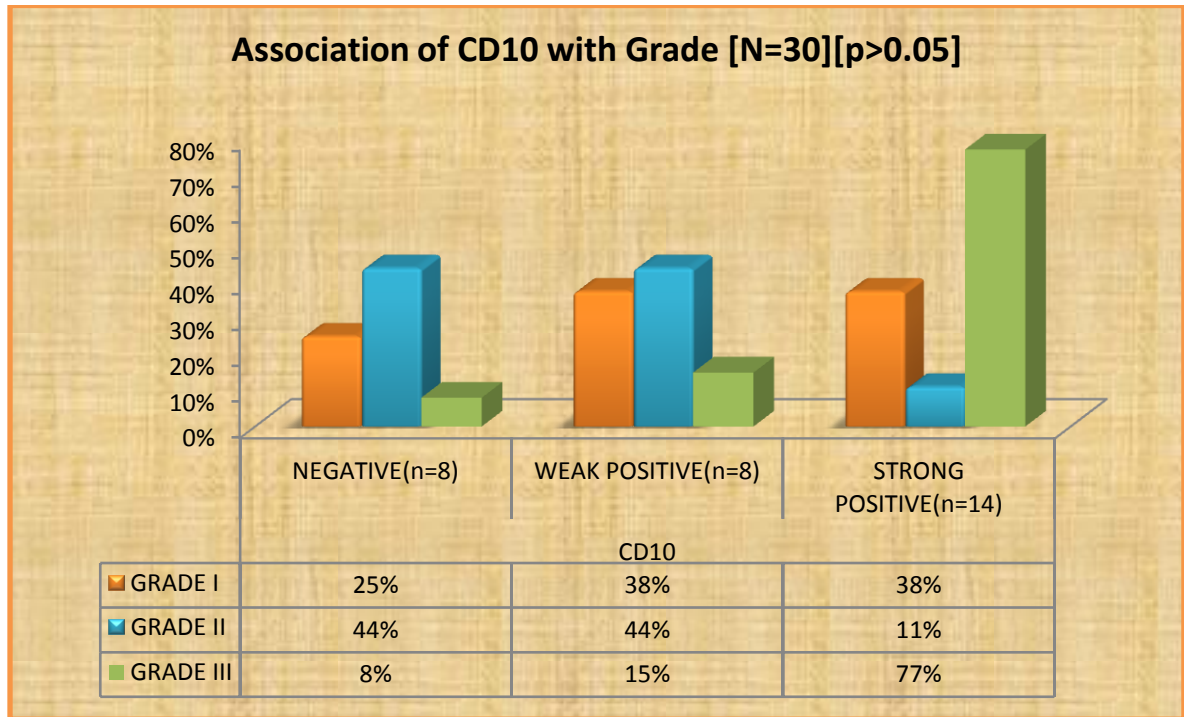
WITH HISTOPATHOLOGICAL GRADE

Association of CD10 with Grade [N=30][p>0.05]

CD10	NEGATIVE(n=8)	WEAK POSITIVE(n=8)	STRONG POSITIVE(n=14)
GRADE I	25%	38%	38%
GRADE II	44%	44%	11%
GRADE III	8%	15%	77%

77%(10 out of 13) of the grade III Invasive ductal carcinoma of breast showed strong stromal CD10 expression. The association is statistically significant, p value is less than 0.05.

TABLE 4: ASSOCIATION OF STROMAL CD10

EXPRESSION WITH ER

ER	CD10			
	NEGATIVE	**WEAK POSITIVE**	**STRONG POSITIVE**	**TOTAL**
NEGATIVE	2	3	13	18
POSITIVE	6	5	1	12
TOTAL	**8**	**8**	**14**	**30**

92% (13 /14) of the strong stromal CD10 positive Invasive ductal carcinoma of breast showed ER negativity . The association is statistically significant, p value is less than 0.05(p value 0.002, Chi- square test).

CHART 4: ASSOCIATION OF STROMAL CD10

EXPRESSION WITH ER

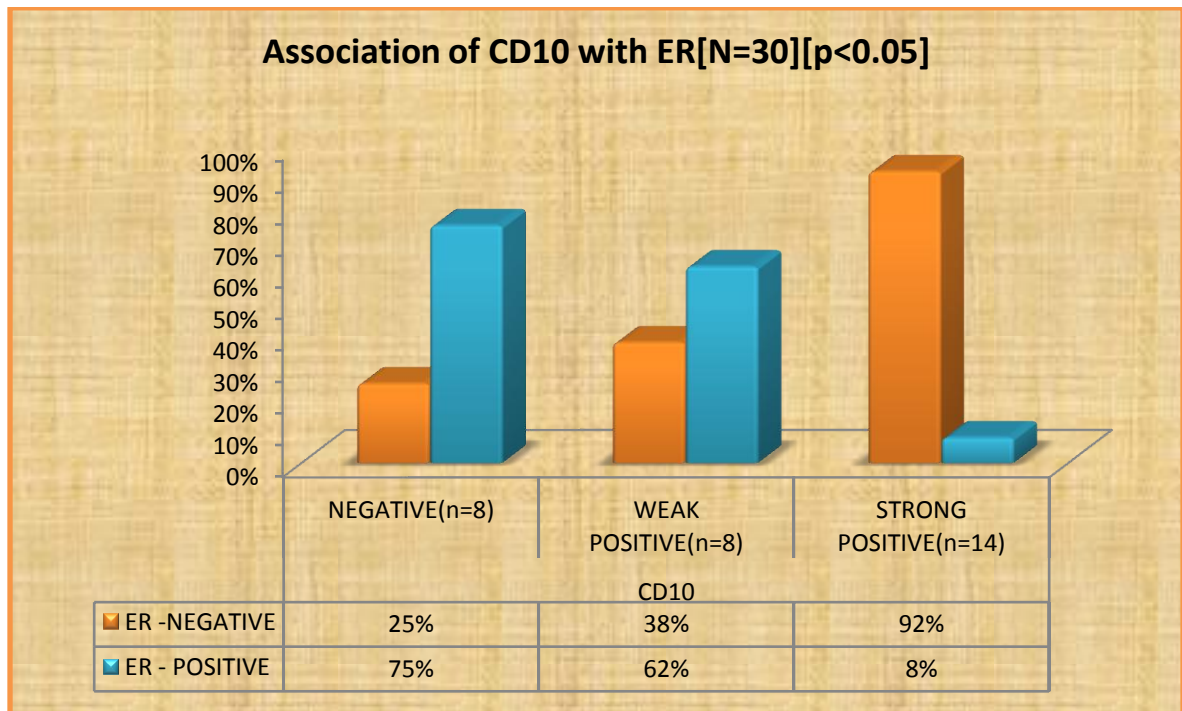**Association of CD10 with ER[N=30][p<0.05]**

CD10	NEGATIVE(n=8)	WEAK POSITIVE(n=8)	STRONG POSITIVE(n=14)
ER -NEGATIVE	25%	38%	92%
ER - POSITIVE	75%	62%	8%

92% (13 /14) of the strong stromal CD10 positive Invasive ductal carcinoma of breast showed ER negativity . The association is statistically significant, p value is less than 0.05(p value 0.002, Chi- square test).

TABLE 5: ASSOCIATION OF STROMAL CD10

EXPRESSION WITH PR

PR	CD10			
	NEGATIVE	WEAK POSITIVE	STRONG POSITIVE	TOTAL
NEGATIVE	3	1	13	17
POSITIVE	5	7	1	13
TOTAL	8	8	14	30

92% (13 /14) of the strong stromal CD10 positive Invasive ductal carcinoma of breast showed PR negativity . The association is statistically significant, p value is less than 0.05 (p value 0.0005, Chi- square test).

CHART 5: ASSOCIATION OF STROMAL CD10

EXPRESSION WITH PR

Association of CD10 with PR[N=30][p<0.05]

CD10	NEGATIVE(n=8)	WEAK POSITIVE(n=8)	STRONG POSITIVE(n=14)
PR -NEGATIVE	38%	13%	92%
PR - POSITIVE	63%	87%	8%

92% (13 /14) of the strong stromal CD10 positive Invasive ductal carcinoma of breast showed PR negativity . The association is statistically significant, p value is less than 0.05 (p value 0.0005, Chi- square test).
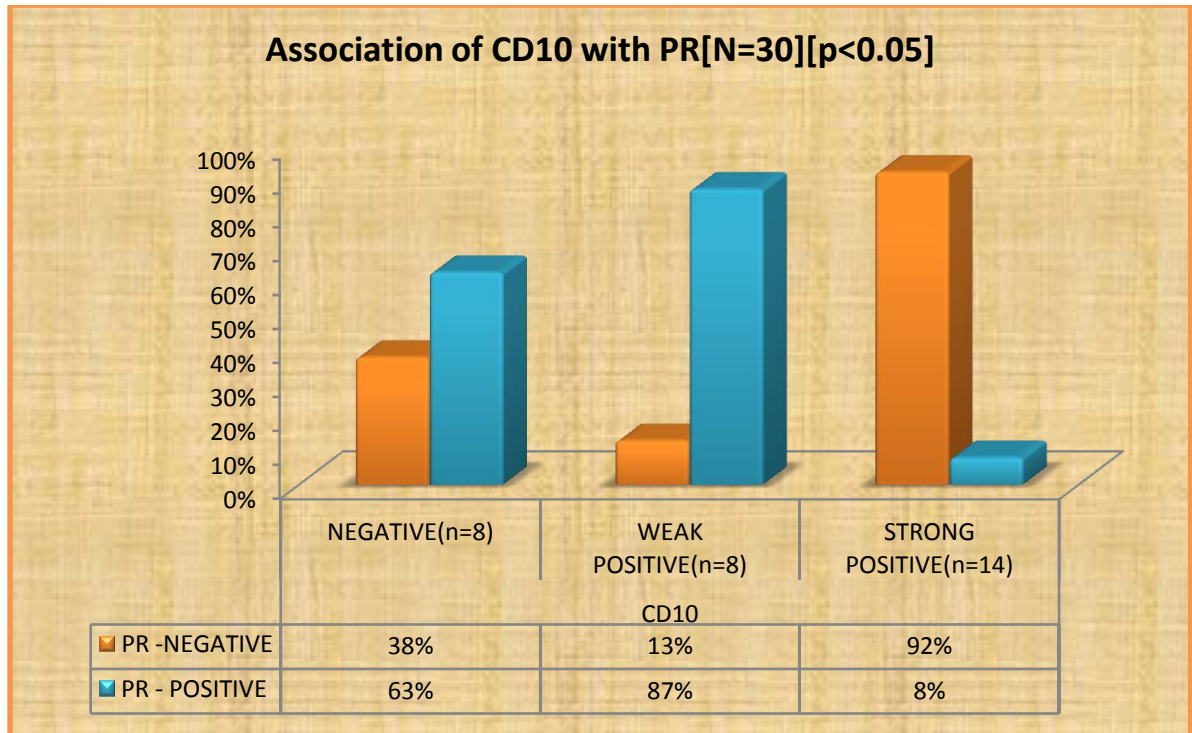
TABLE 6: ASSOCIATION OF STROMAL CD10 EXPRESSION WITH HER2/NEU

HER2/neu	CD10			
	NEGATIVE	WEAK POSITIVE	STRONG POSITIVE	TOTAL
NEGATIVE	8	7	4	19
POSITIVE	0	1	10	11
TOTAL	**8**	**8**	**14**	**30**

71% (10/14) of the stromal CD10 positive Invasive ductal carcinoma of breast showed HER2/neu expression. The association is statistically significant, p value is less than 0.05 (p value 0.0009, Chi- square test).

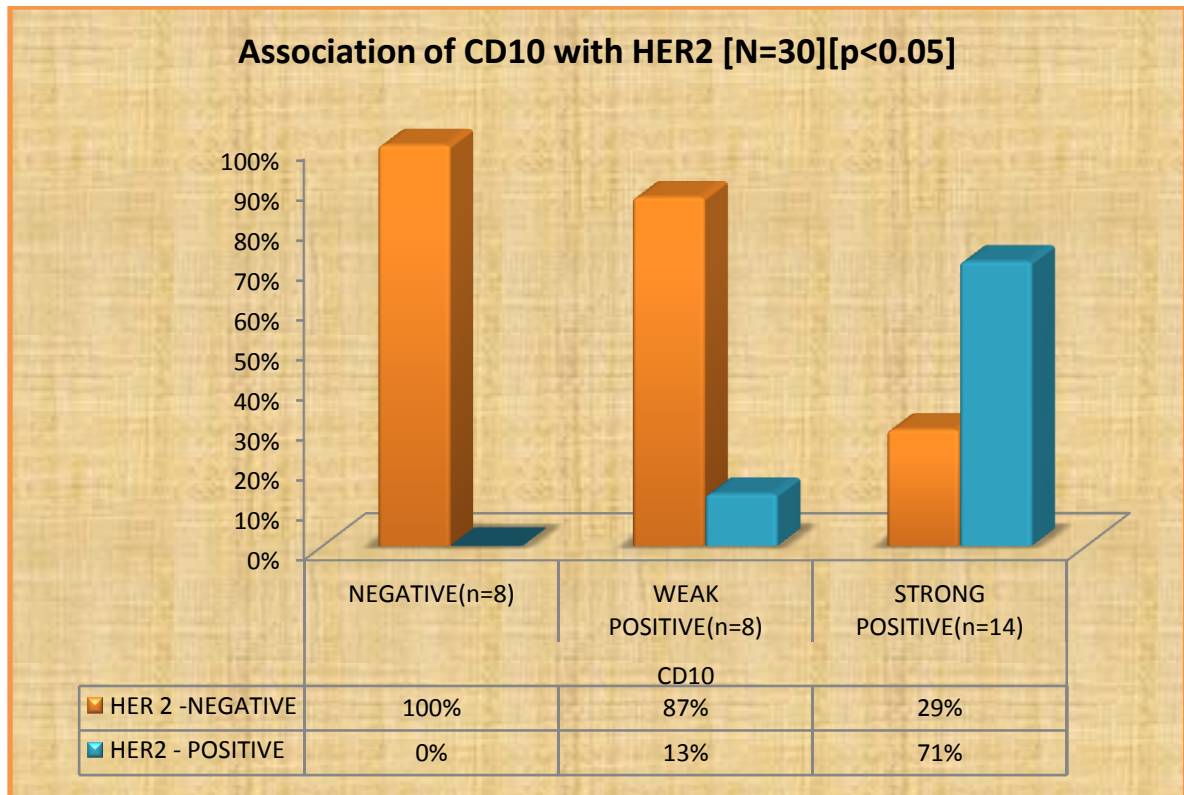# CHART 6: ASSOCIATION OF STROMAL CD10 EXPRESSION WITH HER2/NEU

Association of CD10 with HER2 [N=30][p<0.05]

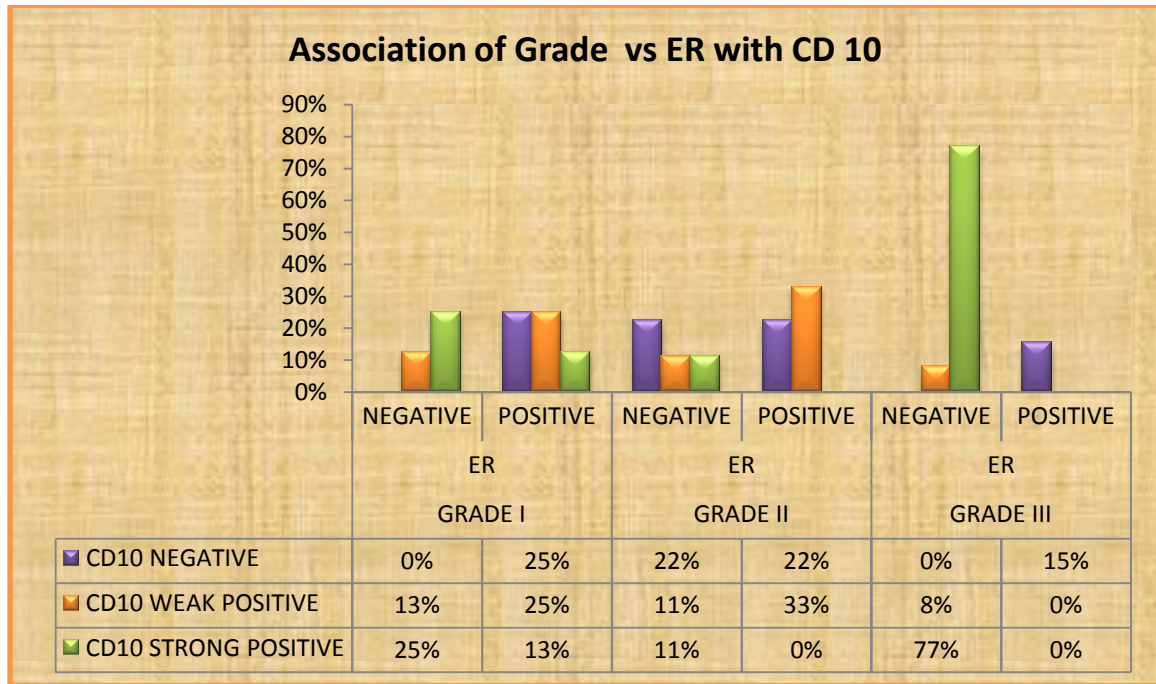| CD10	NEGATIVE(n=8)	WEAK POSITIVE(n=8)	STRONG POSITIVE(n=14)
HER 2 -NEGATIVE	100%	87%	29%
HER2 - POSITIVE	0%	13%	71%

71% (10/14) of the stromal CD10 positive Invasive ductal carcinoma of breast showed HER2/neu expression. The association is statistically significant,   p value is less than 0.05 (p value 0.0009, Chi- square test).

TABLE 7: ASSOCIATION OF CD10 WITH HISTOLOGICAL GRADE AND ER

HISTOLOGICAL GRADE VS ER WITH CD10						
GRADE			CD10			Total
			NEGATIVE	WEAK POSITIVE	STRONG POSITIVE	
GRADE I	ER	NEGATIVE	0	1	2	3
		POSITIVE	2	2	1	5
GRADE II	ER	NEGATIVE	2	1	1	4
		POSITIVE	2	3	0	5
GRADE III	ER	NEGATIVE	0	1	(77%)10	11
		POSITIVE	2	0	0	2

77% of stromal CD10 positive cases associated with histological grade III and ER negativity. Increased stromal CD10 expression is associated with higher tumor grade and reduced ER expression .

CHART 7: ASSOCIATION OF CD10 WITH HISTOLOGICAL GRADE AND ER

Association of Grade vs ER with CD 10

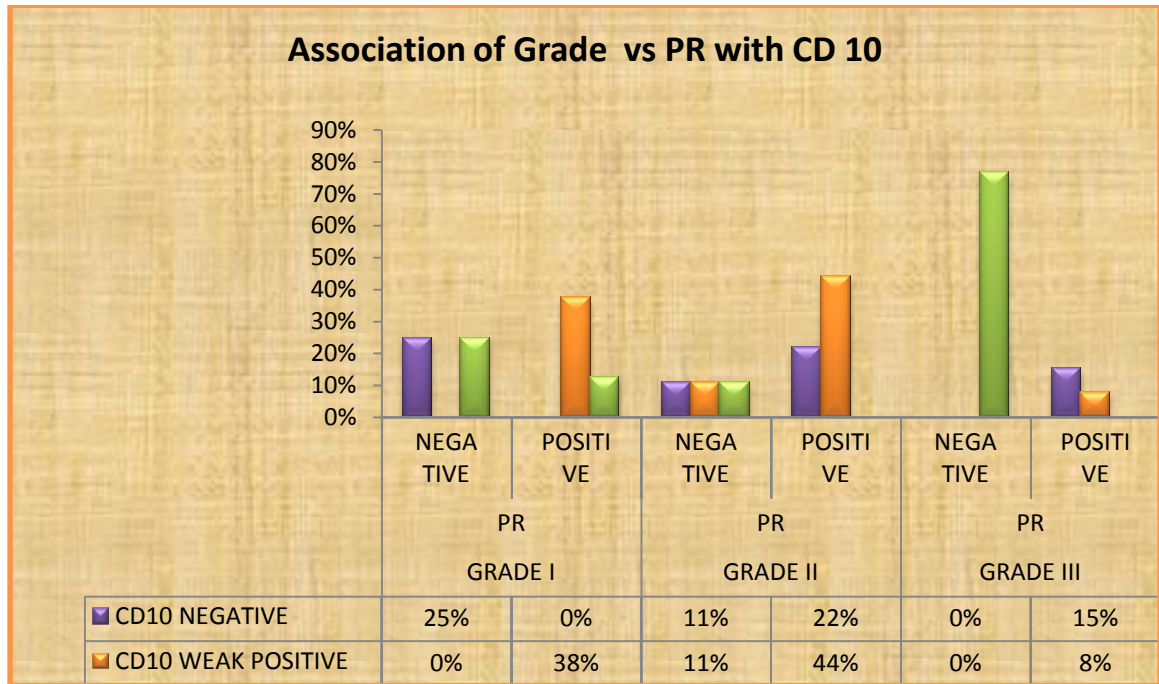|	GRADE I		GRADE II		GRADE III	
	ER		ER		ER	
	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE
CD10 NEGATIVE	0%	25%	22%	22%	0%	15%
CD10 WEAK POSITIVE	13%	25%	11%	33%	8%	0%
CD10 STRONG POSITIVE	25%	13%	11%	0%	77%	0%

77% of stromal CD10 positive cases associated with histological grade III and ER negativity. Increased stromal CD10 expression is associated with higher tumor grade and reduced ER expression .

TABLE 8: ASSOCIATION OF CD10 WITH HISTOLOGICAL GRADE AND PR

HISTOLOGICAL GRADE VS PR WITH CD10						
GRADE			CD10			Total
			NEGATIVE	WEAK POSITIVE	STRONG POSITIVE	
GRADE I	PR	NEGATIVE	2	0	2	4
		POSITIVE	0	3	1	4
GRADE II	PR	NEGATIVE	1	1	1	3
		POSITIVE	2	4	0	6
GRADE III	PR	NEGATIVE	0	0	(77%)10	10
		POSITIVE	2	1	0	3

77% of stromal CD10 positive cases associated with histological grade III and PR negativity. Increased stromal CD10 expression is associated with higher tumor grade and reduced PR expression .

CHART 8: ASSOCIATION OF CD10 WITH HISTOLOGICAL GRADE AND PR

Association of Grade vs PR with CD 10

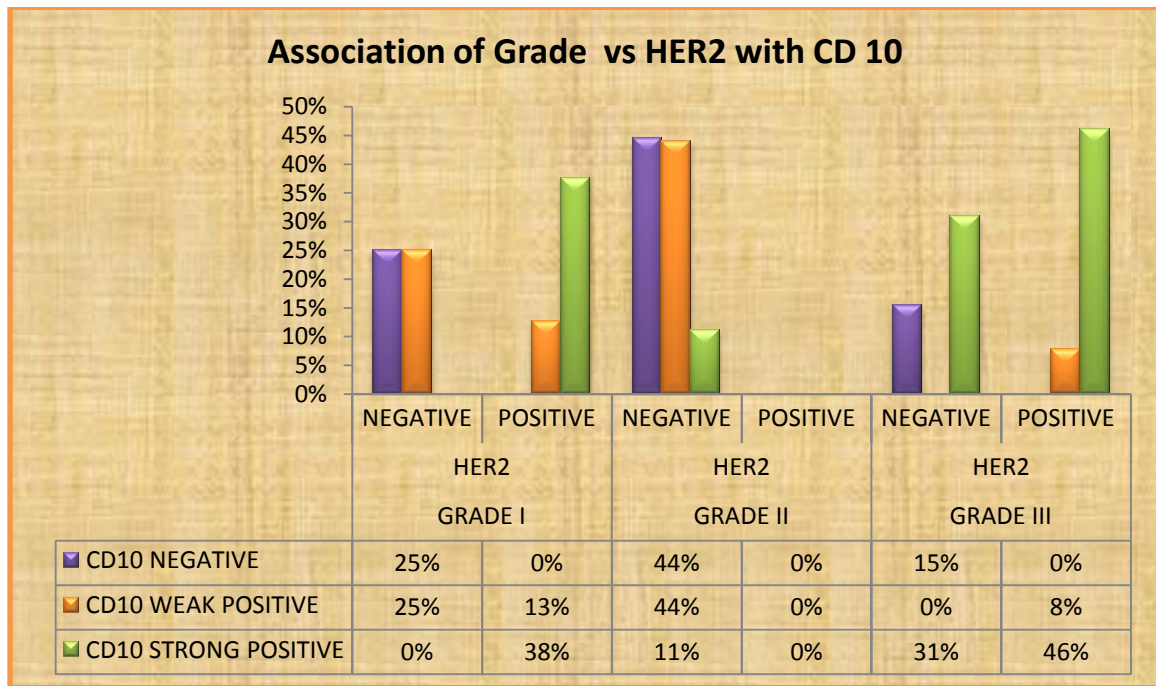|	GRADE I		GRADE II		GRADE III	
	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE
	PR		PR		PR	
CD10 NEGATIVE	25%	0%	11%	22%	0%	15%
CD10 WEAK POSITIVE	0%	38%	11%	44%	0%	8%

77% of stromal CD10 positive cases associated with histological grade III and PR negativity. Increased stromal CD10 expression is associated with higher tumor grade and reduced PR expression .

TABLE 9: ASSOCIATION OF CD10 WITH HISTOLOGICAL GRADE AND HER2

| HISTOLOGICAL GRADE VS HER2 WITH CD10						
GRADE			CD10			Total
			NEGATIVE	WEAK POSITIVE	STRONG POSITIVE	
GRADE I	HER 2	NEGATIVE	2	2	0	4
		POSITIVE	0	1	3	4
GRADE II	HER 2	NEGATIVE	4	4	1	9
		POSITIVE	0	0	0	0
GRADE III	HER 2	NEGATIVE	2	0	4	6
		POSITIVE	0	1	(46%)6	7

46% of stromal CD10 positive cases associated with histological grade III and HER2/neu positivity. Increased stromal CD10 expression is associated with higher tumor grade and increased HER2 expression .

CHART 9: ASSOCIATION OF CD10 WITH HISTOLOGICAL GRADE AND HER2/NEU

Association of Grade vs HER2 with CD 10

	GRADE I		GRADE II		GRADE III	
	HER2		HER2		HER2	
	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE
CD10 NEGATIVE	25%	0%	44%	0%	15%	0%
CD10 WEAK POSITIVE	25%	13%	44%	0%	0%	8%
CD10 STRONG POSITIVE	0%	38%	11%	0%	31%	46%

46% of stromal CD10 positive cases associated with histological grade III and HER2/neu positivity. Increased stromal CD10 expression is associated with higher tumor grade and increased HER2 expression .
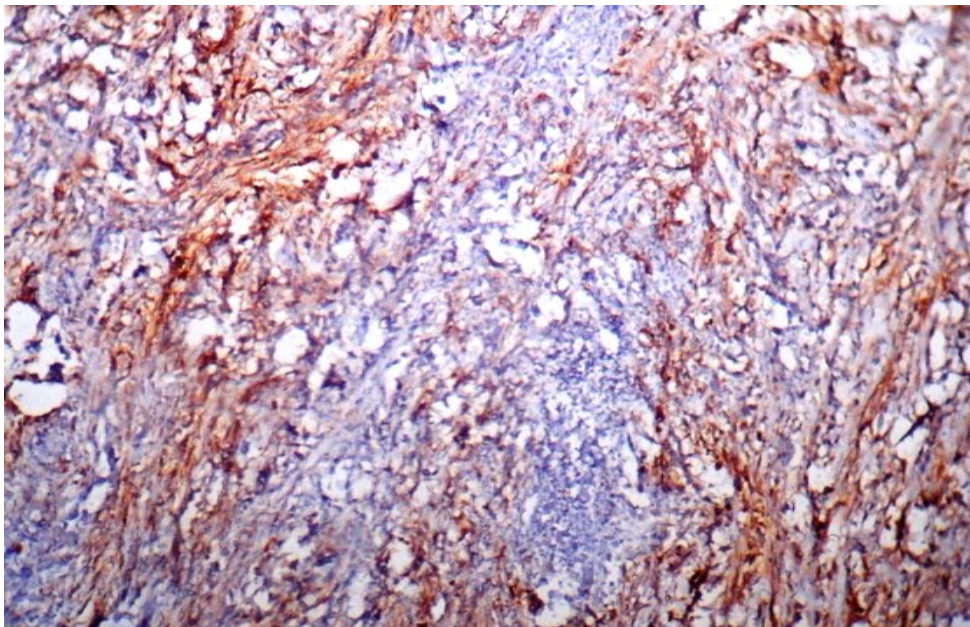
COLOUR PLATES

COLOR PLATE 1

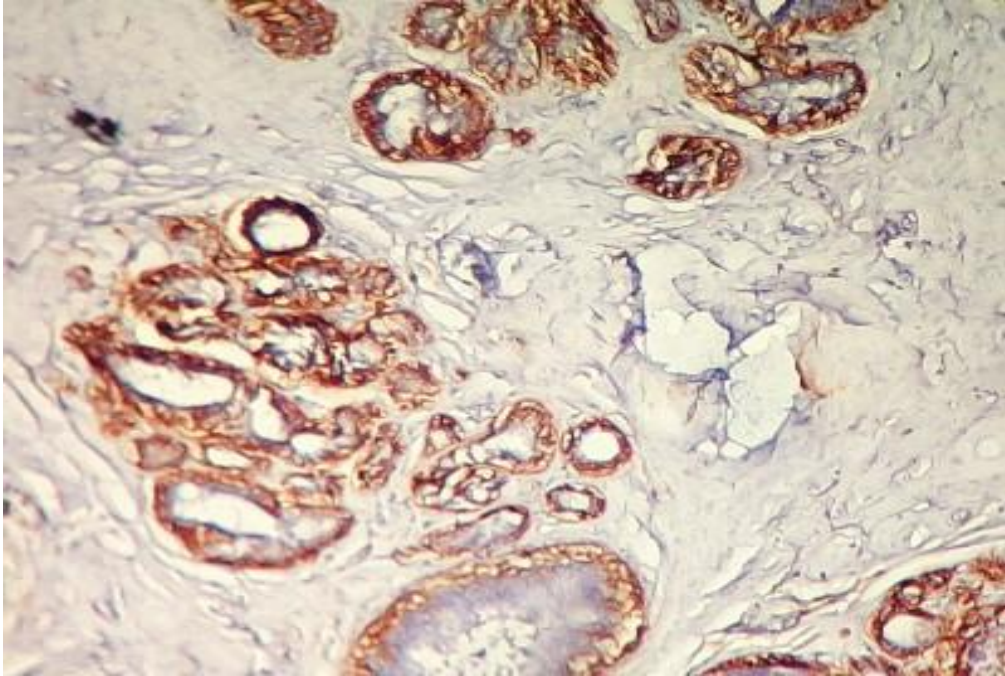## IMMUNOHISTOCHEMISTRY OF STROMAL CD 10
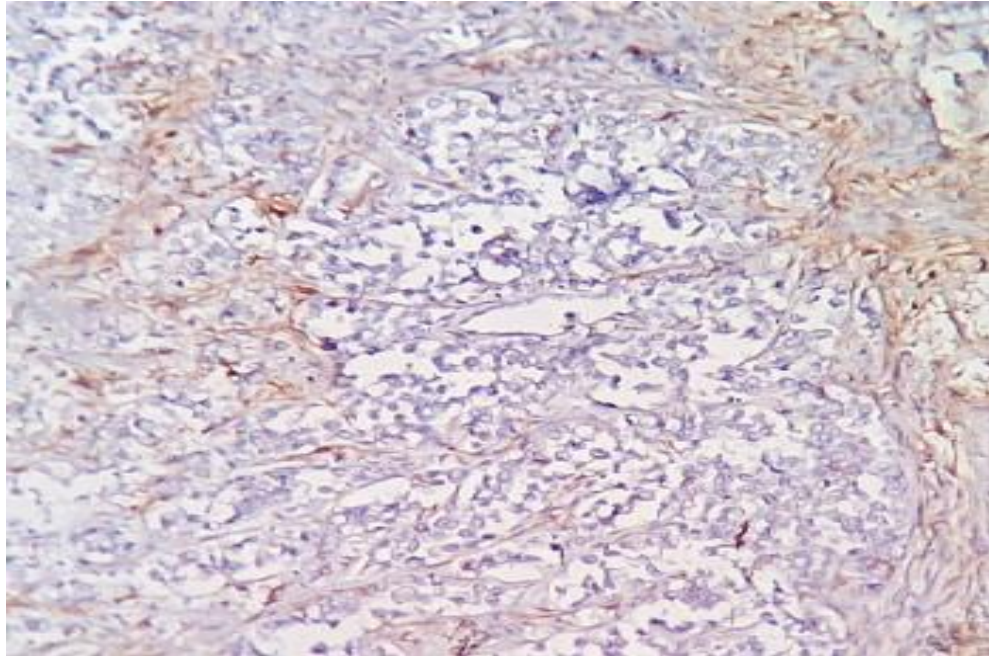
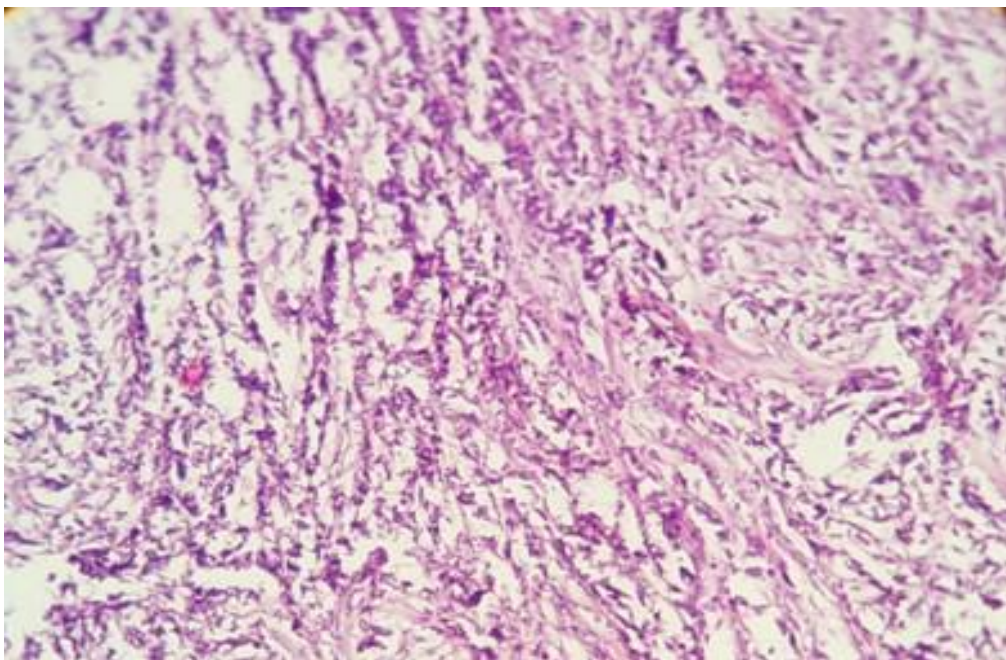**Picture of stromal CD10 cytoplasmic positivity 1+(10%-30% stromal positive cells)**

Picture of stromal CD10 Cytoplasmic positivity 2+(>30% stromal positive cells)

COLOR PLATE 2

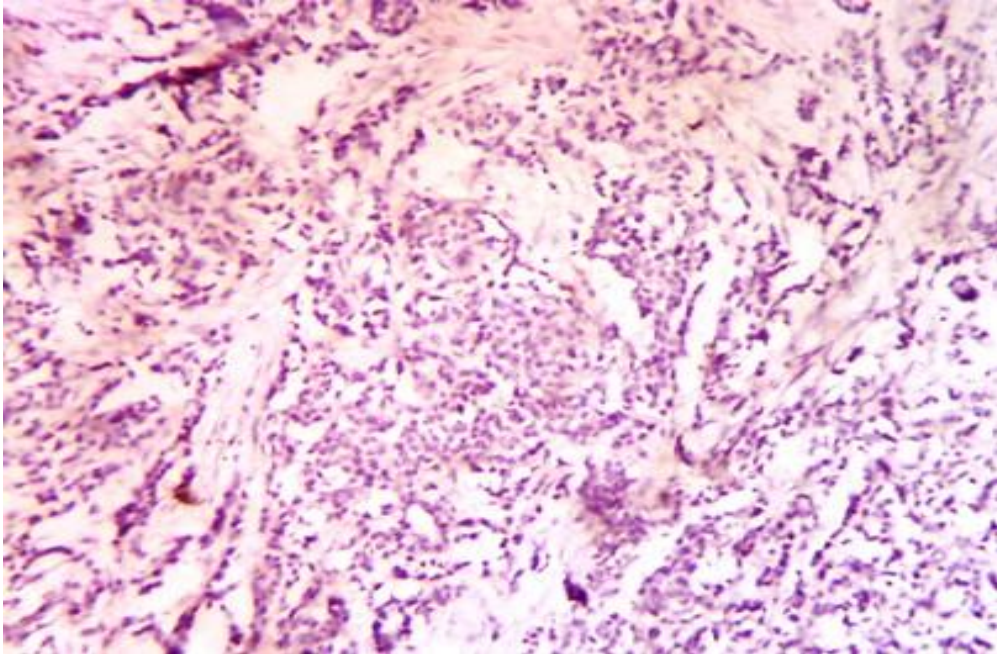## IMMUNOHISTOCHEMISTRY OF CD 10

Picture of stromal CD10 negativity,but myoepithelial cells positive for CD10
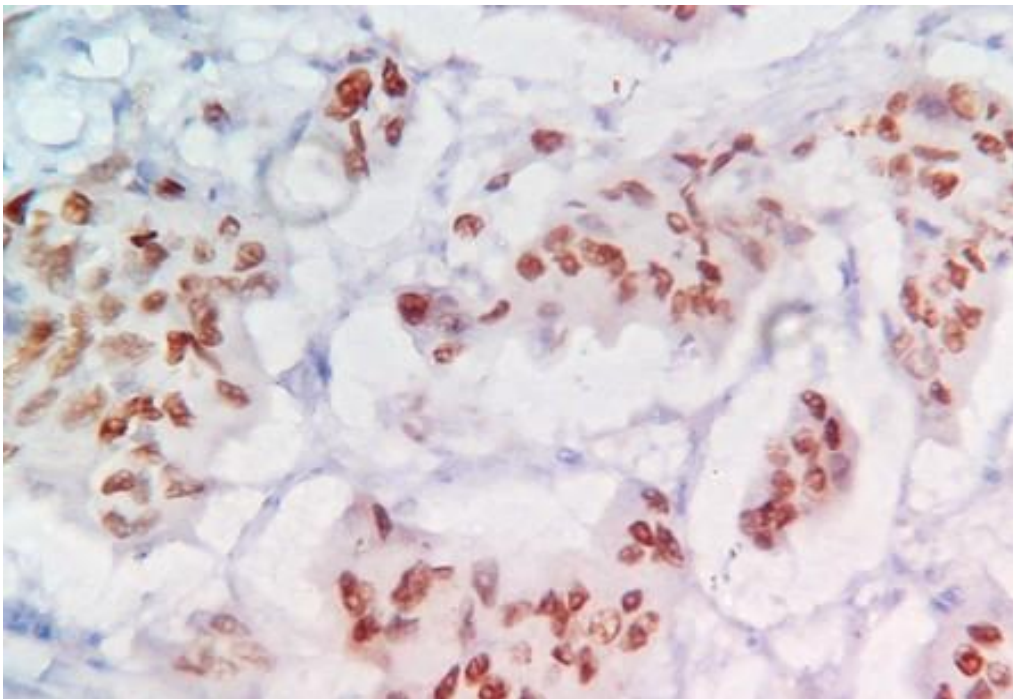

Picture of stromal CD10 negativity

COLOR PLATE 3

IMMUNOHISTOCHEMISTRY OF ER

Picture showing ER negativity

Picture showing ER nuclear positivity(5/8)

COLOR PLATE 4

IMMUNOHISTOCHEMISTRY OF PR

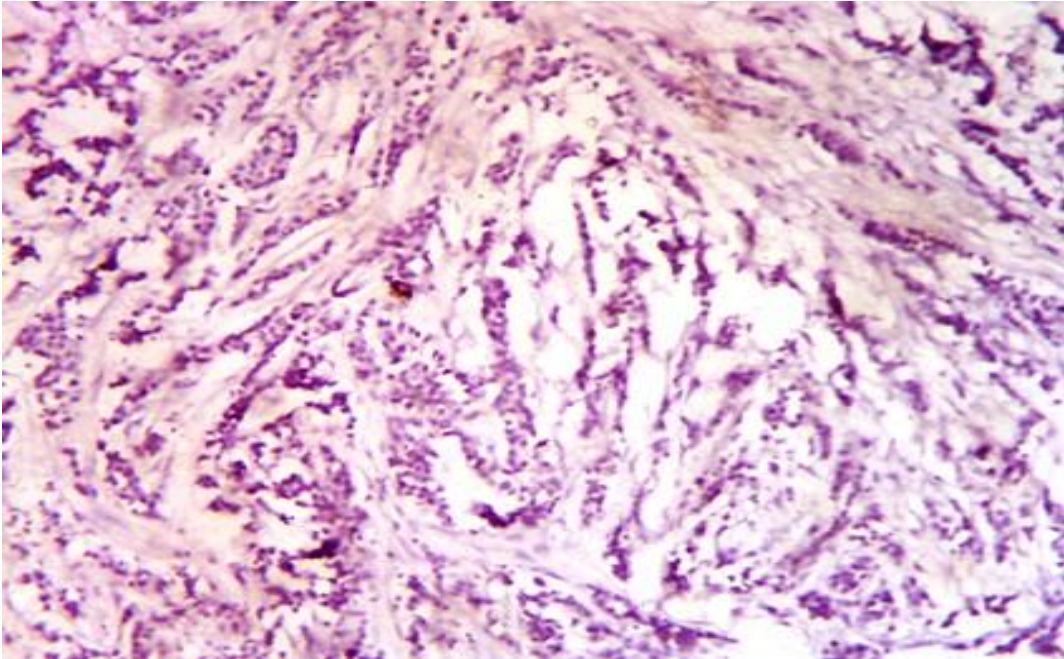Picture showing PR negativity
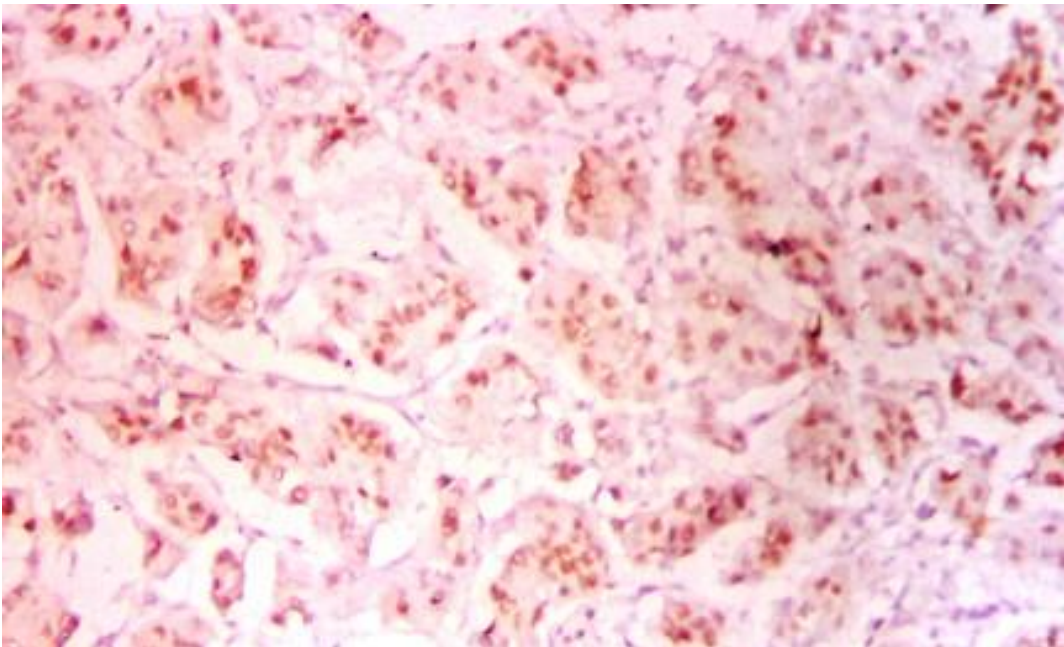
Picture showing PR nuclear positivity (5/8)

COLOR PLATE 5

IMMUNOHISTOCHEMISTRY OF HER2/neu
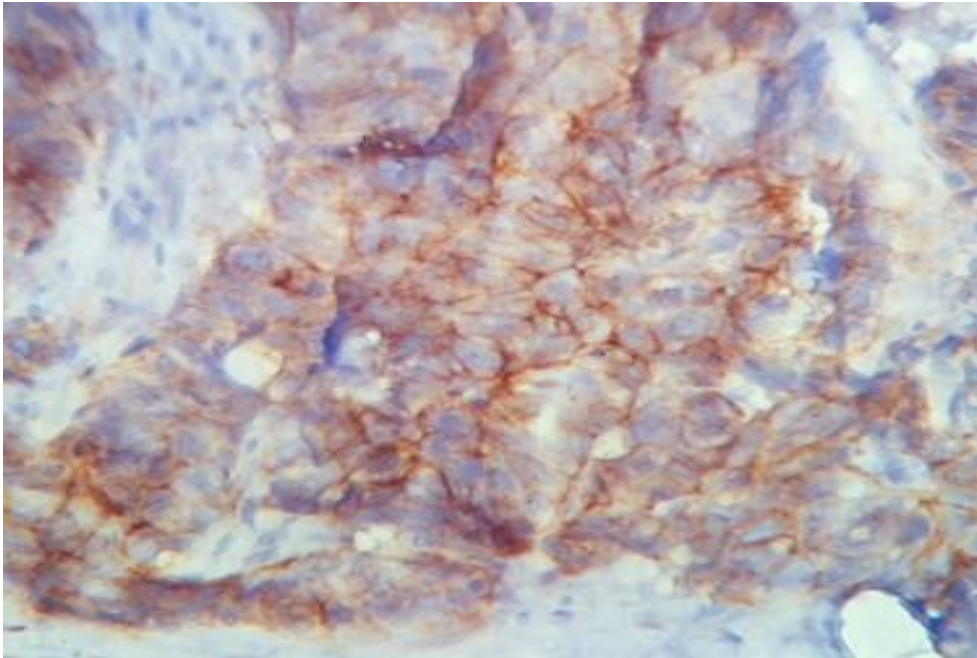
Picture showing HER2/neu Weak to moderate complete membrane staining positivity(2+)
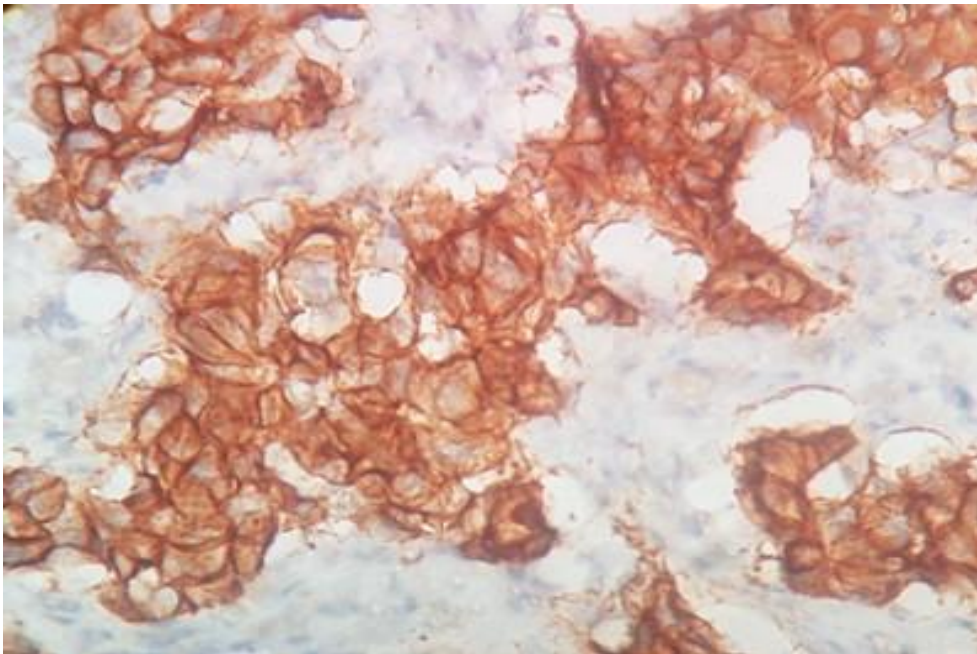
Picture showing HER2/neu Strong complete membrane staining in >30% cells positivity(3+)

COLOR PLATE 6

HEMATOXYLIN &EOSIN STAINING OF IDC BREAST
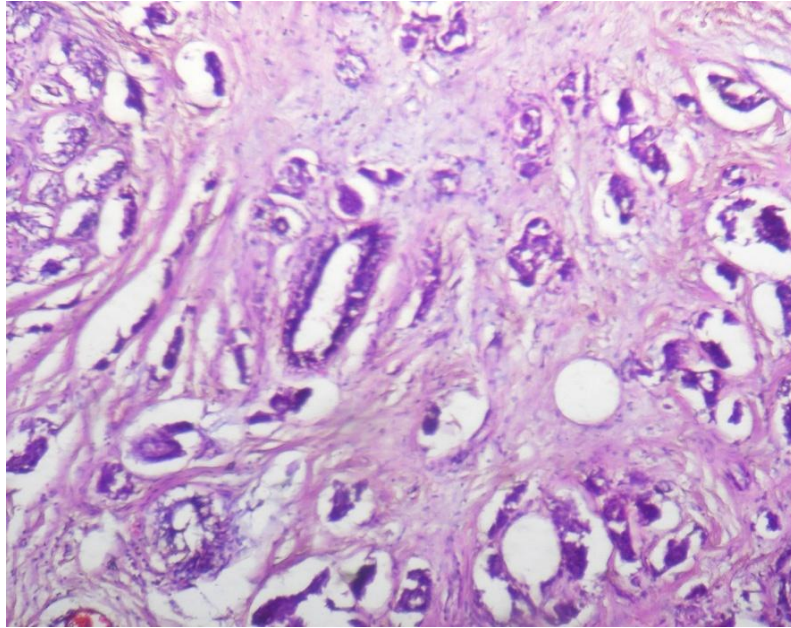
Invasive carcinoma of no special type. Tubules lined by carcinoma cells .(10X)
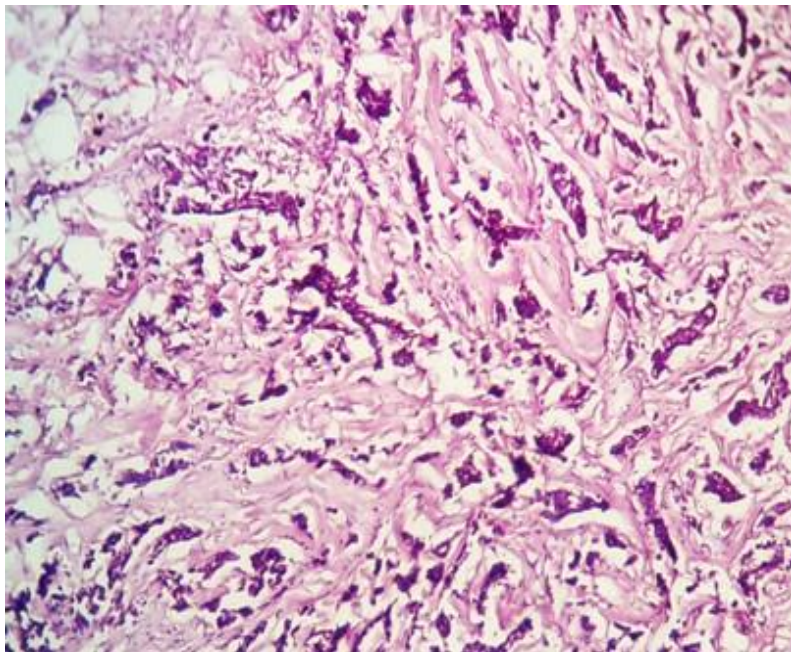
Invasive carcinoma of no special type. Cords of neoplastic cells invading into the stroma.(10X)

COLOR PLATE 7

HEMATOXYLIN &EOSIN STAINING OF IDC BREAST
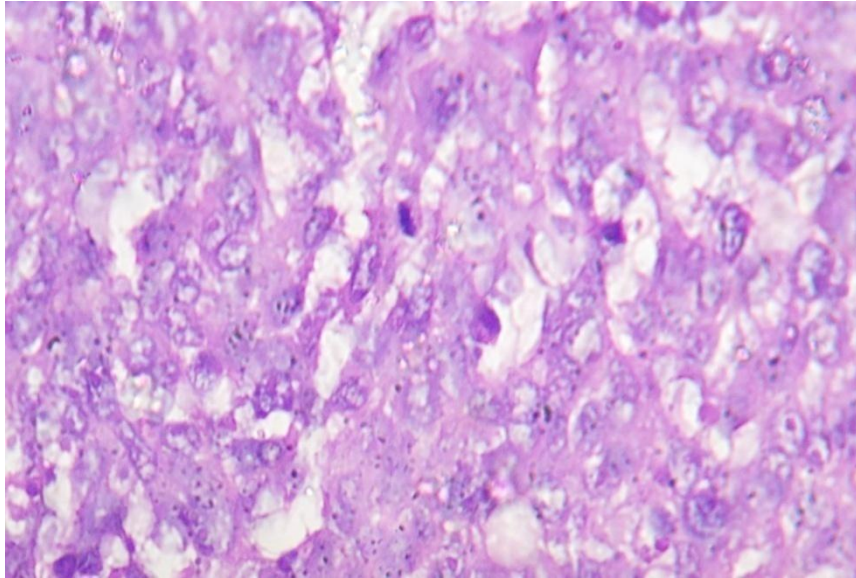
**Invasive carcinoma of no special type. Sheets
of closely packed pleomorphic cells with mitosis(40X)**
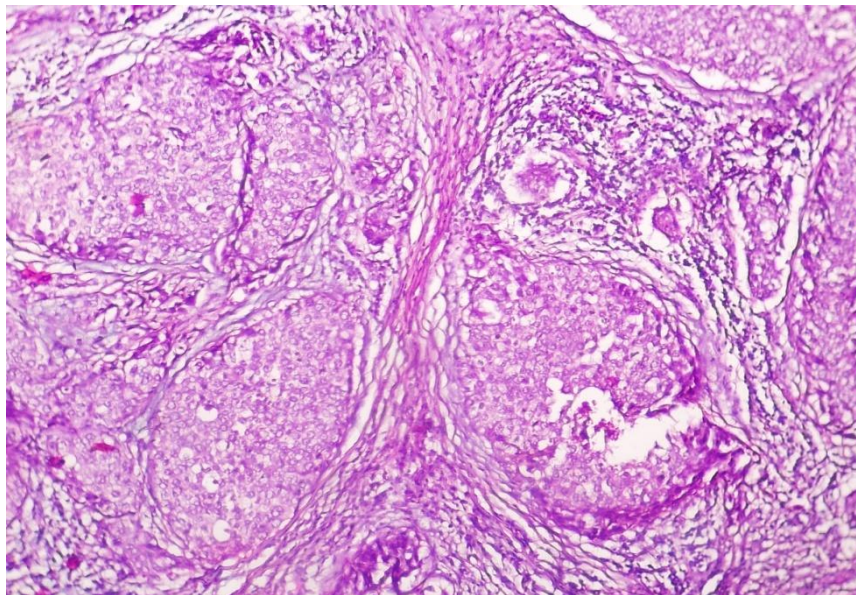
**Invasive carcinoma of no special type. Sheets of closely
packed carcinoma cells(10X)**

DISCUSSION

DISCUSSION

Invasive ductal carcinoma of breast is the third most frequent carcinoma reported in the Department of Pathology, Coimbatore Medical College and is accounting for 10.3% of total malignancies in the year 2014.

In the present study majority of invasive ductal carcinoma of breast cases belong to ages between 41 and 50 years.

The mean age of invasive ductal carcinoma of breast in this study was 48 years. Puri et al found 48.5 years as mean age of patients in their study.[79]

In this study 73% (22 out of 30) of the cases showed positivity for CD10 in the stroma, of which 46%(14) cases were strongly positive and 27%(8) were weakly positive. In a study done by Makretsov et al 79%(205 out of 258) of invasive ductal carcinoma of breast showed stromal CD10 expression.[4] Puri et al also found CD10 expression in 80%(40/50) of invasive ductal carcinoma of breast.[79] Study done by Thomas S et al shows stromal CD10 positivity in 55% (16out of 29) of cases.[81]

In the present study Stromal CD10 positivity was found in 77% cases of grade III invasive ductal carcinoma of breast. Association of CD10 expression with grade III invasive ductal carcinoma of breast is statistically significant with p value <0.05(0.04). Jana SH et al study shows stromal

CD10 positivity in 65% cases of grade III invasive ductal carcinoma of breast and the association is statistically significant.[7] Makretsov et al study shows 59% positivity for stromal CD10 in grade III cases.[4]

TABLE 10: COMPARISON BETWEEN PRESENT STUDY AND OTHER STUDIES

S.No	Name of the Study	Sample size	Direct correlation	Inverse correlation	No correlation
1.	Present study	30	Higher grade, HER2/neu positive	ER, PR	Age
2.	Thomas et al(2013)[81]	29	HER2, Chemotherapy	ER	
3.	Makretsov NA et al (2007)[4]	453	Higher grade, Decreased survival	ER	PR, HER2
4.	Iwaya K et al (2002)[80]	123	Lymph node metastasis		Age, Histological grade
5.	Jana SH et al (2014)[7]	70	Higher grade, HER2, Poor prognosis	ER	PR, Age, Lymphnode status
6.	Puri et al(2011)[79]	50	Higher grade, HER2, Ki67	ER, PR	

In the present study inverse correlation between stromal CD10 expression and hormonal receptors expression was observed.

92%(13/14 cases) of the stromal CD10 positive cases of invasive ductal carcinoma of breast not expressed both ER and PR. This inverse correlation was found to be statistically significant with the p value less than 0.05(0.002 for ER and 0.0005 for PR). Puri et al found correlation between stromal CD10 expression and hormonal receptors negativity. But their results are statistically not significant.[79] Makretsov et al study shows statistically significant correlation between stromal CD10 expression and ER negativity.[4] Jana SH et al study shows no correlation between stromal CD10 expression and PR.[7]

In the present study we obtained direct correlation between stromal CD10 expression and HER2/neu over expression. 71% (10/14 cases) of stromal CD10 positive invasive ductal carcinoma of breast cases showed HER2/neu positive. This correlation is statistically significant with the p value less than 0.05(p value 0.0009). Jana SH et al study also shows correlation between stromal CD10 expression and

HER2/neu over expression.[7] Puri et al study shows statistically significant correlation between stromal CD10 expression and HER2/neu over expression.[79] Makretsov et al does not find statistically significant

correlation between stromal CD10 expression and HER2/neu over expression.[4]

CD10 can be a therapeutic target for managing carcinoma breast since it cleaves the chemotherapeutic agent doxorubicin and results in resistance to chemotherapy. CPI0004Na is a CD10 cleavable peptide prodrug of doxorubicin. Experimental studies show CPI0004Na improves antitumor efficacy and reduces the toxicities of chemotherapeutic agents.[81]

Thus documenting the stromal CD10 status in carcinoma breast cases before and after chemotherapy is important as a possible prognostic and predictive factor.

SUMMARY & CONCLUSION

SUMMARY

A study conducted at Coimbatore Medical College, Coimbatore during the year 2014-2015. The study titled as "TO ASSESS THE STROMAL EXPRESSION OF CD10 IN INVASIVE DUCTAL CARCINOMA OF BREAST AND ITS CORRELATION WITH HISTOLOGICAL GRADE, ER, PR AND HER2 NEU EXPRESSION ". The study consists of 30 cases of invasive ductal carcinoma of breast NOS type. In all the cases immunohistochemistry was done with markers ER, PR, HER2/neu and CD10.

Grading of invasive ductal carcinoma was done according to the Nottingham Modification of the Bloom and Richardson system.

Patients who had underwent preoperative neo adjuvant chemotherapy and radiotherapy was excluded. Statistical analysis was done, results were compared with various available previous studies.

This study showed

1. Majority of invasive ductal carcinoma of breast cases belong to ages between 41 and 50 years.

2. The mean age of invasive ductal carcinoma of breast in this study was 48 years.

3. 73% (22 out of 30) of the cases showed positivity for CD10 in the stroma, of which 46%(14) cases were strongly positive and 27%(8) were weakly positive.

4. Stromal CD10 positivity was found in 77% cases of grade III invasive ductal carcinoma of breast. Association of CD10 expression with grade III invasive ductal carcinoma of breast is statistically significant. p value is < 0.05 (p value 0.04, Chi -square test).

5. Inverse correlation was found between stromal CD10 expression and estrogen receptor expression, p value was < 0.05. As the stromal expression CD10 increases, ER positivity decreases.

6. Inverse correlation was found between stromal CD10 expression and progestrone receptor expression, p value was < 0.05. As the stromal expression CD10 increases, PR positivity decreases.

7. Direct correlation was found between stromal CD10 expression and HER2/neu over expression, p value is < 0.05. As the positivity of HER2 increases there is increasing stromal expression of CD10.

CONCLUSION

To conclude, stromal CD10 expression in invasive ductal carcinoma of breast is directly correlated with higher tumor grade and HER2/neu positivity. It inversely correlates with ER and PR expression. Expression of CD10 in stromal cells of breast cancer leads to inhibition of differentiation of stem cells, thus maintaining the stem cell population. This untargeted, unopposed stem cell population could lead to tumor recurrence even in patients treated with routine chemotherapy.

Hence additional drugs like CPI0004Na, peptide prodrug of doxorubicin which cleaves CD10, could be added to therapy regimen in patients with stromal CD10 positivity. By adding new drugs targeting CD10, the stem cell population can be controlled and tumor recurrence and metastasis can be prevented. This helps in optimal individualized treatment option for each breast cancer patient.

Further studies are needed by involving larger number of patients to assess the stromal CD10 expression, effect of chemotherapy on CD10 status and to develop new therapy targeting CD10.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D,F.GLOBOCAN 2012 v1.O, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No.11

2. NCRP, Three year report of population based cancer registries 2009-2011, ICMR.

3. Surakasula A, Nagarjunapu GC, Raghavaiah KV.A comparative study of pre- and post-menopausalbreast cancer: Risk factors, presentation, characteristics and management. J Res Pharm Pract 2014;3:12-8.

4. Nikita A Makretsov, Malcom Hayes, Beverly A Carter, Shahriar Dabiri, C Blake Gilks and David G Huntsman Stromal CD10 expression in invasive breast carcinoma correlates with poor prognosis, estrogen receptor negativity, and high grade, Modern Pathology (2007) 20, 84-89

5. Cunha GR et al: Role of stromal microenvironment in carcinogenesis. Int J Cancer 107:1, 2003.

6. G et al: Stromal gene expression predicts clinical outcome in breast cancer. Nat Med 14 :518, 2008.

7. Jana SH, Jha BM, Patel C, Jana D, Agarwal A. CD10-A new prognostic stromal marker in breast carcinoma, its utility, limitations and role in breast cancer pathogenesis. Indian J Pathol Microbiol 2014;57:530-6.

8. Mohammadizadeh F, Salavati M, Afshar Moghaddam N. CD10 expression in stromal component of invasive breast carcinoma: A potential prognostic determinant J Res Med Sci 2012; 17(Spec 2):S194-9.

9. Russo J et al: Full-term pregnancy induces a specific genomic signature in the human breast. Cancer Epidemiol Biomarkers Prev 17:51, 2008.

10. Blackwood MA, Weber BL. BRCA1 and BRCA2: from molecular genetics to clinical medicine. J Clin Onco 1998,16: 1969-77.

11. Futreal PA et al, BRCA1 mutation in primary breast and ovarian carcinomas. Science 1994,266: 120-122

12. Rossouw JE et al.:Risks and benefits of estrogen plus progestin in healthy postmenopausal woman: principal results from the women's Health Initiative randomized controlled trial. JAMA 288:321, 2002.

13. Collaborative Group on Hormonal Factors in Breast cancer, Beral V: Breast cancer and breastfeeding : Lancet 360:187, 2002.

14. Tan DS, Marchio C, Reis-Filho JS. Hereditary breast cancer: from molecular pathology to tailored therapies. J Clin Pathol 2008,61:1073-1082.

15. Robson M, Offit K. Management of an inherited predisposition to breast cancer. N Engl J Med 2007, 357 : 154-162.

16. Yager JD, Davidson NE: Estrogen carcinogenesis in breast cancer . N Engl J Med 354: 270, 2006.

17. Mott JD, Werb Z. Regulation of matrix biology by matrix metalloproteinases. Curr opin Cell Biol 2004; 558-64

18. Fidler IJ. The pathogenesis of cancer metastasis: The seed and soil hypothesis revisited. Nat Rev Cancer 2003: 3:453-8.

19. Bremnes RM, Donnem T, Al saad S, Al-Shibli K, Andersen S, Sirera R,et al. The role of tumor stroma in cancer progression and prognosis: Emphasis on carcinoma associated fibroblasts and non small cell lung cancer. J Thorac Oncol 2011:6; 209-17.

20. Jinga DC, Blidaru A, Condrea I, Ardeleanu C,Dragomir C, Szegli G, et al MMP-9 and MMP-2 gelatinases and TIMP-1 and TIMP-2 inhibitors in breast cancer; correlation with prognostic factors. J cell Mol Med 2006:10; 499-510.

21. De Wever O, Mareel M. Role of tissue stroma in cancer invasion. J Pathol 2003: 200: 29-47.

22. Kesse-Adu R, Shousha S. Myoepithelial markers are expressed in at least 29% of oestrogen receptor negative invasive breast carcinoma. Mod Pathol 2004;17: 646-652.

23. Coussens Lm, Fingleton B, Matrisian LM. Matrix metalloproteinase inhibitors and cancer: trials and tribulations. Science 2002;295:2387-2392.

24. Pan C, Cardarelli PM, Nieder MH,et al. CD10 is a key enzyme involved in the activation of tumor activated peptide prodrug CPI-0004Na and novel analogues: implications for the design of novel peptide prodrugs for the therapy of CD10+ tumors. Cancer Res 2003;63:5526-5531.

25. Bachelard-Cascales E, Chapellier M, Delay E, Pochon G Voeltzel T, Puisieux A,et al. The CD10 enzyme is a key player to identify and regulate human mammary stem cells. Stem cells 2010;28: 1081-8.

26. Weil M, Itin A, Keshet E. A role for mesenchyme-derived tachykinins in tooth and mammary gland morphogenesis. Development 1995;121: 2419-28.

27. Kenny AJ, O'Hare MJ, Gusterson BA. Cell-surface peptidases as modulators of growth and differentiation. Lancet 1989;2: 785-7.

28. Maguer-Satta V, Besancon R, Bachelard-Cascales E. Concise review: Neutral endopeptidase(CD10): A multifaceted environment actor in

stem cells, physiological mechanism, and cancer. Stem cells 2011;29:389-96.

29. Susan C. Lester, Robbins and Cotran Pathologic basis of Disease, Eighth edition, page no: 1079.

30. Susan C. Lester, Robbins and Cotran Pathologic basis of Disease, Eighth edition, page no: 1080.

31. Rosai and Ackerman's Surgical Pathology, Tenth edition, volume 2, page no: 1689.

32. Rosai and Ackerman's Surgical Pathology, Tenth edition, volume 2, page no: 1690.

33. NHS Breast Screening Programme 2005 Pathology reporting of breast disease, 3rd ed. NHS Breast Screening Programme publication no 58. NHS Cancer Screening Programmes, Royal College of Pathologists, Sheffield, United Kingdom.

34. Lester S C, Bose S, Chen Y Y et al. 2009 Protocol for the examination of specimens from patients with invasive carcinoma of the breast. Arch Pathol Lab Med 133: 1515-1538.

35. Susan C. Lester, Robbins and Cotran Pathologic basis of Disease, Eighth edition, page no: 1082.

36. Rosai and Ackerman's Surgical Pathology, Tenth edition, volume 2, page no: 1696.

37. Susan C. Lester, Robbins and Cotran Pathologic basis of Disease, Eighth edition, page no: 1083.

38. Susan C. Lester, Robbins and Cotran Pathologic basis of Disease, Eighth edition, page no: 1084.

39. Susan C. Lester, Robbins and Cotran Pathologic basis of Disease, Eighth edition, page no: 1084, Table no: 23-3.

40. Page D L, Anderson T J, Sakamoto G 1987 Infiltrating carcinoma: major histological types. In: Page D L, Anderson T J (eds) Diagnostic histopathology of the breast. Churchill Livingstone, London, p 193-235

41. Fisher E R, Gregorio R M, Fisher B 1975 The   pathology of invasive breast cancer. A syllabus derived from findings of the National Surgical Adjuvant Breast Cancer Project (protocol no. 4). Cancer 36: 1-85.

42. Christopher. D. M Fletcher Diagnostic Histopathology of Tumor, Fourth edition, Vol 1, page no:1088.

43. Tavassoli F, Devilee P 2003 Pathology and genetics of tumours of the breast and female genital organs. World Health Organization classification of tumours. IARC Press, Lyon, France.

44. Pepercorn J et al.: Molecular subtypes in breast cancer evaluation and management: divide and conquer. Cancer Invest 26:1, 2008.

45. Kang SP et al.: Triple negative breast cancer: current understanding of biology and treatment option. Curr opin Obstet Gynecol 20:40, 2008.

46. Reis-Filho JS, Tutt ANJ: Triple negative tumors: a critical review. Histopathology 52:108, 2008.

47. Bempt IV et al.: The complexity of genotypic alterations underlying HER2 positive breast cancer: an explanation for it's clinical heterogeneity. Curr Opin Oncol 19:552, 2007.

48. Ellis I O, Galea M, Broughton N et al. 1992 Pathological prognostic factors in breast cancer. II. Histological type. Relationships with survival in a large study with long-term follow-up. Histopathology 20: 479-489.

49. Simpson P T, Reis-Filho J S, Lambros M B et al. 2008 Molecular profiling pleomorphic lobular carcinomas of the breast: evidence for a common molecular genetic pathway with classic lobular carcinomas. J Pathol 215: 231-244

50. Rakha E A, Green A R, Powe D G et al. 2006 Chromosome 16 tumor-suppressor genes in breast cancer. Genes Chromosomes Cancer 45: 527-535

51. Susan C. Lester, Robbins and Cotran Pathologic basis of Disease, Eighth edition, page no: 1085.

52. Quincey C, Raitt N, Bell J et al. 1991 Intracytoplasmic lumina—a useful diagnostic feature of adenocarcinomas. Histopathology 19: 83-87.

53. Rakha E A, Patel A, Powe D G et al. 2010 Clinical and biological significance of E-cadherin protein expression in invasive lobular carcinoma of the breast. Am J Surg Pathol 34: 1472-1479.

54. Schrader KA et al.: Hereditary diffuse gastric cancer: association with

lobular breast cancer. Fam Cancer 7:73, 2008.

55. McBoyle M F, Razek H A, Carter J L, Helmer S D 1997 Tubular carcinoma of the breast: an institutional review. Am Surg 63:639-644.

56. Carstens P H, Huvos A G, Foote F W Jr et al. 1972 Tubular carcinoma of the breast: a clinicopathological study of 35 cases. Am J Clin Pathol 58: 231-238.

57. Cowan W K, Kelly P, Sawan A et al. 1997 The pathological and biological nature of screen-detected breast carcinomas: a morphological and immunohistochemical study. J Pathol 182: 29-35.

58. Christopher. D. M Fletcher Diagnostic Histopathology of Tumor, Fourth edition, Vol 1, page no:1094.

59. Christopher. D. M Fletcher Diagnostic Histopathology of Tumor, Fourth edition, Vol 1, page no:1095.

60. Komaki K, Sakamoto G, Sugano H et al. 1988 Mucinous carcinoma of the breast in Japan. A prognostic analysis based on morphologic features. Cancer 61: 989-996.

61. Yoder BJ et al.: Molecular and morphologic distinction between infiltrating ductal and lobular carcinoma of breast. Breast J 13: 172, 2007.

62. Christopher. D. M Fletcher Diagnostic Histopathology of Tumor, Fourth edition, Vol 1, page no:1096.

63. Ellis I O, Elston C W, Pinder S E 1998 Papillary lesions. In: Elston C W, Ellis I O (eds) Systemic pathology. The breast. Churchill Livingstone, London, p 133-146.

64. Christopher. D. M Fletcher Diagnostic Histopathology of Tumor, Fourth edition, Vol 1, page no:1097.

65. Siriaunkgul S, Tavassoli F A 1993 Invasive micropapillary carcinoma of the breast. Mod Pathol 6: 660-662.

66. Rakha E A, Gill M S, El-Sayed M E et al. 2009 The biological and clinical characteristics of breast carcinoma with mixed ductal and lobular morphology. Breast Cancer Res Treat 114: 243-250.

67. Rosen P P, Cranor M L 1991 Secretory carcinoma of the breast. Arch Pathol Lab Med 115: 141-144.

68. Schmitt F C, Soares R, Seruca R 1998 Bilateral apocrine carcinoma of the breast. Molecular and immunocytochemical evidence for two independent primary tumours. Virchows Arch 433: 505-509.

69. Tavassoli F, Devilee P 2003 Pathology and genetics of tumours of the breast and female genital organs. World Health Organization classification of tumours. IARC Press, Lyon, France.

70. Wei B, Ding T, Xing Y et al. 2010 Invasive neuroendocrine carcinoma of the breast: a distinctive subtype of aggressive mammary carcinoma. Cancer 116: 4463-4473.

71. Khan H N, Wyld L, Dunne B et al. 2003 Spindle cell carcinoma of the breast: a case series of a rare histological subtype. Eur J Surg Oncol 29: 600-603.

72. Carter M R, Hornick J L, Lester S et al. 2006 Spindle cell (sarcomatoid) carcinoma of the breast: a clinicopathologic and immunohistochemical analysis of 29 cases. Am J Surg Pathol 30: 300-309.

73. Christopher. D. M Fletcher Diagnostic Histopathology of Tumor, Fourth edition, Vol 1, page no:1106.

74. Susan C. Lester, Robbins and Cotran Pathologic basis of Disease, Eighth edition, page no: 1089.

75. Luck AA et al.: The influence of basal phenotype on the metastatic pattern of breast cancer. Clin Oncol20:40, 2008.

76. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer. Histopathology 1991, 19, 403-410.

77. Rosai and Ackerman's Surgical Pathology, Tenth edition, volume 2, page no: 1721.

78. Rosai and Ackerman's Surgical Pathology, Tenth edition, volume 2, page no: 1720.

79. Vandana Puri, Manjula jain, and Shaji Thomas Int Jou of Breast Cancer, 2011, atricle ID 437957.

80. Iwaya K, Ogawa H, Izumi M, Kuroda M, Mukai K. Stromal expression of CD10 in invasive breast carcinoma; a new predictor of clinical outcome. Virchows Arch 2002;440:589-93.

81. Thomas S, Babu RJ,Agarwal K, Puri V, Jain M, et al. Effect of neoadjuvant chemotherapy on stromal CD10 antigens in breast cancer- A prelimnary study. Indain J Cancer 2103;50:46-51.

ANNEXURES

ANNEXURE -I
MASTER   CHART

S.NO:	HPE NO:	AGE	HISTOPATHOLOGICAL GRADING					ER	PR	HER2	CD10
			TUBULE	NUCLEAR	MITOSIS	SCORE	GRADE				
1	563/13	51	3	3	2	8	III	0	1	3	2
2	706/13	51	3	3	2	8	III	0	0	0	2
3	1802/13	42	3	2	3	8	III	1	0	3	2
4	3/14	23	3	3	3	9	III	0	0	0	2
5	3427/14	36	2	2	1	5	I	4	3	2	1
6	1399/14	36	2	3	3	8	III	0	0	2	2
7	4063/14	57	2	1	1	4	I	6	4	1	1
8	670/14	60	2	3	3	8	III	1	0	1	2
9	1252/14	35	2	2	2	6	II	1	1	0	0
10	2279/14	41	2	2	1	5	I	0	5	3	1
11	2039/14	40	2	1	1	4	I	0	0	3	2
12	850/14	42	2	1	2	5	I	5	0	0	0
13	2461/14	52	3	3	2	8	III	6	4	1	0
14	82/15	42	3	3	2	8	III	0	1	3	2
15	222/15	42	2	2	2	6	II	4	4	0	1
16	2676/13	50	3	3	2	8	III	0	4	3	1
17	794/15	50	2	2	2	6	II	1	3	0	0
18	452/15	49	3	3	2	8	III	1	0	3	2
19	1030/15	65	2	3	1	6	II	3	4	1	0
20	2178/13	55	1	1	1	3	I	0	1	3	2
21	1762/13	72	2	2	2	6	II	4	5	0	1
22	2660/14	39	3	2	2	7	II	0	0	0	1
23	1638/11	45	1	1	1	3	I	5	4	3	2
24	637/11	40	1	2	1	4	I	5	1	2	0
25	568/11	55	2	2	2	6	II	4	4	0	1
26	380/11	65	3	3	2	8	III	4	3	2	0
27	10/11	58	2	3	3	8	III	1	0	3	2
28	1448/11	45	2	3	2	7	II	1	1	0	2
29	612/11	50	3	3	2	8	III	0	0	3	2
30	2727/13	55	2	2	2	6	II	4	5	2	0

PROFORMA

Name   age

Ward   IP NO:

Address

Presenting complaints

     Lump in breast

     Pain

     Discharge from nipple

     Skin ulceration

Duration of presenting illness

Past history

History of previous surgeries for breast lump

History of chemotherapy/radiotherapy

History of breast lump in other breast

Family history

Personal history

Diet

Menstrual history

Breast feeding history

General examination

Nourishment

Built

Conscious

Febrile/afebrile

Pallor

Jaundice

Cyanosis

Clubbing

Lymphadenopathy

Edema

Vitals

PR

RR

BP

Local examination of the breast

Side – right/left

Quadrant

Size of the tumor

Fixity to the skin

Fixity to the underlying fascia

Examination of axillary lymph node

Number of node

Mobile/fixed

Size

Group of node: anterior/posterior/lateral/apical

Gross examination of modified radical mastectomy specimen:

Size of the specimen including skin, nipple, areola

Size of the tumor

Margins: infiltrative/circumscribed

Quadrant

Histological diagnosis

Any special type:

Lymph node status – no: of positive nodes/no: of total nodes examined

Histological grading

Tubule formation: 1/2/3

Nuclear pleomorphism:1/2/3

Mitosis:1/2/3Histological grade: I/II/III

ANNEXURE - III

ABBREVIATIONS

AJCC	American joint committee classification
CD	Cluster of Differentiation
CEA	Carcinoembryonicantigen
CI	Confidence Interval
DAB	Diaminobenzidine
DCIS	Ductal carcinoma in situ
DPX	Dextrene polystyrene xylene
ER	Estrogen receptor
GCDFP	Gross Cystic Disease Fluid Protein
HER2	Human epidermal growth factor receptor
IARC	International Agency for Research on Cancer
LCIS	Lobular carcinoma in situ
NOS	Not Otherwise Specified
PR	Progesterone receptor
TBS	Tris buffer solution
WHO	World health organization

ANNEXURE - IV

ஒப்புதல் படிவம்

பெயர்   :

வயது   :

பாலினம்   :

முகவரி   :

        அரசு கோவை மருத்துவக் கல்லூரியில் நோய் குறியியல் மருத்துவ
துறையில் பட்ட மேற்படிப்பு பயிலும் மாணவர் மரு.சு.உலகநாதன் அவர்கள்
மேற்கொள்ளும் "மார்பக புற்றுநோயில் CD10 வின் வெளிப்பாட்டினை அறிதல்"
பற்றிய ஆய்வில் செய்முறை மற்றும் அனைத்து விளக்கங்களையும் கேட்டுக்
கொண்டு எனது சந்தேகங்களை தெரிவுபடுத்திக் கொண்டேன் என்பதை
தெரிவித்துக் கொள்கிறேன்.

        நான் இந்த ஆய்வில் முழு சம்மதத்துடனும், சுய சிந்தனையுடனும்
கலந்து கொள்ள சம்மதிக்கிறேன்.

        இந்த ஆய்வில் என்னைப் பற்றிய அனைத்து விவரங்கள்
பாதுகாக்கப்படுவதுடன் இதன் முடிவுகள் ஆய்விதழில் வெளியிடப்படுவதில்
ஆட்சேபனை இல்லை என்பதை தெரிவித்துக் கொள்கிறேன். எந்த நேரத்திலும்
இந்த ஆய்வில் இருந்து நான் விலகிக் கொள்ள எனக்கு உரிமை உண்டு
என்பதையும் அறிவேன்.

இடம் :   கையொப்பம் / ரேகை

தேதி :