IMMUNOHISTOCHEMICAL EXPRESSION OF CD 10 IN BREAST CARCINOMA AND ITS CORRELATION WITH CLINIC PATHOLOGICAL PARAMETERS

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"IMMUNOHISTOCHEMICAL EXPRESSION OF CD10 IN BREAST CARCINOMA AND ITS CORRELATION WITH CLINICOPATHOLOGICAL PARAMETERS."

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ABSTRACT

INTRODUCTION:

Interaction between stromal and the tumour cells is of crucial importance in breast cancer progression and response to therapy. Stromal cells undergo striking changes during breast cancer progression. Studies have shown that expression of CD10 by stromal cells is associated with higher tumour grade and tumour stage, and thus signifies the biological aggressiveness of various epithelial malignancies. Stromal markers are now becoming apparent as novel markers in evaluating the prognosis of invasive breast cancer and have not been studied substantially till date.

OBJECTIVES:

- To study immunohistochemical expression of CD10 in stromal cells of breast carcinoma.
- To correlate CD10 expression with various clinicopathological prognostic factors such as size of the tumour, histological grade, lymph node status, and ER, PR, HER2-neu status

MATERIAL AND METHODS:

A hospital based cross sectional study was done on 50 mastectomy specimens received in the histopathology section of the Department of Pathology. Tumor size, histological grade, lymph-node status, stage of the tumor was noted. IHC staining for ER, PR, Her2neu and CD10 markers was performed and expression of CD10 was correlated with these clinco-pathological prognostic factors. Results were subjected to statistical analysis.

RESULTS:

Stromal CD10 expression was seen in 40 (80%) cases, with no expression in 10(20%) cases. Stromal expression of CD10 showed stastistically significant correlation with increasing tumor grade, ER negativity and PR negativity. There was no correlation between CD10 and age of the patient, lymph node status, tumor size, stage of the tumor and HER2neu status.

CONCLUSION: Stromal expression of CD10 correlated strongly with well-established negative prognostic marker that is higher tumor grade, ER negative status and PR negative Status and thus it can be used as an independent prognostic marker.

KEY WORDS: Breast cancer, CD10, Prognosis

LIST OF ABBREVATIONS USED

BIRADS : Breast imaging reporting and data system

BRCA : Breast cancer gene

CD10 : Cluster of differentiation

DAB : Diaminobenzidine

DCIS : Ductal carcinoma in situ

DPX : Distyrene, Plasticizer (tricresyl phosphate), xylene

ER : Estrogen receptor

HER2neu : Human epidermal growth factor receptor 2

HPF : High power field

HRP : Horse radish peroxidase

IHC : Immunohistochemistry

MiB1 : Mindbomb 1

PIP3 : Phosphatidylinositol trisphosphate

PTEN : Phosphatase and tensin homolog

PR : Progesterone receptor

TNM : Tumor node metastasis

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Breast cancer is the second most common cancer in the world and has a significant impact on health in both industrialised and developing nations. Breast cancer is the most common cancer that kills women worldwide, with more than a million new cases being identified every year.[1, 2, 3]

Breast cancer poses a serious threat to women's lives and health, and it has drawn attention from a number of sectors.. “Data from the 2018 Global Cancer Survey” show that among women worldwide, carcinoma breast has the highest incidence of malignant tumours, accounts for 15% of all malignant tumor-related mortality, ranks sixth in the death rate of malignant tumours, and exhibits a tendency of rapid increase.[4]

It was predicted that by the year 2021 the incidence of female breast cancer would be 85 per 1,00,000 women.[5]

The peak incidence of breast cancer is above the age of 50 years in developed countries, while in India it is above 40 years.[6]The incidence of breast carcinoma in women in India is 27%, which is highest among all other types of breast carcinoma. The overall incidence and mortality of females diagnosed with breast cancer is highest in Asian countries like India and Pakistan.[5]

The tissue environment in which the tumour is present, as well as the internal environment of the tumour cell themselves, are all considered to be parts of the tumour microenvironment. “The tumour microenvironment is made up of immune and non-immune cells, the non-immune cells including “cytokines”, “macrophages”, “natural killer (NK) cells”, “degenerate cells”, and “fibroblasts”, “endotheliocytes”, and “vascular smooth muscle cells.” The development, progression, and prognosis of cancer are all closely related to the tumour immune microenvironment, which also serves as the site of immunological escape and tumour cell immune surveillance.[5]

"Breast carcinoma development, growth, and metastasis have all been linked to the immune microenvironment, which includes "high expression of vascular endothelial growth factors(VEGF)", "tumor-associated macrophages (TAMs)", "tumor-infiltrating lymphocytes (TILs)", and other molecules that "promote the growth and migration of tumour cells."[5]

The importance of the tissue microenvironment in maintaining survival of the cell, multiplication, relocation, polarisation, and the degree to which cell resembles their cell of origin has been amply demonstrated in the literature.[2]

Well acknowledged conventional clinicopathological prognostic factors like, "histological tumor grade", "lymph node metastase" , "ER and PR" immune status, "HER2-neu status" are customarily studied in every case of carcinoma of breast. Stroma has an important function in modifying and regulating tumour infiltration and metastasis. A deeper comprehension of the role played by the stroma in the development of cancer can help spot particular cues that aid the progress, Tumor cell penetration and aberrant survival finally led to the identification of new therapeutic targets for potential treatment.[7]

Each tumour cell has a unique capacity for invasion, metastasis, and pace of growth. The prognosis of the lesion varies depending on the member and the types of oncogene activated. To evaluate the oncogene's expression and amplification, many tumour markers have been applied. Targeted therapy against the upregulated oncogenes can alter the medical presentation of the tumour.[7]

Breast cancer patients still have a poor prognosis because of the high rates of metastasis and local recurrence, which makes treatment ineffective. This failure can be related to the biological properties and characteristics of the tumor-forming cells. The intervening tumour microenvironment, which interacts with cancer cells to change different characteristics of tumour formation such as tumour growth, vascularity, invasiveness, and metastatic

dissemination, adds to the complexity. Future cancer therapies would depend on anticancer treatment adaption to the heterogeneity of the tumour.[1]

Since they have not been extensively researched up to this point, stromal markers are now showing up as novel markers in assessing the prognosis of invasive breast cancer.[8]

CD10 also known as, "Common Acute Lymphoblastic Antigen", is a "90–110-kDa" "cell surface zinc-dependent metalloproteinase," and is commonly highlighted in "bone marrow", "lymphoid stem cells", "pro-B lymphoblasts", "mature neutrophils", various subtypes of "lymphomas", "renal cell carcinoma", and "endometrial stromal sarcoma."[1, 2]

Stromal CD10 expression is known to be linked with higher tumour grade and tumour stage, and thus signifies the biological assertiveness of various malignancies belonging to lining epithelium.[2]

In gastric carcinoma stromal CD10-positivity shows association with infiltration into blood vessels and tumour metastases. There is high stromal CD10 expression in malignancies, as against borderline benign or phyllodes tumours.[9]

Many studies have shown that the interaction between stromal and the tumour cells is of crucial importance in breast cancer progression and response to therapy. Based on earlier research suggesting that CD10+ stromal cells undergo striking changes during breast cancer progression, there is a need to describe the distinctive nature of this cell population and its clinical relevance more effectively.[10]

The stroma is not as genetically unstable as malignant tumor cells and thus there is a low proabability of these cells to develop drug resistance. Recently many studies stated that, tumour microenvironment can be considered as a potential therapeutic target.[11]

Based on generally established prognostic parameters including, “tumour size”, “histologic tumor grade”, and “axillary lymph node status”, Considering each patient's underlying cancer cells have a different capacity for metastasis, it is difficult to accurately anticipate the course of illness for all patients. Numerous studies have demonstrated that different patient groups have variable life expectancies, demonstrating the heterogeneity of breast tumours and emphasising the need for greater research at molecular level and improved patient categorization. Therefore, primary goal of this study was to find novel prognostic marker that could more accurately reveal the likelihood for metastasis and also can be as an added prognostic component in the therapy algorithm for breast carcinomas. [9,12]

Therefore, the study was conducted to assess the immunohistochemistry expression of CD10 in stromal cells in invasive breast cancer and associate the CD10 expression with several prognostic markers such the age of the patient, size of the tumour, histological grade, lymph node status, stage of the tumor and ER, PR, and HER2-neu status..

OBJECTIVES OF THE STUDY:

- To study immunohistochemical expression of CD10 in stromal cells of breast carcinoma.
- To correlate CD10 expression with various clinicopathological prognostic factors such as size of the tumour, histological grade, lymph node status, stage of the tumor and ER, PR, HER2-neu status

REVIEW OF LITERATURE:

EMBRYOLOGICAL DEVELOPMENT OF BREAST

The interplay of ectoderm and mesenchyme results in the formation of the breast. During the fifth week of intrauterine life, epidermal thickenings begin to grow between the axilla and the crotch, along the ectodermal primitive galactic band. All ridges, with the exception of those at the pectoral region, had vanished by the IX week of intrauterine life.[21]

The thickening at the pectoral region invaginates at weeks of 7-8 weeks of Intrauterine Life, followed by tri dimensional expansion. The mesenchymal cells that make up the nipple and areola's smooth muscle begin to differentiate between 12 and 16 weeks of Intrauterine Life. At 16 weeks of intrauterine development, epithelial cords develop buds and branch to create 15 to 25 cords, which serve as the secretory alveoli. Hair follicle, sebaceous gland, and sweat glands begin to differentiate from one another, but only the sweat glands fully grow, and sebaceous glands do not have hair follicles alongside them. [21]

The hormones enter the fetal circulation at 27 weeks of intrauterine life, causing canalization of the epithelial tissues. As this goes on, 15-25 mammary ducts are formed. The lobuloalveolar structures begin to mature 32 weeks into the pregnancy.[13-20]At birth, the nipple begins to develop from the stratum spinosum, first as a pit.[21]

The ducts and its branches are encircled by fibrous tissue made of the papillary dermis that surrounds the cords. The Astley Cooper suspensory ligaments that connect the breast to the dermis are formed by the reticular dermis. The myoepithelial cells play a crucial role in the branching of glandular tissue as they develop between 23 and 28 weeks of gestation.[22-27]

NORMAL ANATOMY AND HISTOLOGY OF MAMMARY GLAND

The breast or mammary gland is covered by skin and subcutaneous tissue and rests on the pectoralis muscle, from which it is separated by a fascia. The terminal duct-lobular unit (TDLU) and the major duct system are the two main parts of the branching ductal system that radiates from the nipple and makes up the parenchymal tissue that forms up human breasts. The lobule and terminal ductule together make up the TDLU, which is the gland's secretory component. It joins the subsegmental duct, which then links to the segmental duct, the collecting duct, and finally the nipple. The lactiferous sinus is a fusiform dilatation situated beneath the nipple between the collecting and segmental duct.[28]

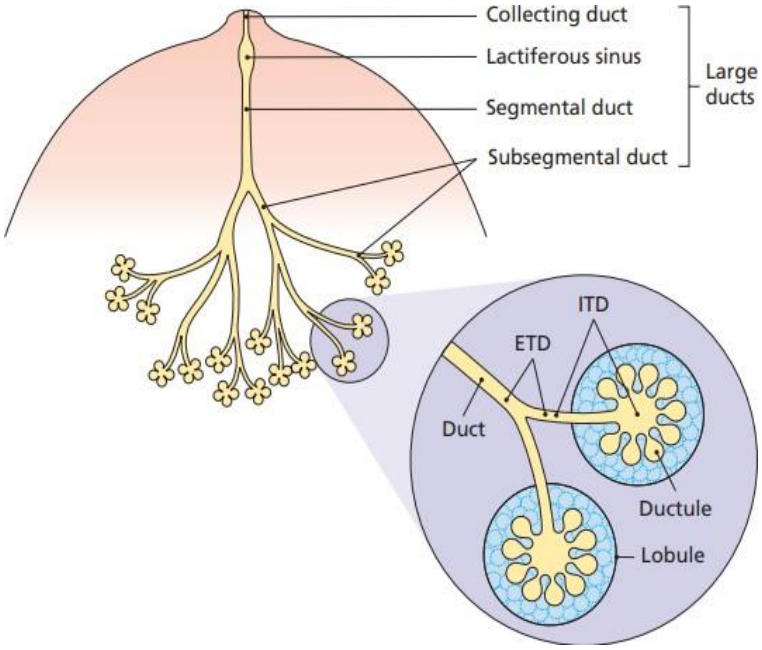


FIG 1- Diagrammatic representation of breast anatomy

Histologically breast parenchyma is comprised of ducts, lobules and stroma. The ducts are lined by 2 layered epithelium comprised of outer myoepthelial cells which are flat and contractile in fuction and inner ductal cell layer. The ductal cellslie above the myoepithelial cell layer. In between the ducts lies stroma composed of fibroblast, collagen fibers, arterioles, venules and adipose tissue.[29]

BREAST CARCINOMA

EPIDEMIOLOGY

One in ten of all new cancer cases diagnosed each year worldwide are female breast cancer cases, making it the most common type of cancer in women in both developing and advanced nations. The WHO estimates that, 107.8 million "Disability-Adjusted Life Years (DALYs)" are associated with malignant neoplasms, of which. "19.6 million" are associated with carcinoma of breast." Over the past three decades, breast cancer has shown an increase in both incidence and fatality rates. Incidence of breast cancer increased by two fold in 60 of 102 cases between 1990 and 2016, while death rate has become twice in 43 of 102 nations. As a result of westernising lifestyles (such as delayed pregnancies, less breastfeeding, early menarche, physical inactivity, and poor dietary habits), improved cancer registration, and cancer detection, it is predicted that the incidence of breast cancer will increase even more in low- and middle-income countries.[30]

With an expected, "2.3 million new cases worldwide", breast cancer is presently most oftenly determined malignancies and the sixth cause of cancer-associated deaths, according to GLOBOCAN 2020 estimates.[31]

The prevalence of breast cancer has started to rise after staying steady for a long time. This is because more cases were discovered with the advent of mammographic screening. The primary goal of screening is the diagnosis of in situ malignancies, primarily invasive carcinomas that are ER positive. Mammography is the primary method for detecting DCIS, which accounts for the rise in DCIS diagnoses since 1980. Because of screening, stage I cancers—small lymph node negative carcinomas—have become more common.[29]

With nearly 0.3 million deaths per year, Cancer is the second most prevalent disease in India and the cause of maximum mortalities. Breast cancer incidence in India have increased over the past rising, with up to 100,000 additional sufferers being detected every year.[32]

According to estimates, the lifetime risk of breast cancer for women who live to the age of 85 is 1 in 9.[33]

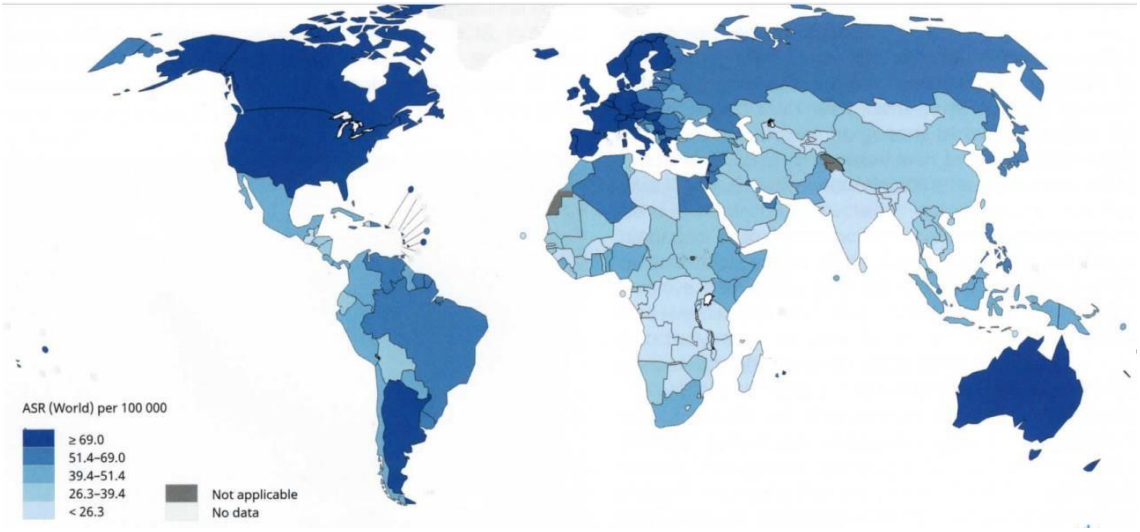


FIG 2- "Estimated age-standardized incidence rates (ASRs; World)", per 100 000 person-years, of "breast cancer in the year 2018"[34]

RISK FACTORS FOR DEVELOPING CARCINOMA:

While some risk factors for breast cancer development have been proven, many others are still debatable. It has been proposed that intense and/or sustained oestrogen stimulation acting on a genetically vulnerable background is the common denominator for the majority of these cancers.

1. **Family background-**A woman's risk of developing breast cancer is two to three times higher than the general population if her first-degree relative had the disease at a young age, was bilaterally afflicted, or both.[35]

2. **Reproductive history and Menstrual history- "** Early onset menarche, nulliparity, late age at first birth, and late menopause are all associated with increased risk." [28.] Women who have undergone bilateral oophorectomy are less likely to develop breast cancer; salpingooophorectomy before the age of 35 reduces the risk by almost 50%.[28,36] Breastfeeding for at least 4 months has been linked to a lower risk of breast cancer in parous women.[28]

3. **Exogenous hormones -** Exogenous hormones have a complicated effect on breast cancer risk that varies depending on the length of treatment and the drugs taken in combination. In short, compared to the use of oestrogen alone, the risk seems to increase with longer duration of use, current use, and the combination of oestrogen and progestins.[28]In postmenopausal women with a hyperandrogenic plasma hormone profile, the chance of developing breast cancer is enhanced.[37,38]

4. **Hormonal contraceptives-** Numerous epidemiologic studies have been done, but none of them have identified any elevated risk, or just a very slight increase, among young long-term users.[39]

5. **Radiation exposure-** Ionizing radiation exposure has been linked to an increased risk of breast cancer, especially if it happened during the time when the breasts were developing in the pubertal age group.[28]

6. **Genetic Predisposition-** Five percent to ten percent of breast cancer cases are caused by familial factors.[40,41] A high lifetime risk of acquiring breast cancer as well as a number of other malignancies, such as ovarian cancer, has been linked to the discovery of two high-penetrance susceptibility genes that are affected by germline mutations..[40] "These are *BRCA1*, located on chromosome 17q21, and *BRCA2*, located on chromosome 13q12.3"[28] By the age of 70, carriers are thought to have a risk of up to 70% to 80% developing breast cancer. The affected person may have to make a difficult decision if the test results are positive for the mutation; the two major options are close follow-up and bilateral preventive mastectomy.[42]

TABLE 1: RELATIVE RISK OF DEVELOPING BREAST CANCER[29]

RISK FACTORS	RELATIVE RISK
Female gender Increasing age Germline mutation of high penetrance Strong family history (>first degree relative, young age multiple cancers) Personal history of breast cancer High breast density	**≥4.0**
Germline mutation of moderate penetrance High dose radiation to chest at young age Family history (>first degree relative)	**2.1-4.0**
Early menarche(<12years of age) Late menopause (>55 years of age) Late first pregnancy(>35 years of age) Nulliparity Absence of breast feeding Exogenous hormone therapy Post menopausal obesity Physical inactivity High alcohol consumption	**1.1-2.0**

PATHOGENESIS OF BREAST CARCINOMA

Breast cancer risk factors can be divided into three categories: hereditary, hormonal, and environmental factors.

- **GENETIC FACTORS-**The typical tumour suppressor properties of BRCA1 and BRCA2 indicate that cancer can only develop when both alleles are inactive or

dysfunctional. For the repair of specific types of DNA damage, BRCA1 and BRCA2 encode proteins.

The genetic mutations involved in development of breast cancers can be divided into familial and sporadic.

The majority of BRCA2 mutations are linked to ER-positive malignancies, whereas the majority of BRCA1 mutations are linked to triple-negative tumours.

The homologous recombination method of DNA damage repair, cell cycle checkpoint regulation, ubiquitylation, chromatin remodelling, and DNA decatenation are only a few of the various roles performed by the BRCA1encoded protein. BRCA2 encodes a protein that functions in DNA repair, cytokinesis, and meiosis. In other words, accurate homologous recombination-mediated repair of DNA double-strand breaks requires both BRCA1 and BRCA2. [41]

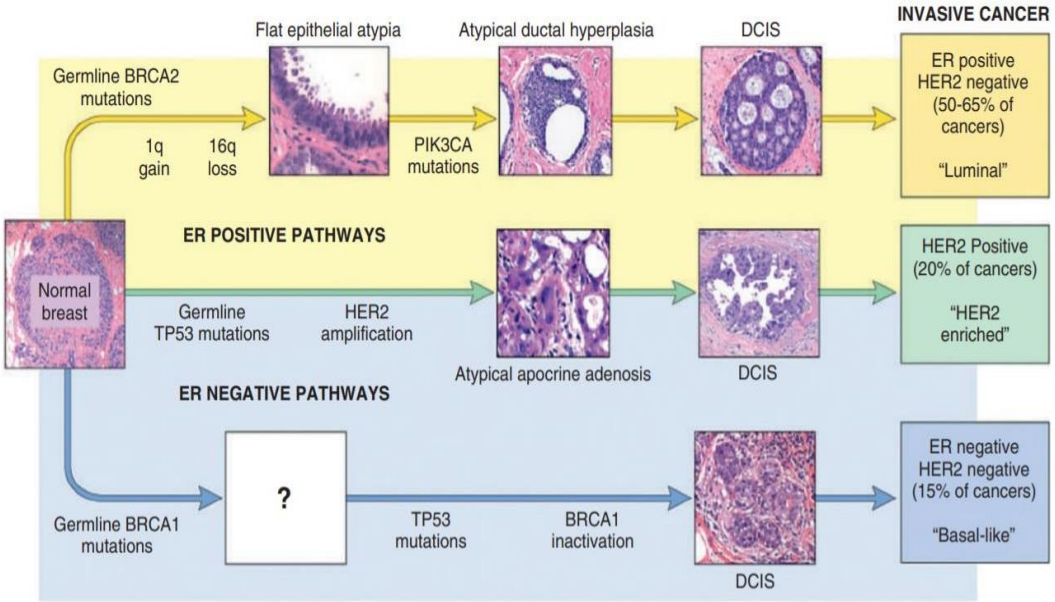


FIG 3- MAJOR PATHWAYS OF BREAST CANCER DEVELOPMENT [37]

Other mutated genes associated with familial breast cancer include TP53 and PTEN.

Somatic TP53 mutations, particularly commonly obsereved in triple-negative and HER2-positive breast tumours, mutations that upregulates, " PI3KAKT" signalling are frequent in sporadic "ER-positive" and "HER2-positive" breast cancers. [29]

- **HORMONAL INFLUENCES**

Growth factors including,"transforming growth factor(TGF)", "platelet-derived growth factor(PDGF)", "fibroblast growth factor(FGF)", and others are produced under the effect of estrogen, and these factors have the potential to promote the growth of tumours through paracrine and autocrine pathways. Numerous more genes, some of which are crucial for the growth or development of tumours, are also regulated by estrogen receptors in an estrogen dependent fashion, likely to be involved in development and growth.[29]

- **ENVIRONMENTAL FACTORS**

Variable breast cancer incidence rates in genetically homogeneous groups point to environmental effects.[29]

CLINICAL FEATURES

Although skin retraction, nipple inversion, nipple discharge, and less frequently a change in the size or form of the breast or a change in the colour or texture of the skin associated with ulceration may be noted, palpable mass is the most prevalent clinical indication of IBC in non-screened population.[43]

DIAGNOSIS OF BREAST CANCER

1. **Mammography-** X-ray images of the breast are used in mammograms.In certain breast screening services, digital mammography has been substituted in place of

conventional mammography. However, the repeated use of mammography calls for careful consideration of any radiation risk. Additionally, false-positive calls result in extra imaging or histological evaluation, primarily percutaneous breast biopsy.[44]

After examining the mammograms, radiologists group their findings into a final assessment category using the BIRADS diagnostic system. The American College of Radiology created the, "Breast Image Reporting And Data System (BIRADS)" to conclude, evaluate and classify so as to standardise mammographic reporting. Continuation suggestions are given in light of the final assessment category.

BIRADS Category 4-6 suggests malignancy on mammographs, with BIRADS 4 suggesting suspicious for malignancy, BIRADS 5 suggesting highly suggestive of malignancy and BIRADS 6 suggesting biopsy proven malignancy.

2. **Magnetic resonance imaging (MRI):** MRI is a powerful imaging device that generates high-resolution images without the use of damaging radiation and is more potent.[45,46]

Results from breast MRIs rely on how amplified lesions become after intravenous contrast injection. Due to neovascularization, the tumour tissue is very permeable, allowing the contrast agent to extravasate there.[44]

"Due to their paramagnetic characteristics, a variety of paramagnetic metal ion complexes of "manganese (Mn)", "iron (Fe)", and "gadolinium (Gd)" have been utilised as MRI contrast agents." Contrast agent use is connected to well-known adverse affects and disadvantages. It has been demonstrated that trans-metallation of gadolinium produce detrimental consequences.[47]

Recent research publications and patents have suggested novel carrier systems and sophisticated targeting methods to improve the efficacy and reduce the toxicity of MRI contrast agents.[44]

3. **Molecular breast imaging (MBI)-** In MBI, a radioactive tracer is used to illuminate breast cancer tissues so they may be seen by a nuclear medicine scanner. "The Miraluma test", "sestamibi test", "scintimammography", or specialised gamma imaging are further names for this procedure. "Tc-99m sestamibi," which is approved for breast cancer imaging, is the major component of MBI. MBI can detect tiny breast lesions with a similar sensitivity to MRI and a greater specificity.[48]

4. **Breast biopsy.** Breast cancer is diagnosed using two different needle biopsies: core needle biopsy(CNB) and fine needle aspiration cytology (FNAC).

As opposed to FNAC, Core Needle Biopsy removes a small cylindrical piece of tissue (a core), typically, three to five cores are extracted, though more can be taken. It is the job of a pathologist to examine the core tissue samples and to look for malignancy.[48]

WHO CLASSIFICATION OF BREAST CARCINOMA[34]

DUCTAL CARCINOMA INSITU

- Intraductal Carcinoma, Non-infiltrating (NOS)
- DCIS of low nuclear grade
- DCIS of intermediate nuclear grade
- DCIS of high nuclear grade

INVASIVE BREAST CARCINOMA

- Infiltrating duct carcinoma NOS
- Invasive breast carcinoma No special Type
- Oncocytic carcinoma
- Lipid-rich carcinoma
- Glycogen-rich carcinoma
- Sebaceous carcinoma
- Lobular carcinoma NOS
- Tubular carcinoma
- Cribriform carcinoma
- Mucinous adenocarcinoma
- Mucinous cystadenocarcinoma NOS
- Invasive micropapillary carcinoma of breast
- Apocrine adenocarcinoma
- Metaplastic carcinoma NOS

NONINVASIVE (IN SITU) CARCINOMA

Noninvasive breast carcinomas can be divided into two morphologic types: "ductal carcinoma in situ (DCIS)" and "lobular carcinoma in situ (LCIS)", Both are limited to basement membrane and does not infiltrate into the surrounding stroma.[29]

Ductal Carcinoma Insitu (DCIS)- has various histologic appearances, DCIS of the "comedo" type, also known as comedocarcinoma, is known to be more aggressive than other kinds of DCIS. The most prevalent subtypes of "non comedo" types of DCIS are-

1. "Solid DCIS": The afflicted breast ducts are totally filled with cancer cells.
2. "Cribriform DCIS": The damaged breast ducts are partially filled with cancer cells, but there are spaces between the cells.
3. "Papillary and micropapillary DCIS": Within the affected breast ducts, the cancer cells arrange themselves in a fern-like pattern, and micropapillary DCIS cells are smaller than papillary DCIS cells.[49]

Lobular Carcinoma Insitu (LCIS)- Usually has a consistent uniform pattern. The monomorphic cells are organised in weakly cohesive clusters and have bland, spherical nuclei. Since these lesions do not exhibit calcifications, unlike DCIS, it is typically an accidental finding. It has been noted that invasive breast cancer develops in one-third of LCIS women. Invasive carcinomas that arise after a diagnosis of LCIS, in contrast to DCIS, can develop in either breast, with two- third arising in the ipsilateral breast and 1/3 in the opposite breast. "LCIS" is a sign of an elevated chance of developing cancer in bilateral breast as well as a straight predecessor to various malignancies..[29]

INVASIVE (INFILTRATING) BREAST CARCINOMA

MACROSCOPY

Most of the Invasive Breast Carcinoma presents as a large grossly perceptible mass having an uneven, stellate border or nodular appearance. The tumor is firm to hard on palpation with the margins usually not well circumscribed. Few tumors may have gritty feel on cutting the tumor tissue with a knife.[34]The tumour typically has a "stab-like" or "stellate"appearance. If the extent of the neoplasm is substantial, "areas of hemorrhage", "necrosis", and "cystic degeneration" may be evident. [28] The gross findings should

always be correlated with the radiographic reports to confirm the size, site, focality etc of the lesion for appropriate tissue sampling.[34]

MICROSCOPY-

In microscopy, the typical development pattern is that of "sheets", "nests", "cords", or "scattered individual cells". It may be completely missing, barely noticeable, or have a well-developed glandular/tubular distinction. The tumour cells can vary in size and shape, but by definition they are bigger and more pleomorphic than the typical form of invasive lobular carcinomas, and they also have more conspicuous nuclei and nucleoli as well as more mitotic figures. Necrosis can occur in some cases, Calcification which are often associated with insitu component has been reported in a few cases, can be seen either as "coarse" or "fine deposit". Foci of "squamous metaplasia", "apocrine metaplasia", or "clear cell changes" may be seen. The stroma can be sparse or abundant, and it can be highly fibrotic or cellular (referred to as "desmoplastic") in appearance. It could be challenging to distinguish the tumour cells in situations with a lot of stroma. There may be regions of "elastosis," which can affect the mainly veins and ducts' wall. Calcification can. Chronic mononuclear inflammatory cell infiltrate is usually seen at the interphase between tumor tissue and the surrounding stroma.[28,29,34]

PREDICTIVE AND PROGNOSTIC FACTORS FOR INVASIVE BREAST CARCINOMA: [29]

1. Distant metastasis(M)- Metastasis beyond regional lymphnode is most important prognostic factor.
2. Regional lymphnodes(N)- Nodal metastasis (including the number of involved nodes) is the second most important prognostic factor.

3. Tumor(T)- Size, involvement of the skin(e.g ulceration, dermal metastases), invasion into the chest wall , and presentation as inflammatory carcinoma are important prognostic factors.
4. Histologic grade- Survival diminishes with higher histologic grade. Modified Scarff-Bloom-Richardson grading system is used which includes 3 features: tubule formation, nuclear pleomorphism and mitotic count to histologically grade the tumor. Well differentiated carcinomas have good prognosis while poorly differentiated carcinomas have poor prognosis

TABLE 2- MODIFIED SCARFF- BLOOM RICHARDSON GRADING OF INVASIVE BREAST CARCINOMA

TUBULE FORMATION	
Score 1	>75% of tumour showing tubules
Score 2	10-75% of tumour showing tubules
Score 3	<10% of tumour showing tubules
NUCLEAR SIZE	
Score 1	Uniform cells with small nuclei similar in size with normal breast epithelial cells.
Score 2	Cells larger than normal, showing moderate pleomorphism with open vesicular nuclei, visible nucleoli
Score 3	Cells with vesicular nuclei, prominent nucleoli showing marked pleomorphism
MITOTIC COUNT*	
Score 1	0-8mitosis/10HPF
Score 2	9-16mitosis/10HPF
Score 3	>17mitosis/10HPF
TOTAL SCORE	
Score 3-5	Well differentiated (Grade I)
Score 6-7	Moderately differentiated (Grade II)
Score 8-9	Poorly differentiated (Grade III)

5. Expression of ER, PR and HER2 neu- Survival is highest for most favourable combination (high ER and PR and absent HER2) and is lowest for least favourable combination (absent ER, PR and HER2)

Other prognostic Factors include:[29]

- Lymphovascular invasion- Tumor cells in vascular spaces at the periphery of carcinomas are poor prognostic factors.
- Special histologic types- Some histologic types of cancer are strongly correlated with very favourable survival (e.g: tubular carcinoma, adenoid cystic carcinoma)
- Gene expression profiling- The most important clinical value of these assay is to detect patients with "antiestrogen- responsive cancers" who do not need chemotherapy
- According to American Joint Committee of Cancer staging system (AJCC) the most important prognostic factors include integration of anatomic staging and molecular characteristic of breast cancer**.**

STAGING OF BREAST CANCER

- "The International Union for Cancer Control (UICC) and the American Joint Commission on Cancer (AJCC) both accepted the clinical staging system for breast carcinoma, which is the one that is most commonly used. It is based on the TNM (Tumor, Nodes, and Metastases) system."

TABLE 3- "(TNM) STAGING OF BREAST CANCER"[34]

(T):	**Primary tumour**
TX:	**Primary tumour cannot be assessed**
T0:	**No evidence of primary tumour**
Tis:	**Carcinoma in situ; DCIS/ LCIS/ Paget's**
T1:	**Tumour size (2 cm or less)**
T1mi:	≤ 0.1cm of microinvasion in its greatest dimensions
T1a:	more than 0.1 cm but less than 0.5 cm in its greatest dimensions
T1b:	more than 0.5cm but less than 1 cm in its greatest dimensions
T1c:	more than 1cm but less than 2 cm in its greatest dimensions
T2:	**Tumour size >2 cm but not more than 5 cm in its greatest dimensions**
T3:	**Tumour size more than 5cm in its greatest dimensions**
T4:	**Tumour of any size with direct extension to chest wall and or to the skin (ulceration or skin nodules)**
T4a:	Extension to chest wall, (does not include pectoralis muscle invasion only)
T4b:	Ulceration and/or ipsilateral satellite skin nodules and/or oedema
T4c:	both of the above (T4a and T4b)
T4d:	Inflammatory carcinoma

(N)	**Regional lymph nodes**
NX:	**(RLN) cannot be assessed**
N0	**No regional lymph node metastasis**
N1	**Metastasis in movable ipsilateral level I.II axillary lymph node(s)**
N2	**Metastasis in ipsilateral level I.II axillary lymph node(s) that are clinically fixed or matted; or in clinically detected ipsilateral internal mammary lymph node(s) in absence of clinically evident axillary lymph node metastasis**
N2a	Metastasis in axillary lymph node(s) that are fixed to one another (matted) to one another
N2b	Metastasis only in clinically detected internal mammary lymph node(s) and in absence of clinically detected axillary lymph node metastasis
N3	**Metastasis in ipsilateral infraclavicular (level III axillary) lymph node(s), with or without level I.II axillary lymph node(s) that are clinically fixed or matted; or in clinically detected ipsilateral internal mammary lymph node(s) with clinically evident level I, II axillary lymph node metastasis or metastasis in ipsilateral supraclavicular lymph node(s) with or without axillary or internal mammary lymph node involvement.**
N3a	Metastasis in infraclavicular lymph node(s)
N3b	Metastasis in internal mammary and axillary lymph node(s)
N3c	Metastasis in supraclavicular lymph node(s)

(M):	Distant metastases
M0:	No distant metastasis
M1:	Distant detectable metastasis as histologically proven larger than 0.2mm

TABLE 4- AMERICAN JOINT COMMISSION ON CANCER (AJCC) STAGING, 8TH EDITION [37)]

STAGE	TUMOR(T)	LYMPH NODES(N)	DISTANT METASTASIS(M)	10 YEAR SURVIVAL
0	Ductal carcinoma in situ	No metastasis	Absent	97
I	Invasive carcinoma ≤2cm	-No metastasis -or only micro metastasis	Absent	87
II	-Invasive carcinoma >2cm -Invasive carcinoma >5cm but ≤5cm	-1-3 positive LNs -0-3 positive LNs	Absent	65
III	-Invasive carcinoma >5cm -Any size Invasive carcinoma -Invasive carcinoma with skin or chest wall involvement or inflammatory carcinoma	-Negative or Positive LNs -≥4 positive LNs -Negative or positive LNs	Absent	40
IV	Any size invasive carcinoma	-Negative or positive LNs	Present	5

MOLECULAR CLASSIFICATION OF BREAST CARCINOMA

Complexity in breast cancer has long been recognised and researched. Beginning in the 1980s, when the illness was initially categorised based on histological features, breast tumours were then separated based on the presence of the oestrogen receptor and later HER2. The microarray revolution, which started in 2000, has shown that the phenotypic differences across breast tumours were caused by their unique mRNA expression profiles.. Utilizing the more recent genetic revolution, this was validated. The molecular subtypes of breast cancer are identified using DNA microarrays/Immunohistochemistry and include:[51]

1. **Luminal A:** ER/PR positive, HER2 negative, Ki-67 protein low [53]

GENE EXPRESSION: Expression of lower molecular weight luminal cytokeratins, E- cadherin and high expression of hormone receptors.

2. **Luminal B:**ER/PR positive and Her2/Neu variable, Ki67Intermediate to high.[53]

   GENE EXPRESSION: Expression of lower molecular weight luminal cytokeratins and moderate expression of hormone receptors.

3. **Basal-like breast cancer:** tumours that are ER, PR, and HER2-negative

   GENE EXPRESSION: Expression of basal epithelial markers such as cytokeratins, usually does not express the "molecular targets" that enable responsiveness to extremely successful "targeted therapy" like tamoxifen and aromatase Inhibitors.[54]

4. **"Triple-negative breast cancer (TNBC)":**tumours that are ER, PR, and HER2-negative. The majority of TNBCs with BRCA1 are basal-like tumours. With minimal chances of distant recurrence, the histological varieties of triple negative also include certain unique medullary and adenoid cystic carcinomas.[47]Tp53 is commonly involved, Ki67 index is high.

5. **HER2+:** (ERBB2+) has amplified HER2/neu. HER-2/ neu status can be analysed by fluorescence in situ hybridization (FISH) assays.

   GENE EXPRESSION: High expression of Her2/Neu and negative for ER and PR and Tp53 positive, likely to be high grade and node positive, Ki67 index is high.[47]10%–20% of breast cancer patients have HER2-positive status. Compared to other types, this type is more agressive and metastatises rapidly.[55]

6. **Claudin low:** A more recent class that is frequently triple negative but stands out due to the low expression of E-cadherin and other cell-cell junction proteins. It is typical to have lymphocyte infiltration.[47]

Genetic predisposition- Analysis of breast cancers growing in BRCA1 mutation carriers revealed a higher proportion of tumours with the basal-like gene expression profile which are more likely to be, “high grade, mitotically active, have a syncytial growth pattern, pushing margins, confluent necrosis, negative for hormone receptors and HER2 ("triple negative"), and be linked to TP53 mutations”[55,56,57,58]

SIGNIFICANCE OF IHC IN BREAST CARCINOMA

“Immunohistochemistry is the most common application of immunostaining” It is a scientific technique that looks for certain antigens (markers) in a tissue sample using antibodies. In most cases, the antibodies are joined to an enzyme or a fluorescent dye.

The enzyme or a dye is activated after the antibodies attach to the antigen in the tissue sample. and a microscope can then be used to view the antigen. Immunohistochemistry (IHC) is used to describe intracellular proteins or various cell surfaces in all tissues. In patients with breast carcinoma distinct biological properties of the tumor are used to determine the prognosis and stipulate appropriate systemic therapy, The use of immunohistochemical markers as prognostic and predictive factors implicated in angiogenesis and apoptosis, along with molecular classification of breast cancer thus aiding the management of the patients are all common uses of these markers.

Different subsets of proteins are expressed by the luminal, basal, and myoepithelial cell types that make up normal glandular breast tissue. The luminal cells express the cytokeratins (CK), oestrogen receptor (ER), progesterone receptor, milk fat globule membrane antigen (MFGM), epithelial membrane antigen (EMA), and -lactalbumin (PR). Myoepithelial cells also express specialised markers like smooth muscle actin, calponin, S100, and p63 while basal cell types show a variety of cytokeratins.

The most often employed therapeutic and prognosis immunohistochemistry markers in cases of Ca breast include p53, Ki-67, human epidermal growth factor receptor-2, oestrogen receptor, progesterone receptor, and human epidermal growth factor receptor.

In situ carcinoma versus invasive malignancy, nonneoplastic proliferative lesions against malignant lesions, and pseudoinvasive lesions (adenosis, radial scar, sclerosing lesions, etc.) against invasive malignancy are among the lesion classifications that often need to be distinguished.

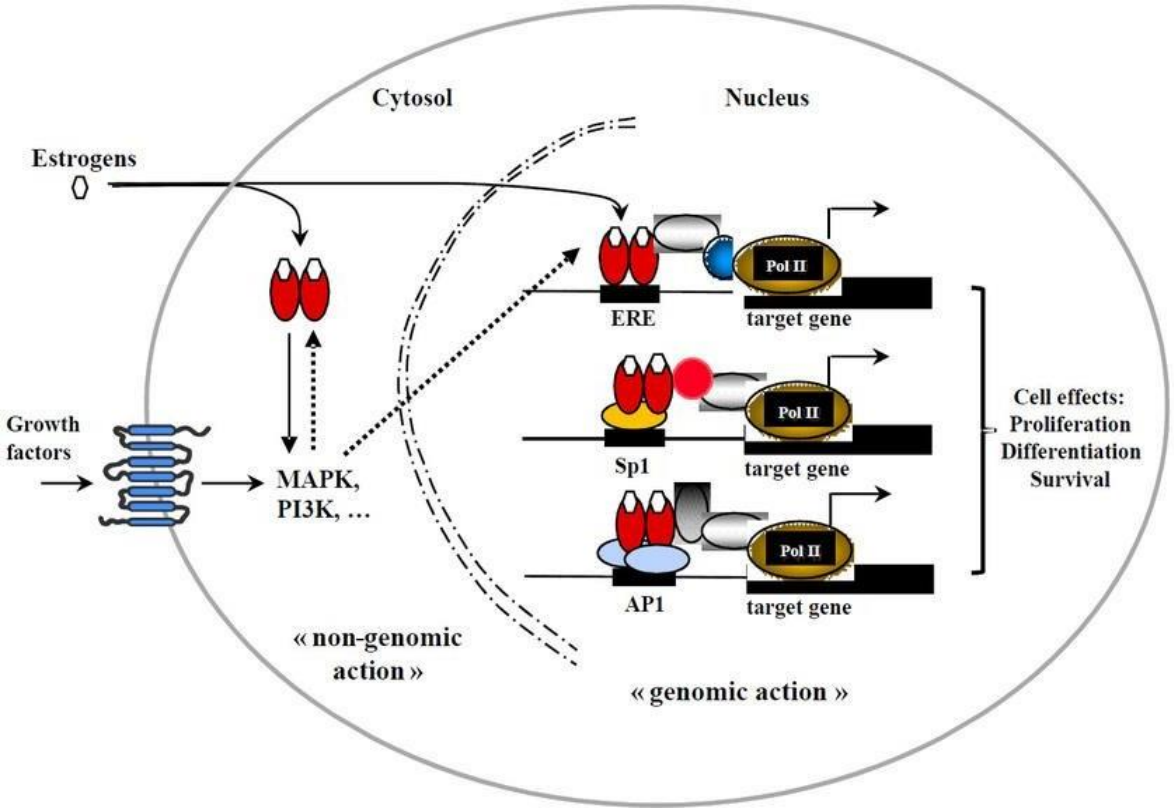
In addition, papillary lesions, microinvasive carcinomas (invasive foci less than or equal to 1 mm in size), and atypical ductal epithelial hyperplasia (ADH) are included. These lesions frequently lend themselves to IHC explanation.[59,60]

Myoepithelial Cells and Assessment of Stromal Invasion-

In addition to being the most common lesions that the surgical pathologist encounters, epithelial lesions of the breast are also the biggest cause for concern when determining whether a lesion is benign or malignant.[59]

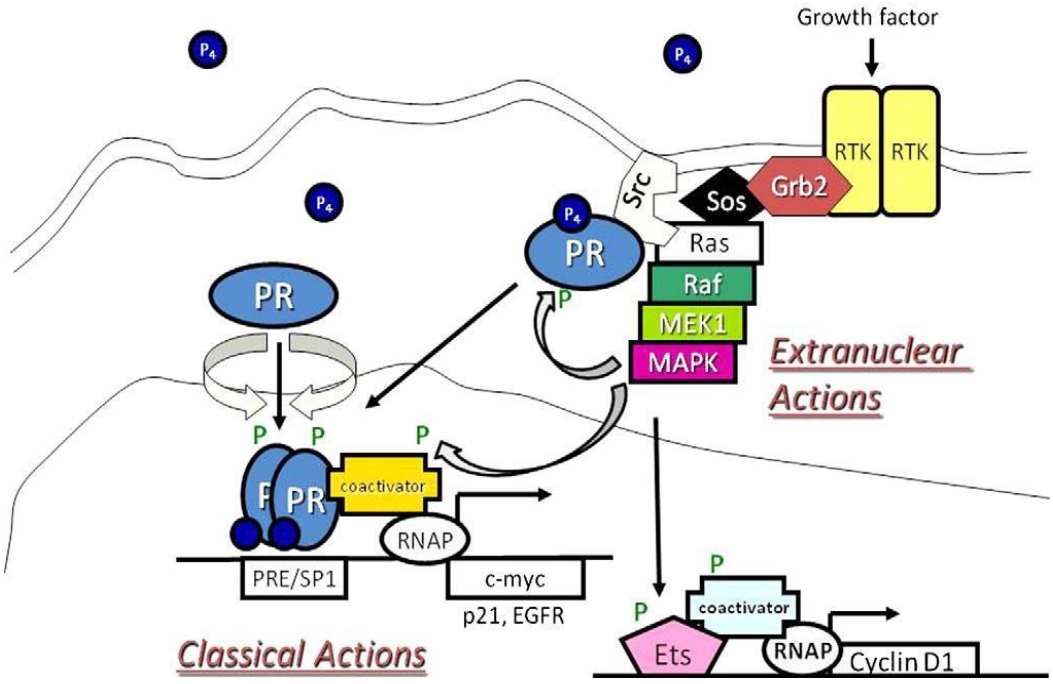
ESTROGEN RECEPTOR

- GPR30 is a G protein-coupled receptor, and ER is a member of the nuclear hormone family of intracellular receptors. When oestrogen receptors are activated, they bind to DNA and control the expression of several genes.
- Located on Chromosome (6q25.1 and 14q23.2).
- The endometrium, breast cancer cells, ovarian stromal cells, and the hypothalamus all have the Erα receptors.
- The kidney, brain, bone, heart, lungs, intestinal mucosa, prostate, and endothelial cells have all been found to express the $ER\beta$ receptors[59,60]



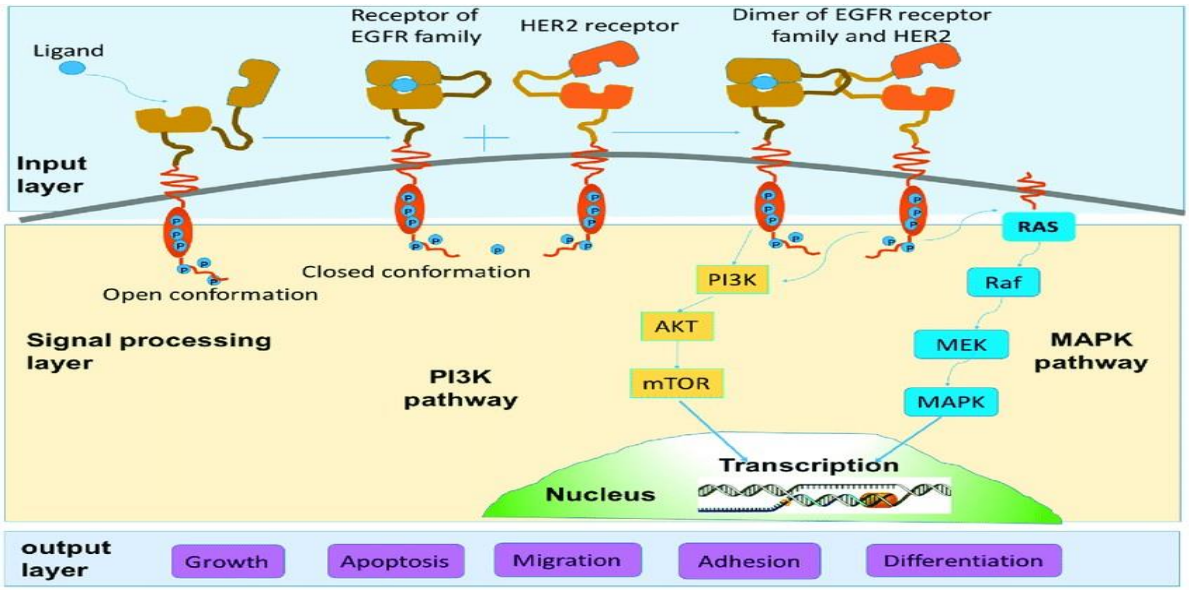
PROGESTERONE RECEPTORS

- "The progesterone receptor (PR)", also known as "Nuclear receptor subfamily 3 (NR3C3)", "group C", "member 3", is a protein found within the cells. It is upregulated by the "steroid hormone progesterone"
- The human PGR gene, which is located on chromosome 11q22, encodes the protein PR. It contains two isoforms, PR-A and PR-B, which differ in their molecular weight. [The PR-B serves to positively regulate the effects of progesterone, whilst the PR-A serves to negatively regulate the PR-B effect.[59,60]



HER2/Neu RECEPTOR

- HER2/neu (c-erbB-2) is an oncogene that encodes a transmembrane glycoprotein with tyrosine kinase activity known as p185, which belongs to the family of epidermal growth factor receptors.
- Its overexpression can be measured by immunohistochemistry or FISH (or its chromogenic equivalent), and a good correlation exists between these methods.[59,60]



CD10 IMMUNO MARKER

CD10 also known as “Common Acute Lymphoblastic Antigen”, is a “90–110-kDa” cell surface “zinc-dependent”metalloproteinase, and is commonly expressed in “bone marrow” “lymphoid stem cells”, “pro-B lymphoblasts”, “mature neutrophils”, various subtypes of “lymphomas”, “renal cell carcinoma”, and “endometrial stromal sarcoma”.[1, 2]

CD10 has also been referred to as "neutral metalloendopeptidase" in the kidney and "enkephalinase" in the brain, or atriopeptidase that specifically degrades atrial natriuretic factor (ANF). Its expression has been studied on the stromal cells of certain breast malignancies and proposed to be over expressed in cancer cells of breast. Various studies have concluded that CD10 could be a potential therapeutic target for new cancer therapies as it causes cleavage of doxorubicin, which is an essential component in many cancer treatment protocols, thus resulting in chemoresistance. The antitumor efficacy of conventional chemotherapeutic regimens can be intensified by inhibiting the enzymatic activity of CD10.[9,10]

MATERIALS AND METHODS:

SOURCE OF DATA

Patients of breast carcinoma admitted to, "BLDE Hospital (Deemed to be University) Shri B. M. Patil Medical College, Hospital and Research Centre", Vijayapura.

Study period: January 2020 to August 2022 (1.5 year- Prospective study, 1year_ Retrospective study).

Study design: Hospital based cross sectional study.

Inclusion criteria: All the mastectomy specimens of primary breast cancer received in the histopathology section of "Department of Pathology" in "BLDE (Deemed to Be University) Shri B.M. Patil Medical College, Hospital and Research Centre", Vijayapura.

Exclusion criteria: Nil

Sample size:

Our study consisted of 50 patients with histologically confirmed diagnosis of invasive ductal carcinoma of breast.

Methodology:

All mastectomy specimens received at the "histopathology" section of the "Department of the Pathology" at "Shri B.M. Patil Medical College, Hospital and Research Centre, BLDE (Deemed to Be University)," Vijayapura, were studied. The specimen were examined grossly, the tissue was preserved in 10% formalin. Representative sections from the specimen were given and processed routinely, slides were prepared and stained.

Five 4 micron thick sections were prepared from the most suitable tumor tissue block. One section was stained with Haematoxylin and Eosin (H & E) for morphologic diagnosis and

modified Bloom-Richardson system of cancer grading. Four sections were mounted on “poly L lysine” coated slides, which were subjected to ER/PR, HER2-neu, and CD10 immunohistochemical staining. The IHC expression of the markers “ER”, “PR”, “HER2-neu”, and “CD10” was studied. The details of patient including the “age”, “disease laterality”, “tumor size”, “histopathological grade”, “lymph node metastasis” were obtained and data was entered in DATA entry form, sample of which has been attached at ANNEXURE II. The data was statistically analysed using “SPSS software version 16.0”Immunohistochemical expression of CD10 in stromal cells were correlated with prognostic factors such as “age”, “tumour size”, “histological grade”, “lymph node status”, “ER”, “PR”, and “HER-2neu” status.

"IMMUNOHISTOCHEMICAL STAINING PROTOCOL"[66]

1. "Cut 4µm sections on charged slides and incubated at 60-70 ºC for 20 min."
2. "Deparaffinize by 2 changes of Xylene 10 minutes each."
3. "Hydrate through absolute alcohol 2 changes, each 5 minutes"
4. "Wash in distilled water, 2 changes, 2 minutes each"
5. "Antigen retrieval for 15 minutes in Pressure cooker 2 whistles with retrieval buffer on TRIS EDTA, maintain pH between 8.5-9.0(preparation- 1 part of concentrated buffer+49 parts of distilled water)"
6. "Add primary antibody and keep for 45 minutes in moist chamber. Then wash in wash buffer two changes, 3 minutes each"
7. "Add Poly-excel Target binder reagent or Secondary antibody and keep for 12 minutes. Wash in wash buffer two changes, 3 minutes each"
8. "Add working Di-amino benzidine (DAB) chromogen (1 ml DAB buffer+1 drop DAB chromogen, mix well) and keep it for 2- 5 minutes"
9. "Wash it with distilled water 2 changes 2 minutes each"
10. "Counterstain with Haematoxylin for 30 seconds, wash with water"
11. "Dehydrate, clear and mount the slide."

TABLE 5- IHC INTERPRETATION OF ER, PR, HER2 NEU AND CD10 MARKERS.

IHC Marker	**Proportion score** PS Range (%)	**Intensity score** IS Type	**Interpretation**
ER/ PR	0 0 1 <1 2 1-10 3 11-33 4 34-66 5 67-100	0 No staining 1+ Weak positive staining 2+ Moderate Positive staining 3+ Strong positive staining	**Allred score= PS +IS** Negative= ≤2 Positive= >2 Maximum score= 8
HER2neu			**ASCO guidelines** **0** = No/faint membrane staining observed in ≤10% of tumor cells. **1+**= Incomplete/faint membrane staining observed in >10% of tumor cells. **2+**= Moderate complete membrane staining observed in >10% of tumor cells **3+**= Circumferential membrane staining i.e. complete, intense and in >10% of tumor cells
CD10			**CD10 (in stromal cells)** CD10 weakly positive - 10–30% of stromal cells positive CD10 strongly positive - >30% stromal cells positive

RESULTS

We studied 50 cases of Invasive breast carcinoma. The age group of the patients with Invasive breast carcinoma varied from 30 years to 80 years with mean age of the patients being 52 years.

In the present study, 21 (42%) cases belonged to histologic grade I, 23 (46%) cases belonged to histologic grade II and 6 (12%) cases were of grade III. Lymph node metastasis was seen in 33(66%) cases. The size of the tumor varied from 0.5 cm to 10 cm.In majority of the cases,ie 32 (64%) cases tumor size was between 2-5 cm(T2) , 11 (22%) cases had tumor size >5 cm (T3), 5 (10%) cases had a tumor size <2cm(T1) and 2 (4%) cases were of T4 showing direct extension to the chest wall and/ skin.

In the present study,majority of the cases i.e.31 (62%) cases were of stage II, 17 (34%) cases were of stage III, and 2 (5%) cases were of stage I. We also assessed the ER, PR and HER2 status of the tumor, we observed that 32 (64%)cases were ER positive, and 18 (36%) cases were ER negative. 26 (48%) cases showed high PR expression and 24(48%) cases did not show PR expression. 26(52%) cases were HER2 negative and 24(48%) cases were HER2 positive.

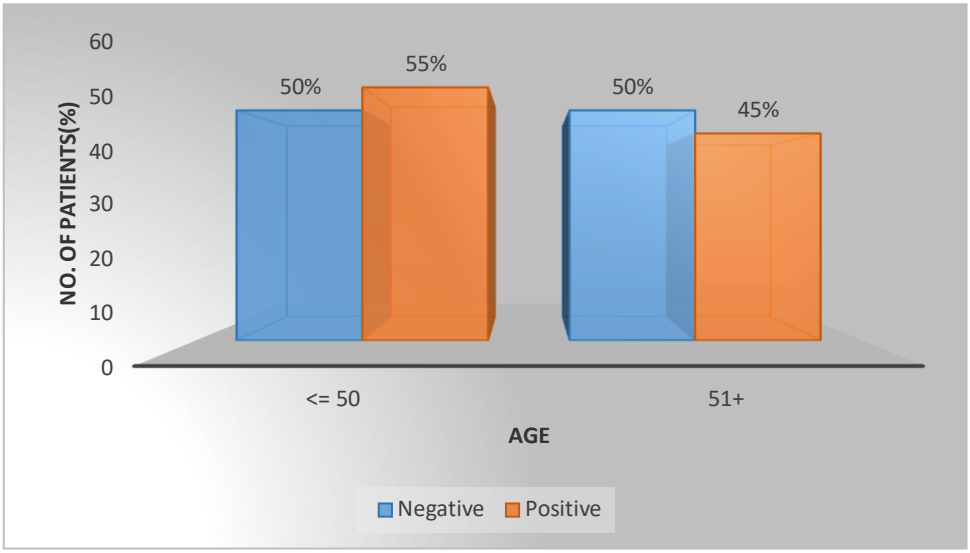
TABLE 6-STROMAL EXPRESSION OF CD10 IN STUDY POPULATION

CD10	No. of patients	Percentage
Negative	10	20.0
Positive	40	80.0
Total	50	100.0

Stromal CD10 positivity was seen in 40 (80%) cases, while 10 (20%) cases were CD10 negative.(Table 6)

Correlation between CD10 expression and the clinicopathological parameters

1. **AGE**



Graph 3: Comparison of CD10 with age (years)

TABLE 7- CORRELATION OF CD10 EXPRESSION WITH THE AGE OF THE PATIENT

CD10	AGE			Chi square test	P value
	≤50	51+	Total		
Negative	5	5	10	0.081	0.777
%	50.0%	50.0%	100.0%		
Positive	22	18	40		
%	55.0%	45.0%	100.0%		
Total	27	23	50		
%	54.0%	46.0%	100.0%		
Statistically insignificant					

In the study, breast carcinoma patients were classified into two groups based on age, those with less than 50 years and more than 50 years of age. Among 50 cases of invasive

breast carcinoma studied, 27 (54%) cases were ≤ 50 years of age and 23 (46%) cases were >50years of age, The highest number of CD10 positivity was observed in study population less than ≤ 50 years of age, with 22 (55%) cases out of 27 cases showing CD 10 positivity, while the study group with more than >51 years of age showed CD10 positivity in 18 (45%) cases out of 23 cases. While 5 cases each in both the groups showed <10% cytoplasmic and membranous staining of stromal cells rendering them CD10 negative. (Graph 1)The p value was 0.007 showing a statistically insignificant association between stromal CD10 expression and Age of the patient. (Table 7).

2. Histologic Tumor grade

TABLE 8- DISTRIBUTION OF CASES ACCORDING TO HISTOLOGICAL GRADE

HISTOLOGIC GRADE	No. of patients	Percentage
I	21	42.0
II	23	46.0
III	6	12.0
Total	50	100.0

In the present study, 21 (42%) cases belonged to histologic grade I, 23 (46%) cases belonged to histologic grade II and 6 (12%) cases were of grade III.(TABLE 8)

TABLE 9- COMPARISON OF CD10 WITH HISTOLOGICAL GRADING

CD10	HISTOLOGIC GRADE				Chi square test	P value
	I	II	III	Total		
Negative	10	0	0	10	17.262	0.0001
%	100.0%	0.0%	0.0%	100.0%		
Positive	11	23	6	40		
%	27.5%	57.5%	15.0%	100.0%		
Total	21	23	6	50		
%	42.0%	46.0%	12.0%	100.0%		
Statistically significant						

Out of 21 (42%) patients with histological gradeI, 11 (27. 5%) cases were CD10 positive, 10 were CD10 negative. Out of 23(46%) patients with histological grade II, All 23(57%) cases were CD10 positive and 6 patients with histological grade III, all 6(15%) cases showed CD10 positivity. The p value was 0.0001 showing a statistically significant correlation between stromal CD10 expression and histologic grade of the tumor. (Table 9)

3. SIZE OF THE TUMOR

TABLE 10- DISTRIBUTION OF CASES ACCORDING TO THE SIZE OF THE TUMOR

TUMOR SIZE	No. of patients	Percentage
T1	5	10.0
T2	32	64.0
T3	11	22.0
T4	2	4.0
Total	50	100.0

The size of the tumor varied from 0.5 cm to 10 cm.In majority of the cases,ie 32 (64%) cases tumor size was between 2-5 cm(T2) , 11 (22%) cases had tumor size >5 cm (T3), 5 (10%)

cases had a tumor size <2cm(T1) and 2 (4%) cases were of T4 showing direct extension to the chest wall and/ skin.(Table 10)

TABLE 11- CORRELATION OF CD10 EXPRESSION AND TUMOR SIZE

CD10	TUMOR SIZE					Chi square test	P value
	T1	T2	T3	T4	Total		
Negative	1	6	3	0	10	0.895	0.827
%	10.0%	60.0%	30.0%	0.0%	100.0%		
Positive	4	26	8	2	40		
%	10.0%	65.0%	20.0%	5.0%	100.0%		
Total	5	32	11	2	50		
%	10.0%	64.0%	22.0%	4.0%	100.0%		
Statistically Insignificant							

The size of the tumor varied from 0.5 cm to 10 cm. In majority of the cases, i.e 32 (64%) cases the tumor size was between 2-5 cm(T2), out of these 26 (65%) cases showed CD10 positivity . 11 (22%) cases had tumor size >5 cm (T3), of these 8(20%) cases showed positive stromal CD10 expression. 5 (10%) cases had tumor size <2cm (T1), 4(10%) cases of which showed CD10 positivity. and 2 (4%) cases were of T4 showing direct extension to the chest wall and/ skin, both of these cases were positive for CD10 immunostaining. The p value was 0.827 showing no statistically significant association between stromal CD10 expression and size of the tumor. (Table10,11)

4. LYMPH NODE STATUS

TABLE 12- DISTRIBUTION OF CASES ACCORDING TO LYMPH NODE STATUS

LYMPHNODE STATUS	No. of patients	Percentage
Negative	17	34.0
Positive	33	66.0
Total	50	100.0

Lymph node metastasis was seen in 33(66%) cases.(Table 12)

TABLE 13- CORRELATION OF CD10 EXPRESSION WITH LYMPH NODE STATUS

CD10	LYMPH NODE STATUS			Chi square test	P value
	NEGATIVE	POSITIVE	Total		
Negative	5	5	10	1.426	0.232
%	50.0%	50.0%	100.0%		
Positive	12	28	40		
%	30.0%	70.0%	100.0%		
Total	17	33	50		
%	34.0%	66.0%	100.0%		
Statistically Insignificant					

In the present study out 50 cases of invasive breast carcinoma, 33(66%) cases had positive nodal status, of these 28 (70%) were CD10 positive and 5 cases were CD 10 negative. 17 (34%) cases did not show lymph node metastases, of these 12(30%) cases showed CD10 stromal expression. P value was 0.232 showing no statistical significance on comparision of stromal CD10 expression with lymph-node status.(Table 12,13)

5. TNM STAGE OF THE TUMOR

TABLE 14- DISTRIBUTION OF CASES ACCORDING TO TNM STAGING

TNM STAGE OF THE TUMOR	No. of patients	Percentage
I	2	4.0
II	31	62.0
III	17	34.0
IV	00	00
Total	50	100

In the present study,majority of the cases i.e.31 (62%) cases were of stage II, 17 (34%) cases were of stage III, and 2 (5%) cases were of stage I.(table 14)

TABLE 15- CORRELATION OF CD10 WITH TNM STAGING OF THE TUMOR

CD10	TNM STAGE					Chi square test	P value
	I	II	III	IV	Total		
Negative	0	6	4	00	10	0.640	0.726
%	0.0%	60.0%	40.0%	00	100.0%		
Positive	2	25	13	00	40		
%	5.0%	62.5%	32.5%	00	100.0%		
Total	2	31	17	00	50		
%	4.0%	62.0%	34.0%	00	100.0%		
Statistically Insignificant							

We observed that 31 (62%) cases were of stage II, out of which 25 (62.5%) cases showed positive CD10 staining. 13(32.5%) cases out of the 17(34%) cases belonging to stage III showed CD10 stromal expression.Whereas all stage I cases i.e 2 cases (5%) showed CD10 immunostaining.P value was 0.726 showing statistically insignificant association between Expression of CD10 by stromal cells and stage of tumor. (Table 14,15)

6. ESTROGEN RECEPTOR STATUS

TABLE 16- ESTROGEN RECEPTOR EXPRESSION OF BREAST CARCINOMA CASES

ER	No. of patients	Percentage
Negative	18	36.0
Positive	32	64.0
Total	50	100.0

Among 50 cases of invasive breast carcinoma**,** 32 cases (64%) were ER positive and 18 cases (36%) were ER negative.

TABLE 17-COMPARISON OF CD10 WITH ESTROGEN RECEPTOR STATUS

CD10	ER STATUS			Chi square test	P value
	NEGATIVE	POSITIVE	Total		
Negative	1	9	10	3.668	0.045
%	10.0%	90.0%	100.0%		
Positive	17	23	40		
%	42.5%	57.5%	100.0%		
Total	18	32	50		
%	36.0%	64.0%	100.0%		
Statistically Significant					

Out of 32 ER positive cases, 23 (57.5%) cases showed ER and CD10 positivity. Out of 18 cases with negative ER expression, 17(42.5%) cases showed Stromal expression of CD10. P value was 0.045 showing statistically significant correlation between expression of CD10 by stromal cells and ER status of the tumor. (Table 16,17)

7. PROGESTERON RECEPTOR STATUS

TABLE 18- PROGESTERONE RECEPTOR EXPRESSION OF BREAST CARCINOMA CASES

PR	No. of patients	Percentage
Negative	24	48.0
Positive	26	52.0
Total	50	100.0

Among 50 cases of invasive breast carcinoma**,** 26 cases (52%) were PR positive and 24 cases (48%) were PR negative.

TABLE 19-COMPARISON OF CD10 WITH PROGESTERONE RECEPTOR STATUS

CD10	PR STATUS			Chi square test	P value
	NEGATIVE	POSITIVE	Total		
Negative	2	8	10	3.926	0.048
%	20.0%	80.0%	100.0%		
Positive	22	18	40		
%	55.0%	45.0%	100.0%		
Total	24	26	50		
%	48.0%	52.0%	100.0%		
Statistically Significant					

Out of 26 PR positive cases, 18 (45%) cases showed PR and CD10 positivity. Out of 24 cases with negative PR expression, 22 (55%) cases showed Stromal expression of CD10. P value was 0.048 showing statistically significant correlation between expression of CD10 by stromal cells and PR status of the tumor. (Table 18,19)

8. HER 2 NEU STATUS

TABLE 20-HER2 NEU RECEPTOR EXPRESSION OF BREAST CARCINOMA CASES

HER2 NEU	No. of patients	Percentage
Negative	26	52.0
Positive	24	48.0
Total	50	100.0

In present study out of 50 cases of invasive breast carcinoma**,** 26 cases (52%) were Her2 negative and 24 cases (48%) were Her2 positive.

TABLE 21-CORRELATION OF CD10 WITH HER 2 NEU STATUS

CD10	HER2 NEU STATUS			Chi square test	P value
	NEGATIVE	POSITIVE	Total		
Negative	3	7	10	2.424	0.119
%	30.0%	70.0%	100.0%		
Positive	23	17	40		
%	57.5%	42.5%	100.0%		
Total	26	24	50		
%	52.0%	48.0%	100.0%		
Statistically Insignificant					

Out of 26 negative cases, 23 (57.5%) cases showed CD10 positivity. Out of 24 cases with positiveHer2 neu expression, 17 (42.5%) cases showed Stromal expression of CD10. P value was 0.119 showing no statistically significant correlation between expression of CD10 by stromal cells and Her2 neu status of the tumor. (Table 20,21)

Table 22: Comparision of CD10 with various clinicopathological parameters

PARAMETERS	CD10		CHI SQUARE TEST	P VALUE
	NEGATIVE	POSITIVE		
	NO OF CASES (%)	NO OF CASES (%)		
AGE				
<50 YEARS	**5 (50%)**	**22 (55%)**	**0.081**	**0.777**
>50 YEARS	**5 (50%)**	**18 (45%)**		
HISTOLOGIC GRADE				
I	**10 (100%)**	**11 (27.5%)**	**17.262**	**0.00001**
II	**0 (00%)**	**23 (57.5%)**		
III	**0 (00%)**	**06 (51%)**		
LYMPH NODE STATUS				
NEGATIVE	**5 (50%)**	**12 (30%)**	**1.462**	**0.232**
POSITIVE	**5 (50%)**	**28 (70%)**		
TUMOR SIZE				
T1	**1 (10%)**	**4 (10%)**	**0.895**	**0.827**
T2	**6 (60%)**	**26 (65%)**		
T3	**3 (30%)**	**8 (20%)**		
T4	**0 (00%)**	**2 (5%)**		
STAGE				
I	**0 (00%)**	**4 (10%)**	**0.640**	**0.726**
II	**6 (60%)**	**26 (65%)**		
III	**4 (40%)**	**8 (20%)**		
IV	**0 (00%)**	**2 (5%)**		
ER STATUS				
NEGATIVE	**1 (10%)**	**9 (90%)**	**3.668**	**0.045**
POSITIVE	**17 (42.5%)**	**23 (57.5%)**		
PR STATUS				
NEGATIVE	**2 (20%)**	**8 (80%)**	**3.926**	**0.048**
POSITIVE	**22 (55%)**	**18 (45%)**		
HER 2 NEU				
NEGATIVE	**3 (30%)**	**7 (70%)**	**2.424**	**0.119**
POSITIVE	**23 (57.5%)**	**17 (42.5%)**		

Tumor grade, ER negativity, and PR negativity were all found to be substantially correlated with stromal expression of CD10. There was no correlation between CD10 and Age of the patient, lymph node status, tumor size, stage of the tumor and HER2 status

PHOTOMICROGRAPHS

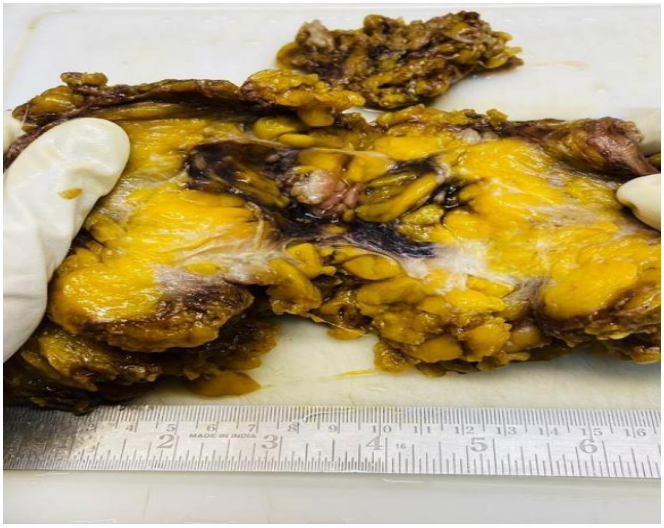


Fig 4: Gross Photograph of Invasive Breast Carcinoma – showing solid, Pale white growth with irregular borders

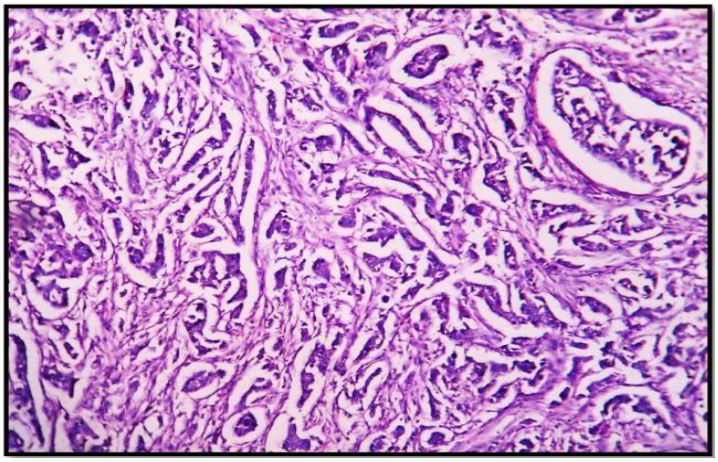


Fig 5- Photomicrograph showing invasive breast carcinoma showing tubule formation (H&E STAIN, 200X)

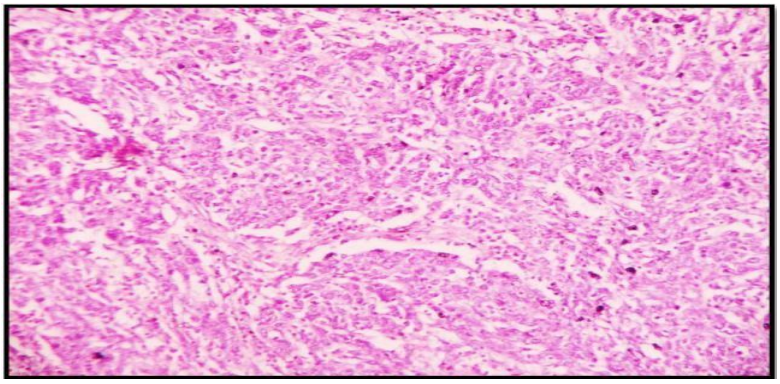


Fig 6- photomicrograph of invasive breast carcinoma showing solid nests of tumor (H&E STAIN, 200X)

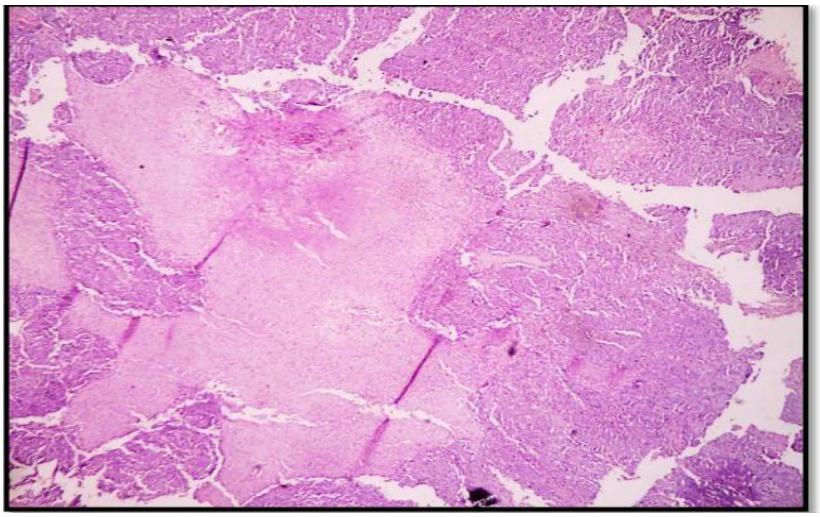


Fig 7 - photomicrograph of invasive breast carcinoma showing solid tumor tissue and desmoplastic stroma (H&E STAIN, 200X)

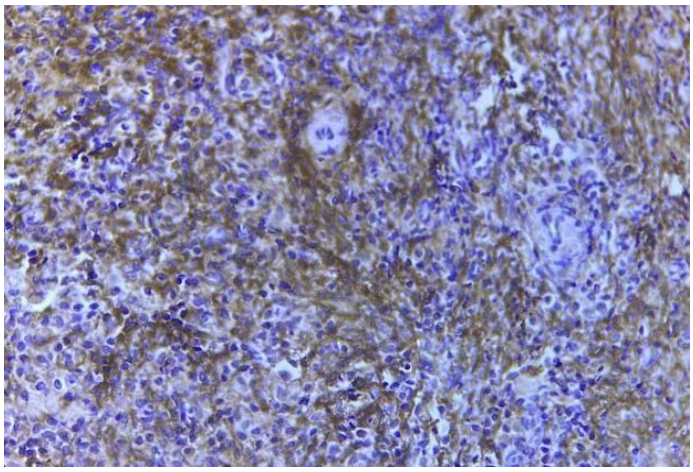


Fig 8- Photo micrograph of IHC marker CD10 showing Strong cytoplasmic expression in stromal cells

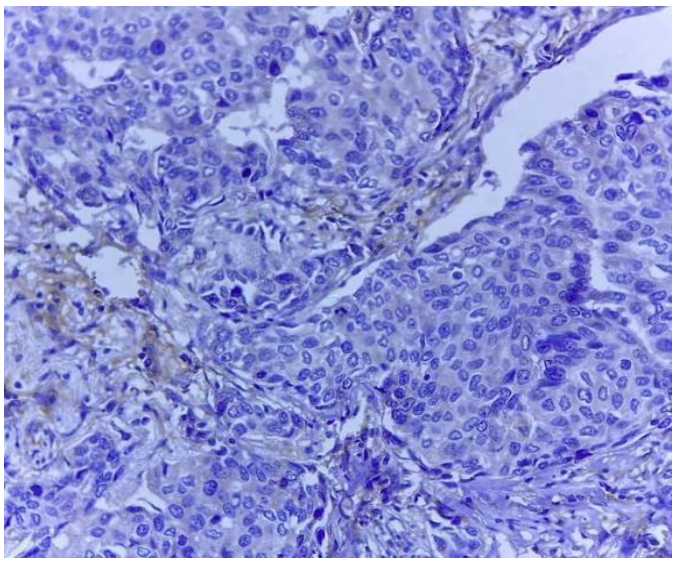


Fig 9- Photo micrograph of IHC marker CD10 showing negative immune staining in stromal cells

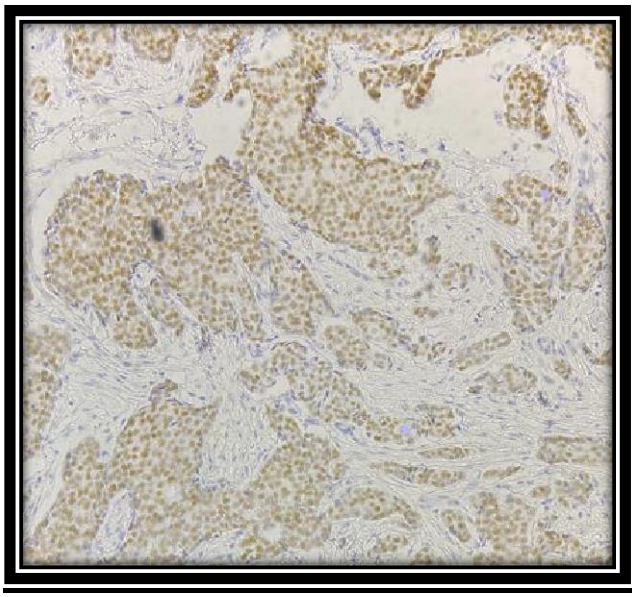


Fig 10- Photo micrograph of IHC marker ER showing nuclear positivity

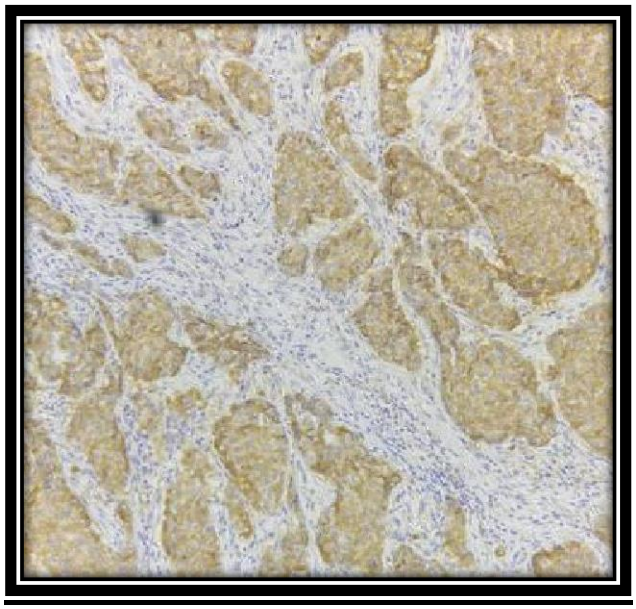


Fig 11- Photo micrograph of IHC marker HER 2 neu showing membranous positivity

DISCUSSION

Breast cancer in women places a significant cost on global health. It accounts for more than one million of the estimated 10 million neoplasms identified worldwide each year in both sexes, making it the most common cause of cancer in women in both high- and low-resource settings. It also accounts for the majority of cancer-related fatalities among women worldwide, killing over 375,000 people in 2000.[30]

The awareness campaigns have started a drift towards a younger age at diagnosis[61] but what is more concerning is the prevalence of aggressive tumours, "ER -ve", "PR -ve", "HER2/neu +ve" or "triple negative tumours" in this age group. Given their rising prevalence, a focus on these aggressive tumours is urgently required.[62,63]

However, there has been significant advancement in the diagnosis and treatment of carcinoma breast, including, "Breast conservation surgeries(BCS)", "Neo adjuvant chemotherapy", "tumour classification" based on overexpression of the HER2/neu protein and the oestrogen, progesterone, and HER2 receptors, and incorporation of these into standard treatment protocols."

Greater knowledge of the molecular causes of metastatic disease would have applications in the medical fields of diagnosis, treatment, and prognosis because metastatic disease is the cause of the mortality linked to breast cancer.[64]

It has recently been clearly established that the interplay between the malignant cells and their microenvironment facilitates the growth of tumours. Several variables that affect the signalling pathways involved in tumour invasion and metastatic spread engage in this interaction. Understanding how stromal cells and cancer cells interact in the tumour microenvironment may help in the search for new treatment targets and prospective indicators.[65]

We examined CD10 expression in stromal cells in the current investigation to determine whether CD10 is connected to a specific clinicopathological feature of breast cancer.

1. **Distribution of the study population based on Age and its correlation with stromal expression of CD10**

   Our study had included a total of 50 cases of invasive breast carcinoma with age group of patients ranging from 30 years to 80 years with a mean age of 52 years. The majority of the patients were classified into two groups ie ≤50 years of age group and >50 years. 27 (54%) cases belonged to age group ≤50 years and among these 27 cases, 22 cases showed CD10 positivity. We also observed that 23 (46%) cases belonged to >50 years of age group and among them, 18(43%) cases showed CD10 positivity. Despite the fact that the number of positive cases rose with age**, t**he p value was 0.777 indicating that there was no statistically significant association between patients age when compared with CD10 positivity in the stromal cells.

   This finding was similar to the findings of studies conducted by Puri et.al,[7] Saayantan H.Jana et. al,[8] Dhande et al**.**[2]

2. **Correlation of Stromal expression of CD10 with Histological grade of the tumor**

TABLE- 23 COMPARISION OF STATISTICAL RESULTS OF HISTOLOGICAL GRADE WITH OTHER STUDIES

AUTHOR	SAMPLE SIZE	P-VALUE FOR HISTOLOGIC GRADE
Present study	50	0.0001
Keiichi Iwaya	110	0.488

et al		
Nikita A Makretsov *et al*	258	0.01
Puri *et al*	50	0.139
Taghizadeh-Kermani A *et al*	100	<0.001
Sayantan H. Jana *et al*	70	0.04
Dhande *et al*	60	<0.01

In the present study, out of 50 cases, 21 (42%) cases belonged to grade I, 23 (46%) cases belonged to grade II and 6 (12%) cases belonged to grade III. On comparing the stromal expression of CD10 with the histological grade of tumor, 11(27.5%) cases of out of 21 cases of grade I carcinoma showed CD10 positivity while all the cases of grade II and grade III carcinoma showed CD10 positivity. Thus the expression of stromal CD10 was higher with higher histologic grade. The p value was 0.0001 showing statistically significant association between CD10 expression and higher tumor grade.

This finding was similar to the findings of studies conducted by Makrestov et.al. [9]Taghizadeh-Kermani A *et al*,[67] Saayantan H.Jana et. al,[8] Dhande et al.[2]

In a study conducted in 2007 by Nikita A. Makretsov et al., 68 patients (26.4%) were of grade 3 and 62 of these showed positive CD10 expression, P value was 0.02 showing statistically significant association with CD10.

A study done in 2014 by sayantan et. al, 28 cases were of grade 3, 26 cases of these 28 cases showed positive immunostaining with CD10 marker, 15 cases of these 26 CD10 positive cases showed strong CD10 positivity. They observed a statistically significant association between higher histologic grade and CD10 expression with p value of 0.0413.

Dhande, et al. compared the stromal expression of CD10 with other prognostic factors in 60 cases of diagnosed breast cancer, they discovered a statistically significant correlation between CD10 and increasing tumour grade.

3. Comparision of Size of the tumor with Stromal CD10 expresion.

In our study we analysed the tumor size based on TNM staging of the breast carcinoma, Maximum number of cases, i.e 32(64%) cases belonged to T2 category, followed by 11(22%) cases, 5(10%) cases and 2(4%) cases belonging to T3, T1 and T4 category respectively. Stromal CD10 expression was observed in 26 cases of T2, 8 cases of T3, and both cases of T4. The P value was 0.827 showing no statistical correlation between stromal expression of CD10 and Size of the tumor.

This finding was similar to the findings of studies conducted by Iwaya K et.al,[12] Makrestov et.al,[9] Nikita Puri et.al,[7]Taghizadeh-Kermani et.al,[67]Saayantan H.Jana et. al,[8] Dhande et al.[2]

4. Correlation of CD10 expression with Lymph-node metastases

In our study we analysed lymph node metastasis by evaluating total number of lymph nodes involved. Present study included 50 cases of invasive breast carcinoma, 33 (66%) cases showed lymph node metastasis and out of these 33 cases, 28(70%) cases showed CD 10 immune reactivity.17 cases out of 50 cases showed negative lymphnode status and among these 17 cases, 12(30%) cases showed positive CD10 expresion. The P value was 0.232 showing no statistically significant association between lymph node status and CD10 expression.

This finding was similar to the findings of studies conducted by Iwaya K et.al, Saayantan H.Jana et. al,[8] Dhande et al.[2]

5. Correlation of stage of tumor with CD10 stromal expression

In the present study we included 50 cases of invasive breast carcinoma and noted the stage of the carcinoma. we observed that the highest number of cases i.e 31(62%) cases belonged to stage 2, followed by 17(34%) cases of stage 3 and 2(4%) cases belonging to stage 1.

We correlated the stromal expression of CD10 in these cases.Out of 31 cases of stage 2 breast carcinoma, 25cases (62.5%) showed CD10 positivity, Similarly 13 cases (32.5%) of stage 3, and both the cases of stage 1 breast carcinoma showed CD10 positivity. The P value was 0.726 showing no statistically significant association between Stroml CD10 expression and stage of the tumor.

This finding was similar to the findings of studies conducted by Makrestov et.al,[9] Nikita Puri et.al,[7] Saayantan H.Jana et. al,[8] Dhande et al.[2]

6. COMPARISION OF CD10 STROMAL EXPRESSION WITH ER, PR AND HER2 STATUS

TABLE- 24 COMPARISION OF STATISTICAL RESULTS OF ER/PR AND HER 2 NEU EXPRESSION WITH OTHER STUDIES

AUTHOR	SAMPLE SIZE	ER STAUS		P-VALUE FOR ER STATUS	PR STATUS		P-VALUE FOR PR	HER2 NEU STATUS		P-VALUE FOR HER2 neu STATUS
		+VE	**-VE**		**+VE**	**-VE**		**+VE**	**-VE**	

							STATUS			
PRESENT STUDY	50	32	18	0.045	26	24	0.048	24	26	0.119
Puri *et al*	50	15	35	0.188	30	20	>0.050	-	-	0.0001
Sayantan H. Jana *et al*	70	37	33	0.0001	20	50	0.1902	45	25	0.0057
Dhande *et al*	60	19	41	<0.05	20	40	0.438	39	21	<0.01

Among the 50 cases studied for stromal expression of CD10 in invasive breast carcinoma, we observed a statistically significant correlation of CD10 in relation with ER negative and PR negative status with a p value 0.045 and p value 0.048 respectively. The P value was 0.119 showing statistically insignificant association between stromal CD10 expression and HER2 neu status.

Studies conducted by Makrestov et. al, Puri *et al*, Sayantan H. Jana *et al*, Dhande *et al* showed statistically significant correlation with CD10 stromal expression and ER and PR status which is in concordance with present study.

In a study conducted by Puri *et al*. in 50 breast cancer patients on correlation of stromal expression of CD10 with well-known prognostic markers such as, ER, PR, HER-2neu and Ki67, they found a correlation of stromal CD10 with PR and HER-2neu, but did not find correlation between stromal CD10 expression and ER status of the tumor .[7]

Sayantal *et al*. studied the role of CD10 stromal marker in breast cancer in 70 cases of breast cancer and they found strong association of stromal CD10 with ER and HER-2neu, but did not find correlation between stromal CD10 expression and PR status of the carcinoma.[8]

Dhande, *et al.* compared stromal expression of CD10 in 60 cases of diagnosed breast Cancer with various prognostic parameters like the size of the tumour, histological grade, lymph node status, ER, PR, HER2neu, ki67 status . They found strong association of stromal CD10 with ER and HER-2neu, but did not find correlation between stromal CD10 expression and PR status of the carcinoma.[8]

SUMMARY

- A hospital-based retro-prospective cross-sectional study was conducted. The study included mastectomy specimens of primary breast cancer received in the histopthology section of Department of Pathology from 1st Jan 2021 to 31st August 2022.
- The histopathological diagnosis of all cases included in this study were based on routine microscopic examination on H&E stain.
- The IHC markers, Estrogen receptor, Progesteron receptor, Her2 neu and stromal CD10 were studied in all the cases of invasive breast carcinoma.
- Data regarding age of the patient, tumor size, histologic grade, lymph node status, stage of the tumor was obtained.
- Stromal expression of CD10 was then compared with various prognostic factors like age of the patient, tumor size, histologic grade, lymph node status, stage of the tumor, ER, PR and HER2 status.
- Age group of the patients in the study varied from 30 years to 80 years with mean age of patient being 51 years.
- Stromal CD10 positivity was seen in 40 (80%) cases among the 50 cases studied and 10 (20%) cases showed CD10 negative immune reactivity.
- Stromal expression of CD10 was strongly associated with higher tumor grade, ER negativity and PR negative status.
- There was no statistically correlation between CD10 expression by stromal cells and the age of the patient, lymph node metastases, size of the tumor, stage of the tumor and HER2 neu status.

CONCLUSION

- The mastectomy specimens were studied for IHC expression of CD10 in stromal cells in 50 cases of invasive breast carcinomas.
- The expression of stromal CD10 was compared with various prognostic parameters ie age of the patient, tumor size, histologic grade, lymph node status, stage of the tumor, ER, PR and HER2 status.
- Stromal expression of CD10 was strongly associated with higher tumor grade, ER negativity and PR negative status indicating CD 10 as a poor prognostic factor.
- CD10 should be mentioned in every standard histopathological report because it can be utilised independently as a prognostic marker.
- CD10 could act as a potential target for newer drug development.

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ANNEXURE-I

IEC/NO- 09/2021
Date- 22/01/2021

B.L.D.E. (DEEMED TO BE UNIVERSITY)
(Declared vide notification No. F.9-37/2007-U.3 (A) Dated 29-2-2008 of the MHRD, Government of India under Section 3 of the UGC Act, 1956)
The Constituent College
SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Institutional ethical committee of this college met on 11-01-2021 at 11-00 am to scrutinize the synopsis of Postgraduate students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has been accorded Ethical Clearance

Title: Immunohistochemal expression of CD10 in breast carcinoma and its correlation with clinicopathological parameters

Name of PG student: Dr Shraddha Barate, Department of Pathology

Name of Guide/Co-investigator: Dr V S Patil , Associate Professor of Pathology

DR S.V.PATIL
CHAIRMAN, IEC
Institutional Ethical Committee
D E (Deemed to be University)
Shri B.M. Patil Medical College,
VIJAYAPUR-586103 (Karnataka)

Following documents were placed before Ethical Committee for Scrutinization:

1. Copy of Synopsis / Research project
2. Copy of informed consent form
3. Any other relevant documents.

10

ANNEXURE-II

B.L.D.E (DEEMED TO BE UNIVERSITY) SHRI B.M.PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTER, VIJAYAPURA-586103

INFORMED CONSENT FOR PARTICIPATION IN DISSERTATION/RESEARCH

I, the undersigned,_____, S/O D/O W/O_____, aged_____years, ordinarily resident of_____do hereby state/declare that Dr _____of _____ Hospital has examined me thoroughly on _____ at _____ (place) and it has been explained to me in my own language that I am suffering from_____disease (condition) and this disease/condition mimic following diseases . Further Doctor informed me that he/she is conducting dissertation/research titled_____under the guidance of Dr_____requesting my participation in the study. Apart from routine treatment procedure, the pre-operative, post-operative and follow-up observations will be utilized for the study as reference data.

Doctor has also informed me that during the conduct of this procedure adverse results may be encountered. Among the above complications, most of them are treatable but are not anticipated hence there is chance of aggravation of my condition and in rare circumstances, it may prove fatal despite the anticipated diagnosis and best treatment made available. Further Doctor has informed me that my participation in this study will help in the evaluation of the results of the study which is useful reference to the treatment of other similar cases in near future, and also I may be benefited in getting relieved of suffering or cure of the disease I am suffering.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept secret and not assessed by the person other than me or my legal hirer except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on the information given by me, I can ask any clarification during the course of treatment/study related to diagnosis, the procedure of treatment, result of treatment or prognosis. At the same time, I have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can terminate me from the study at any time from the study but not the procedure of treatment and follow-up unless I request to be discharged.

After understanding the nature of dissertation or research, diagnosis made, mode of treatment, I the undersigned Shri/Smt_____under my full conscious state of mind agree to participate in the said research/dissertation.

Signature of the patient:

Signature of doctor:

Witness: 1.

2.

Date:

Place:

ANNEXURE III

PROFORMA

NAME **:** OP/IP No.**:**

AGE **:**

SEX **:** D.O.A **:**

RELIGION **:** D.O.D **:**

OCCUPATION **:**

RESIDENCE **:**

Presenting Complaints :

Past history :

Personal history :

Family history :

Treatment history :

USG :

Examination :

VITALS: PR: RR:

BP: TEMPERATURE:

WEIGHT:

SPECIMEN: Radical mastectomy/ biopsy

Tumour size

Lymph node status

HPR Finding:

SCARFF BLOOM RICHARDSON GRADE:

IHC:

ER pattern:

PR pattern:

HER2-neu pattern:

CD10 pattern:

KEY TO MASTER CHART

Sr. No.- Serial number

HPR NO- Histopathology number

ER-Estrogen receptor immuno staining

PR- Progesterone receptor immuno staining

HER2 neu-Herceptin receptor immuno staining

CD10- CD10 receptor immuno staining

AGE(yrs)- Age in years

HISTOLOGIC GRADE- Modified Scarff bloom Richardson grade.

TUMOR SIZE- (T)

STAGE- TNM staging of breast carcinoma

Sr no.	HPR no	ER	PR	Her-2-Neu	CD10	Age (yrs)	HISTOLOGIC GRADE	TUMOR SIZE	LYMPHNODE STATUS	STAGE	IMPRESSION
1	920/19	Positive	Positive	Positive	Positive	45	II	T2	Positive	II	INVASIVE CARCINOMA NST
2	1526/19	Negative	Negative	Negative	Positive	62	III	T2	Negative	II	INVASIVE CARCINOMA NST
3	2159/19	Negative	Negative	Negative	Positive	55	II	T3	Positive	III	INVASIVE CARCINOMA NST
4	3797/19	Negative	Negative	Positive	Positive	57	II	T4	Positive	III	INVASIVE CARCINOMA NST

5	**4922/19**	**Positive**	**Negative**	**Negative**	**Positive**	**50**	**II**	**T3**	**Positive**	**III**	**INVASIVE LOBULAR CARCINOMA**
6	**5062/19**	**Positive**	**Positive**	**Negative**	**Negative**	**55**	**I**	**T3**	**Positive**	**III**	**INVASIVE LOBULAR CARCINOMA**
7	**6720/19 male**	**Positive**	**Positive**	**Positive**	**Positive**	**80**	**II**	**T2**	**Negative**	**II**	**INVASIVE CARCINOMA NST**
8	**6834/19**	**Positive**	**Positive**	**Positive**	**Negative**	**45**	**I**	**T2**	**Positive**	**II**	**INVASIVE CARCINOMA NST**
9	**489/20**	**Positive**	**Positive**	**Negative**	**Positive**	**50**	**I**	**T2**	**Positive**	**II**	**INVASIVE CARCINOMA NST**
10	**7009/19**	**Negative**	**Negative**	**Positive**	**Positive**	**50**	**II**	**T2**	**Positive**	**II**	**INVASIVE CARCINOMA NST**

11	**490/20**	**Positive**	**Negative**	**Positive**	**Negative**	**61**	**I**	**T2**	**Negative**	**II**	**INVASIVE CARCINOMA NST**
12	**1900/20**	**Positive**	**Negative**	**Positive**	**Positive**	**32**	**III**	**T2**	**Positive**	**II**	**INVASIVE CARCINOMA NST**
13	**1110/20**	**Negative**	**Negative**	**Negative**	**Positive**	**71**	**II**	**T2**	**Negative**	**II**	**INVASIVE DUCTAL CARCINOMA NST**
14	**1069/20**	**Positive**	**Positive**	**Negative**	**Positive**	**42**	**II**	**T3**	**Positive**	**III**	**INFILTRATING LOBULAR CARCINOMA NST**
15	**660/20**	**Positive**	**Positive**	**Negative**	**Positive**	**80**	**II**	**T2**	**Positive**	**II**	**MUCINOUS CARCINOMA**

16	**872/20**	**Positive**	**Positive**	**Positive**	**Positive**	**50**	**I**	**T2**	**Positive**	**II**	**INVASIVE CARCINOMA NST**
17	**3481/20**	**Positive**	**Positive**	**Positive**	**Negative**	**45**	**I**	**T2**	**Negative**	**II**	**INFILTRATING DUCTAL CARCINOMA**
18	**3649/20**	**Negative**	**Negative**	**Negative**	**Positive**	**37**	**II**	**T2**	**Negative**	**II**	**INFILTRATING DUCTAL CARCINOMA**
19	**4284/20**	**Positive**	**Positive**	**Negative**	**Positive**	**35**	**I**	**T3**	**Positive**	**III**	**INVASIVE DUCTAL CARCINOMA NST**
20	**1407/20**	**Negative**	**Negative**	**Negative**	**Positive**	**65**	**II**	**T2**	**Positive**	**II**	**INVASIVE DUCTAL CARCINOMA NOS**

21	**2472/20**	**Positive**	**Positive**	**Negative**	**Positive**	**71**	**II**	**T2**	**Positive**	**II**	**INVASIVE DUCTAL CARCINOMA NST**
22	**1450/21**	**Negative**	**Negative**	**Positive**	**Positive**	**58**	**II**	**T2**	**Negative**	**II**	**INFILTRATING DUCTAL CARCINOMA NOS**
23	**1460/21**	**Negative**	**Negative**	**Positive**	**Positive**	**70**	**II**	**T4**	**Positive**	**III**	**INFILTRATING DUCTAL CARCINOMA NOS**
24	**3437/20**	**Negative**	**Negative**	**Positive**	**Negative**	**52**	**I**	**T2**	**Negative**	**II**	**INFILTRATING DUCTAL CARCINOMA NOS**
25	**4216/22**	**Negative**	**Negative**	**Negative**	**Positive**	**35**	**I**	**T3**	**Negative**	**II**	**INVASIVE DUCTAL**

											CARCINOMA NST
26	**3518/22**	**Positive**	**Positive**	**Positive**	**Positive**	**70**	**II**	**T2**	**Positive**	**II**	**INFILTRATING DUCTAL CARCINOMA NST**
27	**3458/22**	**Negative**	**Negative**	**Positive**	**Positive**	**52**	**III**	**T2**	**Positive**	**III**	**INFILTRATING DUCTAL CARCINOMA NOS**
28	**1719/22**	**Negative**	**Negative**	**Negative**	**Positive**	**54**	**II**	**T1**	**Negative**	**I**	**INFILTRATING DUCTAL CARCINOMA NOS**
29	**4848/22**	**Positive**	**Positive**	**Positive**	**Negative**	**45**	**I**	**T3**	**Positive**	**III**	**INFILTRATING DUCTAL CARCINOMA NOS**

30	**4340/22**	**Positive**	**Negative**	**Negative**	**Positive**	**56**	**I**	**T2**	**Negative**	**II**	**INVASIVE DUCTAL CARCINOMA NST**
31	**2625/22**	**Positive**	**Positive**	**Positive**	**Positive**	**33**	**I**	**T2**	**Positive**	**III**	**INVASIVE LOBULAR CARCINOMA**
32	**3900/21**	**Negative**	**Positive**	**Positive**	**Positive**	**59**	**II**	**T2**	**Negative**	**II**	**INVASIVE DUCTAL CARCINOMA NST**
33	**1591/22**	**Negative**	**Negative**	**Positive**	**Positive**	**45**	**II**	**T2**	**Positive**	**II**	**INVASIVE DUCTAL CARCINOMA NST**
34	**4560/21**	**Positive**	**Positive**	**Positive**	**Positive**	**35**	**III**	**T1**	**Negative**	**I**	**INVASIVE BREAST CARCINOMA NST**

35	**5375/21**	**Positive**	**Positive**	**Positive**	**Positive**	**45**	**II**	**T2**	**Positive**	**II**	**INVASIVE BREAST CARCINOMA**
36	**4859/22**	**Positive**	**Positive**	**Negative**	**Negative**	**50**	**I**	**T2**	**Positive**	**III**	**INFILTRATING DUCTAL CARCINOMA NOS**
37	**5004/21**	**Positive**	**Positive**	**Negative**	**Positive**	**42**	**III**	**T3**	**Positive**	**III**	**INVASIVE DUCTAL CARCINOMA OF NST**
38	**4067/22**	**Positive**	**Positive**	**Negative**	**Positive**	**30**	**II**	**T2**	**Positive**	**II**	**INFILTRATING DUCTAL CARCINOMA**
39	**4228/22**	**Negative**	**Negative**	**Negative**	**Positive**	**55**	**I**	**T2**	**Positive**	**II**	**INVASIVE DUCTAL CARCINOMA OF NST**

40	**1890/22**	**Positive**	**Positive**	**Negative**	**Positive**	**55**	**II**	**T1**	**Positive**	**II**	**INVASIVE DUCTAL CARCINOMA OF NST**
41	**2811/21**	**Positive**	**Positive**	**Negative**	**Positive**	**50**	**III**	**T1**	**Positive**	**III**	**INFILTRATING DUCTAL CARCINOMA NOS**
42	**2258/22**	**Positive**	**Positive**	**Negative**	**Negative**	**40**	**I**	**T1**	**Positive**	**III**	**INVASIVE BREAST CARCINOMA NST**
43	**184/22**	**Positive**	**Positive**	**Positive**	**Positive**	**44**	**I**	**T2**	**Negative**	**II**	**INFILTRATING DUCTAL CARCINOMA NOS**
44	**4238/21**	**Positive**	**Positive**	**Positive**	**Negative**	**63**	**I**	**T2**	**Negative**	**II**	**INVASIVE DUCTAL**

											CARCINOMA OF NST
45	**2035/21**	**Negative**	**Negative**	**Negative**	**Positive**	**46**	**II**	**T3**	**Positive**	**III**	**NVASIVE BREAST CARCINOMA NST**
46	**5823/21**	**Positive**	**Positive**	**Positive**	**Negative**	**70**	**I**	**T3**	**Negative**	**II**	**INFILTRATING DUCTAL CARCINOMA NOS**
47	**4003/21**	**Positive**	**Negative**	**Negative**	**Positive**	**75**	**I**	**T2**	**Positive**	**II**	**INVASIVE DUCTAL CARCINOMA NST**
48	**4211/21**	**Positive**	**Negative**	**Positive**	**Positive**	**43**	**I**	**T2**	**Positive**	**III**	**INVASIVE DUCTAL CARCINOMA NST**

49	**4452/21**	**Negative**	**Negative**	**Negative**	**Positive**	**35**	**II**	**T2**	**Negative**	**II**	**INFILTRATING DUCTAL CARCINOMA NOS**
50	**5907/21**	**Positive**	**Negative**	**Negative**	**Positive**	**48**	**I**	**T3**	**Positive**	**III**	**INFILTRATING DUCTAL CARCINOMA NOS**

STROMAL CD10 EXPRESSION IN CA BREAST

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