"ASSESSMENT OF EXPRESSION OF IMMUNOHISTOCHEMICAL MARKER CD44 IN CARCINOMA BREAST"

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Dissertation submitted to the

BLDE (DEEMED TO BE UNIVERSITY)

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

DOCTOR OF MEDICINE IN PATHOLOGY

Under the Guidance of

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ABSTRACT

INTRODUCTION:

Breast cancer accounted for approximately 2 million cases and 6 million cancer-related deaths, ranking second in incidence and fourth in mortality among cancer types in most countries worldwide. According to the literature, it was observed that CD44 expression is associated with Basal-like epithelial markers in the tumor cells, advanced T stage and metaplastic variants. It was observed that there was a no correlation between CD44 expression and Oestrogen receptor negativity. Since CD44 is a recently identified marker, further research is necessary to comprehend its immunoexpression and its relationship to hormone receptor status and other prognostic factors that contribute to patient management in therapy. There was no significant association between overall survival, disease-free survival and CD44 expression. Hence, this study was conducted to assess the expression of CD44 in breast tissue.

OBJECTIVES:

1. To assess the expression of the CD44 marker on tumor cells of carcinoma breast.
2. To correlate the expression of the CD44 marker with major prognostic factors like size of tumor, Lymph node metastasis and expression of ER, PR and Her-2-Neu receptors.

MATERIAL AND METHODS:

A hospital-based cross-sectional study was conducted on 60 mastectomy specimens received in the histopathology section of the Department of Pathology. The patient's age, tumor size, histological type, histological grade, and lymph node status were recorded. IHC staining for ER, PR, HER2/neu and CD44 markers was performed and expression of CD44 was correlated with these clinico-pathological and prognostic parameters. The results were subjected to statistical analysis.

RESULTS:

CD44 expression was observed in 51 out of 60 cases (85%). A statistically significant correlation was found between the expression of CD44 with patient's age, tumor size, histological grade and HER 2neu hormonal marker. No statistically significant correlation was found between other parameters like, lymph node status and ER and PR expression.

CONCLUSION:

Expression of CD44 correlated strongly with well-established poor prognostic markers that is Her2/neu status and histological grade thus expression of CD44 suggest aggressive tumor biology and it can be used as an independent prognostic and therapeutic marker.

KEY WORDS: Breast cancer, CD44, Prognosis

LIST OF ABBREVATIONS USED

AJCC: American Joint Commission of Cancer

ASIR: Age Standardized Incidence Rate

BIRADS : Breast Imaging Reporting And Data System

BRCA : Breast Cancer Gene

DAB : Diaminobenzidine

EPS : Encapsulated Papillary Carcinoma

PR: Progesterone Receptor

ER : Estrogen Receptor

HER2/neu : Human Epidermal Growth Factor Receptor 2

IDC NOS : Infiltrating Ductal Carcinoma- Not Otherwise Specified

IHC : Immunohistochemistry

ILC : Infiltrating Lobular Carcinoma

IPC : Invasive Papillary Carcinoma

MBI : Molecular Breast Imaging

CD44: Cluster of Differentiation 44

TNBC : Triple Negative Breast Cancer

TNM : Tumor Node Metastasis

TIL : Tumor Infiltrating lymphocyte

EMT : Epithelial-to-Mesenchymal Transition

BHGI : The Breast Health Global Initiative

CSC : Cancer stem cell

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Breast cancer is one of the most commonly diagnosed cancers and the prime cause of cancer-related deaths among women[1]. Every year, over a million new cases of breast cancer are identified, making up more than 23% of all cancers that affect women worldwide[1].

Less developed region of Southern and Eastern Asia, as well as sub-Saharan African, have lesser rates of breast cancer[2]. Many sectors have taken notice of breast cancer because it is a major threat to women's lives and health[2]. Breast cancer ranks second in incidence and fourth in mortality in most countries worldwide, accounting for about 2 million cancer cases and 6 million cancer deaths, according to GLOBOCAN 2022 estimates[3]. According to the World Health Organization (WHO) 2022, there are significant differences in breast cancer rates based on human development worldwide. In nations that have an extremely high Human Development Index (HDI), for example, 1 in 12 women will receive a breast cancer diagnosis in their lifetime, and 1 in 71 will succumb to the disease[3].

The incidence of breast carcinoma in India has increased more than half in last three decades. The number of cases of breast carcinoma in India in 2016 was 1,18,000, and the prevalent cases were 5,26,000[4].

The etiology and pathophysiology of breast cancer interact, making it a complex illness influenced by a number of genetic, hormonal, and environmental factors[5]. Etiological factors include a positive family history, weight gain, cigarette smoking, consumption of alcohol, early menarche, delayed menopause, a sedentary lifestyle, nulliparity, and hormone replacement therapy[6]. Numerous variables, such as tumor size, histologic type, histologic grade, age at diagnosis, pTNM staging, lymph node metastasis, chemotherapy response and molecular profile (p53 gene), HER2/neu, and others, affect the prognosis of breast cancer[1,7]. The classical

and standard immunohistochemical markers are estrogen receptor (ER), progesterone receptor(PR)[7]. Breast cancer is divided into four main groups based on gene expression profiling studies: HER2 type, basal-like/triple negative, luminal A and luminal B. These subgroups differ greatly in terms of prognosis and treatment goals[8]. Clinical oncology uses prognostic indicators to predict the likelihood of tumor recurrence and metastasis and to aid choose tailored therapy[7]. Patients with breast cancer have a bad prognosis due to the high rates of local recurrence and metastasis, which renders treatment ineffective. This failure may be linked to the biological traits and attributes of the cells that become tumors[9]. The complexity is increased by the intervening tumor microenvironment, which associate with cancer cells to alter various aspects of tumor formation, including tumor progression, invasiveness, and metastatic dissemination[9].

Currently, the Breast Health Global Initiative (BHGI) is developing appropriate policies and procedures to offer the highest level of breast cancer prevention in the world[10]. Due to differences in DNA genetic makeup, the molecular subtyping of breast cancer leads to distinct biomarker expression, which greatly facilitates precise patient stratification for selecting the most suitable treatment in personalized breast cancer care. This knowledge can be used to investigate novel biomarkers that may aid in focusing breast cancer treatment.

Cancer stem cells (CSCs) are presently considered as the precursors for any malignancy. There are several CSCs identified in solid epithelial malignancies, such as breast cancer. CD24, CD44, and aldehyde dehydrogenase are a few examples. CSCs are defined as CD44+ CD24- positive cells in breast cancer[11].

CD44 is a transmembrane glycoprotein that crosses the cell membrane and binds to hyaluronic acid resulting in the activation of various cascades. Its expression depends on various subtypes of malignancy. Its activation leads to cellular proliferation, adhesion and migration. It will

maintain the stemness of tumor cells as good promotion of tumorigenesis[12]. Additionally, CD44 engages in growth factor signalling by sequestering growth factors. It's unknown how CD44 relates to tumor-infiltrating lymphocytes (TIL), EMT and metastasis[12].

There are 20 exons in the CD44 gene. Its N-terminal and C-terminal domains are encoded by constant exons 1–5 and 16–20, respectively. Every member of the CD44 family shares this pattern. The most prevalent type CD44, has ten constant exons[13].

CD44 in tumor cells of breast cancer when compared it with Luminal A, B, Her-2neu+ and Basal-like classification, it was observed that the Basal-like variant showed more expression of CD44 when compared to almost Nil expression of CD 44 in Luminal A as well Luminal B. Hence, CD44 expression suggests of poor prognosis[14].

Studies shows that CD44 expression is more in stage 3 when compared to stage 1breast carcinoma. Among triple-negative breast carcinoma, the expression of CD44 is seen in 94% of cases. The CD44 expression is not significantly associated with the grade of the tumor, estrogen receptor positive status, proliferation index, tumor size and patient's age. It was observed that there was a no correlation between CD44 expression and estrogen receptor negativity[15].

It was also observed that CD44 expression is associated with Basal-like epithelial markers in the tumor cells, advanced T stage and metaplastic variants[16].

High CD44 expression is seen in a higher stage of the disease and no significant association was seen between overall survival, disease-free survival and CD44 expression[17].

Increased expression of CD44 is seen in carcinoma breast with metastasis to lymph nodes and triple-negative breast malignancy. It has not affected the overall survival as well as disease-free survival of patients[18].

AIMS AND OBJECTIVES OF THE STUDY

1. To assess the expression of the CD44 marker on tumor cells in breast carcinoma.
2. To correlate the expression of the CD44 marker with major prognostic factors like Tumor size, Lymph node metastasis and expression of estrogen, progesterone and Her-2-Neu receptors.

REVIEW OF LITERATURE

EMBRYOLOGICAL DEVELOPMENT OF BREAST

The mesenchyme and ectoderm are attributed with the formation of the breast in both men and women. While the ectoderm develops the vessels and alveoli, the mesenchyme gives rise to the connective tissue and its associated blood vessels[19]. The thickening of the epidermis that first develops on the anterior surface of a five week-old embryo and eventually develops into the breast is called a mammary ridge, or milk line. Beginning in the tenth week of pregnancy, the center (pectoral) part of the milk lines which give rise to the breast will atrophy[20]. Mesenchymal condensation occurs around the breast bud, an epithelial stalk at the spot of mammary development on the chest wall, during the fifteenth week of pregnancy[20]. The ectodermal thickening of the breast primordium penetrates the dermis. Furthermore, during the fifth month of pregnancy, 16 to 24 firm cords of ectodermal cells develop within the underlying mesoderm (dermis)[21]. The alveoli and lactiferous ducts will eventually be produced by the canalization of these buds. The epidermal surface of the developing nipple begins as a superficial indentation during the last 3 months of pregnancy and then protrudes after delivery. When ducts reach puberty, they develop acini at the ends[21].

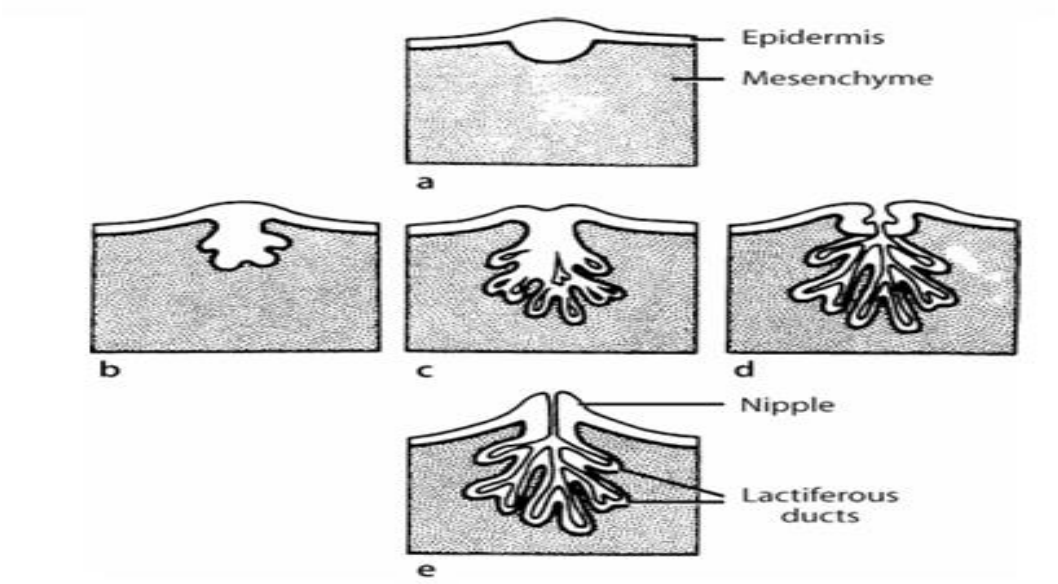

Figure1 :Development of the breast. a–d Phases in the development of the duct system and glandular tissue from the epidermis. Connective tissue septa are derived from mesenchyme of the dermis. e Eversion of the nipple near birth. (From Skan dalakis et al)

ANATOMY AND HISTOLOGY OF ADULT BREAST

A well-organized collection of distinct glands of various sizes makes up the mammary gland. Under the headings of differentiation and development, they undergo a number of changes[22]. The breast's peripheral anatomic borders are only loosely defined at the deep surface where the gland lies over the pectoralis fascia. Its weight, form and size might all be more uniform[7]. From the 2nd to the 6th rib at the midclavicular line, it has a distinctive conical protuberance that extends from the side boundary of the sternum to the anterior axillary line, frequently reaching to the axilla. A skin-to-chest wall attachment is made possible by suspensory (cooper) ligaments[23]. It is a complicated tubulo-alveolar gland with 15 to 25 unusual lobes that radiates from the nipple. The stratified squamous epithelium is joined to each lobe by a lactiferous duct, which has a diameter of 2 to 4 mm. The duct opens at the nipple and has an uneven angular

definition. Each duct under the areola eventually manifests as a 0.4-5 to 0.7-mm take-off at the end of the nipple. The ducts are continually attached to the milk-producing terminal duct lobular units (TDLU). The lobules of different orders make up each lobe; the smallest and longest tubules are the alveolar ducts, and the alveoli are covered by microscopic saccular evaginations. Despite its density, the interlobular connective tissue is almost exclusively composed of tiny collagenous fibres, enlarged cells and almost no fat[19,20,21,22,23].(Fig 2)

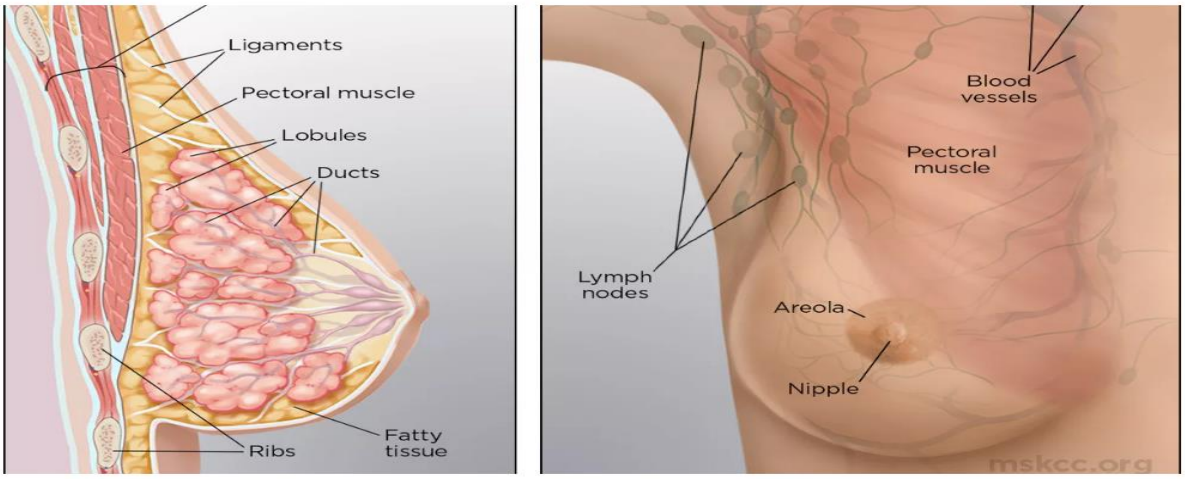

Figure 2: Anatomy of breast

Histologically, breast parenchyma is comprised of ducts, lobules and stroma. The epithelium that lines the ducts has two layers: the outer layer consists of cuboidal cells in the inner ducts and flat, contractile myoepithelial cells on the outside. The stroma is situated in between the ducts and is made up of fibroblasts, collagen fibres, arterioles, venules, and adipose tissues[23].

BREAST CARCINOMA

Epidemiology:

Breast cancer accounts for 19.6 million of the 107.8 million Disability-Adjusted Life Years (DALYs) that the World Health Organisation (WHO) evaluation are caused by malignant neoplasms in women globally[24]. The Human Development Index and age-standardized incidence rates of breast cancer are firmly and favourably correlated, according to the 2018

GLOBOCAN figures[25,26]. The ASIR was highest in countries with extremely high HDI (75.6 per 100,000), whereas it was more than 200% lesser in medium and low HDI countries (27.8 per 100,000 and 36.1 per 100,000, respectively) based on data from 2020[25,26]. Breast cancer claims 684,996 lives globally, with a 13.6/100,000 age-adjusted death rate. In-spite of having the highest prevalence rates in wealthy countries, Asia and Africa, they were responsible for 63% of all fatalities worldwide in 2020[24]. The prevalence and mortality rates of breast cancer have risen over the past three decades[25]. Current projections indicate that by 2030, there will be 0.87 million fatalities worldwide and 2.7 million new cases annually[25]. It is believed that the elements for the above cause to rise are changes in social practices that raise the risk of breast cancer[27].

Risk factors: A considerable variety of factors, both modifiable and non-modifiable, increases the likelihood of developing breast cancer[10]. (Table 1)

Table 1- MODIFIABLE AND NON MODIFIBALE RISK FACTOR

Non-Modifiable Factors	Modifiable Factors
Female sex	Exogenous hormones
Older age	Diethylstilbestrol
Family history (of breast or ovarian cancer)	Physical activity
Genetic mutations	Overweight/obesity
Race/ethnicity	Alcohol intake and Smoking
Pregnancy and breastfeeding	Insufficient vitamin supplementation
Menstrual period and menopause	Excessive exposure to artificial light
Density of breast tissue	Intake of processed food
Previous history of breast cancer	Exposure to chemicals
Non-cancerous breast diseases	Other drugs
Previous radiation therapy	

Important risk factors are:

(i) Female sex- Women are more susceptible to breast cancer because of higher hormone stimulation. Women's breast cells are sensitive to hormones, particularly progesterone and estrogen, and any abnormalities in their concentrations. Less than 1% of incidences of breast cancer impact men[28].

(ii) Older age- Although over 40% of people with breast cancer are older than 65, almost 80% of patients with the disease are over 50.[29] The aggressive, resistant subtype of triple-negative breast cancer is most frequently diagnosed in individuals under 40 years of age, whereas in patients over 70, the luminal A subtype is more prevalent[29].

(iii) Family history- If a first-degree relative had BC at an early age, was bilaterally impacted, or both, their risk of getting the disease is two to three times higher than the general population[10].

(iv) Genetic Mutations- BRCA1 (found on chromosome 17) and BRCA2 (found on chromosome13) are two essential genes with a high penetrance. Other highly extensive breast cancer genes involved are TP53, CDH1, PTEN, and STK11[30].

(v) Reproductive history and menstrual history- An increased risk is associated with nulliparity, late menarche, late menopause, and late age of first birth.[11,30] In parous women, breastfeeding for at least four months has been related with a decreased risk of breast cancer[30].

(vi) Previous Radiation Therapy- There is a direct correlation between age and radiation therapy-induced cancer; individuals who get radiation-therapy, age before thirty years are at a higher chances of developing breast cancer[31].

(vii) Exogenous hormones- Exogenous hormones have a complex impact on the risk of breast cancer that changes based on the period of treatment and the mix of medications. In conclusion, the risk seems to be higher for combination estrogen and progestin use, longer duration of use and current use than for estrogen use alone[20,27].

Pathogenesis of breast cancer-

Breast cancer risk factors can be categorized into three groups: hereditary, hormonal and environmental factors.

GENETIC FACTORS- According to the normal tumor suppressor characteristics of BRCA1 and BRCA2, cancer can only arise when both alleles are dormant or dysfunctional. For the repair of specific types of DNA damage, BRCA1 and BRCA2 encode proteins. There are two types of hereditary alterations that give rise to the development of breast cancer: familial and sporadic. Most BRCA1 mutations are associated with triple-negative tumors, while most BRCA2 mutations are associated with ER-positive cancers. The BRCA1-encoded protein performs a variety of functions, including ubiquitylation, chromatin remodelling, DNA decatenation, cell cycle checkpoint regulation, and the homologous recombination mechanism of DNA damage repair. BRCA2 encodes a protein that functions in DNA repair, cytokinesis, and meiosis. In other words, both BRCA1 and BRCA2 are necessary for precise homologous recombination-mediated repair of DNA double-strand breaks[32].

Other genes with mutations linked to familial breast cancer are PTEN and TP53. Mutations that upregulate "PI3KAKT" signalling are common in sporadic ER-positive and HER2-positive breast cancers, while somatic TP53 mutations are very frequently observed in triple-negative and HER2-positive breast tumors [33].

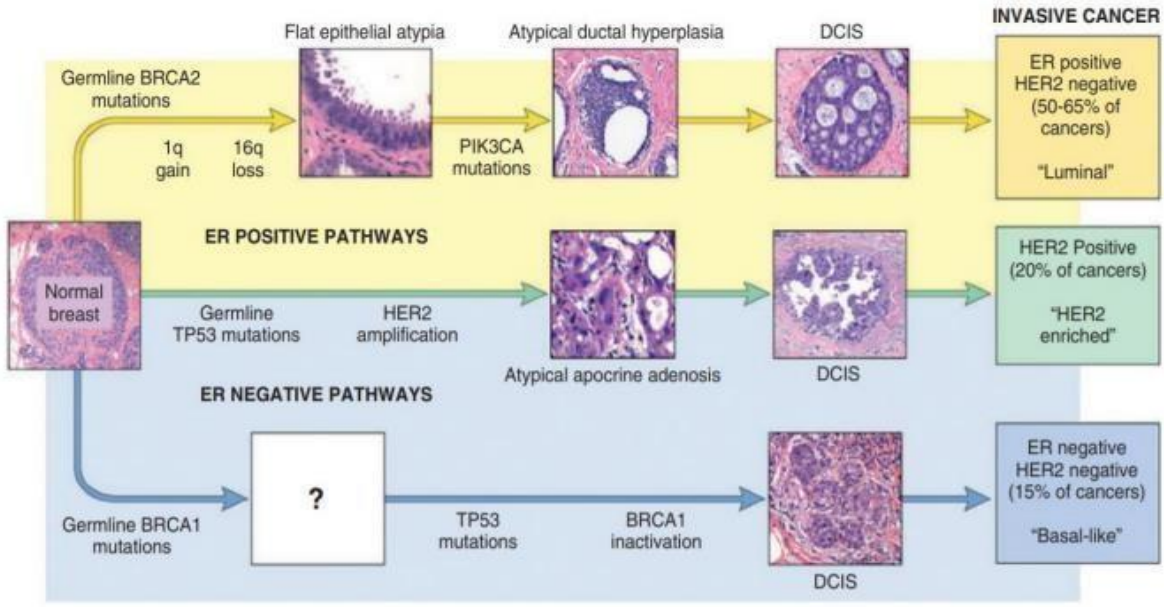

Figure 3: Principal pathways involved in the development of breast cancer [27]

HORMONAL INFLUENCES- Growth factors, including platelet-derived growth factor (PDGF), transforming growth factor (TGF), and fibroblast growth factor (FGF), are produced in response to estrogen. These factors have the ability to stimulate tumor growth via autocrine and paracrine pathways.

Estrogen receptors also control a large number of other genes, some of which are essential for tumor growth or development and these genes are probably involved in development and growth[33].

ENVIRONMENTAL FACTORS- Variable breast cancer incidence rates in genetically homogeneous groups point to environmental effects[33].

Molecular classification

Different clinical outcomes are associated with Luminal A and B subtypes of Luminal-like cancers, distinguished by proliferation-related and luminal-regulated pathways.

(i) Luminal A- (i) The presence of progesterone receptors (PR) and estrogen receptors (ER) and the absence of HER2 are feature of luminal A. Genes that are characteristic of the luminal epithelium lining the mammary ducts are triggered by the ER transcription factors in this subtype. Additionally, it manifests as decreased expression of genes linked to cell growth. Clinically, they have the best prognosis and are low-grade and slow-growing[10,34].

(ii) Luminal B- They are higher grade and have poor prognosis. PR-negative, HER2-positive, ER-positive, or both may be present. Furthermore, it has elevated expression of genes linked to proliferation[10,27,34].

(iii) HER2-enriched breast cancer is attributed by the overexpression of HER2, along with the absence of ER and PR. This subtype primarily expresses proteins and genes associated with cell proliferation (e.g., ERBB2/HER2 and GRB7)[10,27,34].

(iv) Basal-like/Triple-Negative Breast Cancer- A diverse group of breast tumors with the characteristics of being ER-negative, PR-negative, and HER2-negative is known as Triple-Negative Breast Cancer (TNBC). They make up around 20% of all cases of breast cancer. TNBC is more prevalent in women below the age of 40, particularly in African-American women. Although BRCA1 or BRCA2 germline alteration are found in around 80% of breast tumors resulting from BRCA1 germline mutations, TNBC makes up 11–16% of all TNBCs. TNBC is linked to a poor prognosis and has a tendency to be biologically aggressive[2,35].

(v) Claudin-Low Breast Cancer- They are tumors with a poor prognosis, primarily characterized as estrogen receptor (ER) negative, progesterone receptor (PR) negative, and human epidermal growth factor receptor 2 (HER2) negative. CL tumors account for 7–14% of invasive breast cancer cases. The CL sub-type is defined by low expression of genes associated with cell-cell adhesion, that include occludin, E-cadherin, and claudins 3, 4, and 7[36].

CLINICAL FEATURES - The most common clinical sign of IBC in the unscreened population is palpable mass, even though other symptoms like skin retraction, nipple inversion, nipple discharge, and less frequently a change in the breast's size or form or a change in skin's color or texture linked to ulceration may be observed[37].

Diagnosis of breast cancer

1. **Mammography**- X-ray images of the breast are used in mammogram. Digital mammography has been used instead of traditional mammography in several breast screening programs. However, any radiation risk must be carefully considered when using mammography repeatedly. Additionally, false-positive calls result in extra imaging or histological evaluation, primarily percutaneous breast biopsy[38]. Following the examination of the mammograms, radiologists use the BIRADS diagnostic method to classify their results into a conclusive evaluation category. The Breast Imaging Reporting and Data System (BIRADS) was created by the American College of Radiology to identify, assess, and categorize in order to standardize mammography reporting. Continuation suggestions are given in light of the final assessment category. BIRADS Category 4-6 implies that the mammograms show cancer, BIRADS-4 shows a suspicious finding for malignancy, BIRADS-5 shows a very suggestive finding for

malignancy, and BIRADS-6 shows a diagnosis of malignancy that has been confirmed by biopsy.

2. **Magnetic resonance imaging** (MRI)- An increasingly potent imaging technique, MRI produces high-resolution images without the use of hazardous radiation [39,40]. The way that intravenous contrast injection amplifies lesions affects the results of breast MRIs. Because of neovascularization, the tumor tissue is very permeable, allowing the contrast agent to diffuse there[33]. Due to their paramagnetic characteristics, a number of iron (Fe), gadolinium (Gd), and manganese (Mn) paramagnetic metal ion complexes have been employed as MRI contrast agents The use of contrast agents is associated with recognized negative effects and limitations. Research had shown that the trans-metalation of gadolinium can lead to adverse outcomes[42,41].

Novel-carrier systems and sophisticated targeting techniques have been offered in recent research publications and licensed to increase the effectiveness and reduce the harmful effects of MRI contrast agents[38] .

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 monitor. This process is also known as the Miraluma test, sestamibi test, scinti-mammography, or specialized gamma-imaging. The main ingredient in MBI is Tc-99m sestamibi, which is authorized for imaging breast cancer. MBI has a higher specificity and comparable sensitivity to MRI in detecting small breast lesions[43].

4. **Breast biopsy**- Two distinct needle biopsies are used to identify breast tumor: fine-needle aspiration cytology (FNAC) and core-needle biopsy (CNB). A little cylindrical piece of tissue called a core is removed during a Core Needle Biopsy, as opposed to FNAC. Usually, 3-5 cores are removed, though more can also be taken. A pathologist's responsibility is to analyse core tissue samples and look for malignancy[43].

WHO CLASSIFICATION OF BREAST TUMORS- 5th edition[44]

Ductal carcinoma in situ (DCIS):

- Intraductal carcinoma, non-infiltrating, NOS
- DCIS of low nuclear grade
- DCIS of intermediate nuclear garde
- DCIS of high nuclear grade

Invasive breast carcinoma:

- Infiltrating duct carcinoma NOS
- Oncocytic carcinoma
- Lipid- rich carcinoma
- Glycogen- rich carcinoma
- Sebaceous carcinoma
- Lobular carcinoma NOS
- Tubular carcinoma
- Cribriform carcinoma NOS
- Mucinous adenocarcinoma
- Mucinous cystadenocarcinoma NOS
- Invasive micropapillary carcinoma of the breast
- Apocrine adenocarcinoma
- Metaplastic carcinoma NOS

NONINVASIVE (IN SITU) CARCINOMA

Lobular carcinoma in-situ (LCIS) and ductal carcinoma in-situ (DCIS) are the two morphologic variants of non-invasive breast cancers. Both are restricted to the basement membrane and do not infiltrate the surrounding stroma[33]. Ductal Carcinoma Insitu (DCIS) can present with a variety of histologic features, but the "comedo" kind, often mentioned as comedo-carcinoma,

is considered to be more hostile than other types of DCIS. The most prevalent subtypes of "non comedo" types of DCIS are

1. Solid DCIS: Cancer cells fully occupy the breast ducts that are affected.

2. Cribriform DCIS: Cancer cells are partially present in the damaged breast ducts, although there are spaces between the cells.

3. Papillary and Micro-papillary DCIS: The cancer cells form a frond-like arrangement inside the impacted breast canal, and micro-papillary DCIS cells are smaller than papillary DCIS cells.[45]

Lobular Carcinoma Insitu (LCIS)- Usually has a consistent uniform pattern. The monomorphic cells have bland, spherical nuclei and are arranged in weakly cohesive clusters. Unlike DCIS, these lesions do not show calcifications, hence the discovery is usually accidental finding. It has been shown that one-third of women with LCIS acquire invasive breast cancer.

Unlike DCIS, invasive carcinomas that develop following an LCIS diagnosis can occur in either breast, with two-thirds developing in the ipsilateral breast and one-third in the opposite side of breast. "LCIS" is a direct precursor to a number of cancers and an indication of an increased risk of bilateral breast cancer [33].

INVASIVE (INFILTRATING) BREAST CARCINOMA

MACROSCOPY

Invasive breast cancer typically manifests as a big, easily noticeable lump with a nodular or uneven, stellate border. Palpation reveals a firm to hard tumor with typically poorly defined borders. A few tumors could feel gritty when the tissue is sliced with a knife[44]. Usually, the tumor appears stellate or stab-like. If the tumor is significantly large, areas of bleeding, tissue

death, and cystic degeneration may be apparent.[47] For proper tissue sample, the size, location, focality, and other details of the lesion should always be verified by comparing the gross findings with the radiography results[44].

MICROSCOPY

The typical development patterns observed in microscopy include sheets, nests, cords, or scattered individual cells, which may sometimes be absent, hardly noticeable or exhibit a well-developed glandular/tubular differentiation. Although the size and shape of the tumor cells might vary, they are by definition larger and more pleomorphic than the typical form of invasive lobular carcinomas. They also have more mitotic figures and more evident nuclei and nucleoli. Some cases may result in necrosis and calcification which is frequently linked to insitu components—has been documented in a small number of cases. It can manifest as a coarse or fine deposit. Areas of squamous metaplasia, apocrine metaplasia, or clear cell alterations may be observed. Both sparse and abundant stroma may be strongly fibrotic or cellular (sometimes called desmoplastic). It could be challenging to distinguish the tumour cells in situations with a lot of stroma. Elastosis may occur in some areas, primarily affecting the walls of veins and ducts. The interphase between the surrounding stroma and tumor tissue is typically where chronic mononuclear inflammatory cell infiltration is observed[33,44,47].

PREDICTIVE AND PROGNOSTIC FACTORS FOR INVASIVE BREAST CARCINOMA: [33]

1. Distant metastasis (M)- The most significant prognostic predictor is metastasis outside of the local lymph node.

2. Regional lymph nodes (N)- Nodal metastasis, which includes the number of nodes involved, is the second most important predictor of outcome.

3. Tumour (T)- Tumor size, skin involvement (e.g., ulceration or dermal metastases), invasion of the chest wall and presentation as inflammatory carcinoma are important prognostic factors.

4. Histologic grade- With increasing histologic grade, survival decreases. Three characteristics- tubule development, nuclear pleomorphism, and mitotic count are employed in the modified Scarff Bloom-Richardson grading system to histologically assess the tumor. The prognosis is better for carcinomas that are well differentiated and worse for those that are poorly differentiated.

Histological grading of breast carcinoma [44]

Table 2- MODIFIED SCARFF-BLOOM RICHARDSON HISTOLOGICAL GRADING

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	0-8 mitosis/10HPF
Score 2	9-16 mitosis/10HPF
Score 3	>17 mitosis/10HPF
TOTAL SCORE	
Score 3-5	Well differentiated (Grade I)
Score 6-7	Moderately differentiated (Grade II)
Score 8-9	Poorly differentiated (Grade III)

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

Additional prognostic factors consist of:[33]

- Lympho-vascular invasion: Tumor cells in the vascular spaces around carcinomas are poor prognostic markers.
- Unique histologic kinds: Adenoid cystic carcinoma and tubular carcinoma are two cancer histologic categories that are strongly associated with remarkably high survival rates.
- Gene expression profiling: These tests are mostly used in clinical settings to identify patients with cancers that respond to estrogen and do not require chemotherapy.
- The clinical staging system for breast cancer has been endorsed by the American Joint Commission on Cancer (AJCC) and the International Union for Cancer Control (UICC).

Staging of breast carcinoma[44]

The most widely used breast cancer staging approach, clinical staging, is recognized by the American Joint Commission on Cancer 8th edition (AJCC) and the International Union for Cancer Control (UICC). It is based on the TNM (Tumor, Nodes, and Metastases) system.

Table 3- STAGING OF BREAST CARCINOMA

(T):	**Primary tumour**
TX:	**Primary tumour cannot be assessed**
T0	**No e evidence of primary tumour**
Tis	**Carcinoma in situ; DCIS/LCIS/Paget's**
T1:	**Tumour size (2 cm or less)**
T1 mi	<= 0.1 cm 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 0.5 cm but less than 1 cm in its greatest dimensions.
T1c:	More than 1 cm but less than 2 cm in its greatest dimensions
T2:	**Tumour size > 2 cm but not more than 5 cm in its greatest dimension**
T3:	**Tumour size more than 5 cm in its greatest dimension**
T4:	**Tumour of any size with direct extension to chest wall and or to the skin (ulceration or skin nodule)**
T4a:	Extension to chest wall, (does not include pectoralis muscle invasion only)
T4b:	Ulceration and/or ipsilateral satellite skin nodule and/or oedema
T4c:	Both of the above (T4a and T4b)
T4cd:	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 internally 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 metastasis
M0	No distance metastasis
M1	Distance detectable metastasis as histologically proven larger than 0.2 mm

Table 4 – AMERICAN JOINT COMMISSION ON CANCER (AJCC) STAGING, 8th EDITION

STAGE	TNM classification	Description	10 Year survival
0	Tis N0 M0	Carcinoma in situ (DCIS or LCIS)	98-100%
I	T1 N0 M0	Tumor </= 2cm, no lymph node involvement, no metastasis	85-95%
IIA	T0/T1 N1 M0 or T2 N0 M0	Tumor </=2cm with 1-3 axillary nodes OR 2-5cm with no nodes	75-85%
IIB	T2 N1M0 or T3 N0 M0	Tumor 2-5 cm with 1-3 axillary nodes OR > 5 cm with no nodes	65-75%
IIIA	T0/T1/T2 N2 M0 or T3 N1/N2 M0	Tumor</=5 cm with 4-9 axillary nodes OR >5cm with 1-9 nodes	50-60%
IIIB	T4 N0-N2 M0	Tumor of any size with chest wall or skin involvement	40-50%
IIIC	Any T N3 M0	Tumor of any size with >/= 10 axillary nodes or internal mammary/ supraclavicular nodes	35-45%
IV	Any T Any N M1	Distant metastasis (bones, lungs, liver, etc)	10-20%

Role of immunohistochemistry in breast carcinoma

Immunostaining is most frequently used in immunohistochemistry. Using antibodies, this scientific method searches for certain antigens (markers) in a tissue sample. The antibodies are often attached to a fluorescent dye or an enzyme. The antigen in the tissue sample can be observed under a microscope once the antibodies have bound to it and an enzyme or dye has been activated. In all tissues, intracellular proteins or different cell surfaces are described using immunohistochemistry (IHC). The prognosis and recommended systemic therapy for patients with breast cancer are determined by specific biological characteristics of the tumor. Immunohistochemical markers are frequently used as prognostic and predictive factors linked to angiogenesis and apoptosis, as well as for molecular classification of breast cancer to help

with patient care[47]. Normal glandular breast tissue is composed of luminal, basal, and myoepithelial cell types, each of which expresses a distinct array of proteins. The cytokeratins (CK), oestrogen receptor (ER), progesterone receptor, epithelial membrane antigen (EMA) and -lactalbumin (PR) are all expressed by the luminal cells.

Specialized markers such as smooth muscle actin, calponin, S100, and p63 are also expressed by myoepithelial cells, whereas different cytokeratins are displayed by basal cell types. p53, Ki-67, human epidermal-growth factor receptor-2, oestrogen receptor, progesterone receptor, and human epidermal growth factor receptor are the most often used therapeutic and prognostic immunohistochemical markers in cases with Ca breast. Among the lesion classifications that frequently need to be differentiated are in situ carcinoma vs invasive malignancy, nonneoplastic proliferative lesions against malignant lesions, and pseudo invasive lesions (adenosis, radial scar, sclerosing lesions, etc.) against invasive malignancy. There are also papillary lesions, atypical ductal epithelial hyperplasia (ADH), and micro-invasive carcinomas (invasive foci smaller than or equal to one mm). These lesions often impart themselves to explanation by IHC marker[48]. Stromal Invasion Assessment and Myoepithelial Cells, the most frequent lesions that surgical pathologists encounter are breast epithelial lesions which also raise the most concerns when assessing whether a disease is benign or malignant[49].

ESTROGEN RECEPTORS

• ER belongs to the intracellular receptor family of nuclear hormones. Activation of oestrogen receptors causes them to attach to DNA and regulate the expression of many genes.

• Located on Chromosome (6q25.1 and 14q23.2). The endometrium, breast cancer cells, ovarian stromal cells, and the hypo-thalamus all have the Erα receptors.

• The ERβ receptors are expressed by the kidney, brain, bone, heart, lungs, intestinal mucosa, prostate, and endothelial cells [48,49].

PROGESTERONE RECEPTORS

• Progesterone receptor (PR) Nuclear receptor sub-family 3 (NR3C3), group C, member 3 is another name for the protein found in cells. It increases due to the steroid hormone progesterone.

The protein is encoded by the human PGR gene, located on chromosome 11q22. Two of its isoforms, PRPR-A and PR-B, differ in their molecular weights. The PR-B positively regulates the effects of progesterone, while the PR-A negatively regulates the PR-B effect[48,49.]

HER2/Neu RECEPTOR

• The transmembrane glycoprotein p185, which is related to the family of epidermal-growth factor receptors and contains tyrosine kinase activity, is encoded by the oncogene HER2/neu (c-erbB-2). Its over production can be assessed by immunohistochemistry or FISH (or its chromogenic counterpart), and there is a strong association between the two techniques[48,49].

CD44 Immunohistochemical Marker:[50]

The CD44 family of single span transmembrane glycoproteins is non-kinase and is expressed at different amounts on connective tissues, bone marrow and embryonic stem cells. A biological marker for cancer stem cells (CSC), CD44 expression is also elevated in subpopulations of cancer cells. Ten of the 19 exons that encode human CD44 are identical across all isoforms.

CD44s and various CD44v iso-forms have both overlaying and definite functional roles. Growth factors on the cell surface can be bound or sequestered by CD44v isoforms, which then present them to their particular receptors to function as co-receptors.

STRUCTURE OF CD44:

Exon-structural and functional domains of CD44

CD44 a trans-membrane glycosaminoglycan-protein complex, is a monomeric chain glycoprotein encoded by a single gene found on a specific chromosome locus 11p131. The 19 exons that make up CD44 are widely expressed throughout the body. The smallest iso-form of CD44 (85–95 kDa), known as the CD44 standard (CD44s), is encoded by the first five and last five exons, which are constant. The 10 exons that make up the CD44 standard isoform can be combined with the middle nine exons, which are known as CD44 variant isoforms (CD44v). The extracellular domain, transmembrane domain, and intracellular/cytoplasmic domain are the three major domains of CD44. While the transmembrane domain offers a channel for communication with co-factors and adaptor proteins, the extracellular domain engages with the external surroundings. With roles in transcription mediation and nuclear localization, the CD44 intracellular domain (CD44-ICD) is a short-tail and long-tail protein.

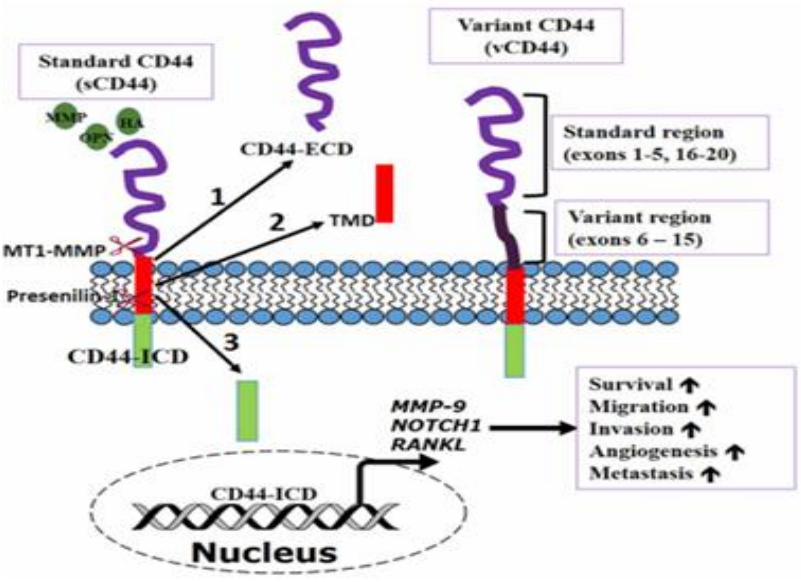

Figure 4: CD44, a multifunctional receptor, plays a crucial role in regulating biological processes related to cancer cell spreading and metastasis. CD44 can be successively cleaved by membrane type 1 matrix metalloproteinase (MT1-MMP) and subsequently by presenilin-1/γ-secretase, which is activated by the

binding of ligands osteopontin (OPN), hyaluronic acid (HA). This splitting process produces (1) extracellular domain (ECD) fragment. (2) CD44β like peptide or trans-membrane domain (TMD), and (3) CD44 intracellular domain (ICD) fragment. CD44ICD enters the nucleus to initiate the transcription of genes that play a crucial role in metastasis and cell survival.

CD44 ligands and interacting molecules

CD44 binds to a number of ligands, such as collagen, fibronectin, chondroitin, osteopontin (OPN) [23], hyaluronic acid (HA) and serglycin/sulphated proteoglycan. For CD44 activation, HA is the most selective ligand. All similar forms of CD44 have the HA-binding domain present at the N-terminal portion of the extracellular domain (Fig. 1). Both stromal and tumor cells express HA, an essential extracellular matrix component.

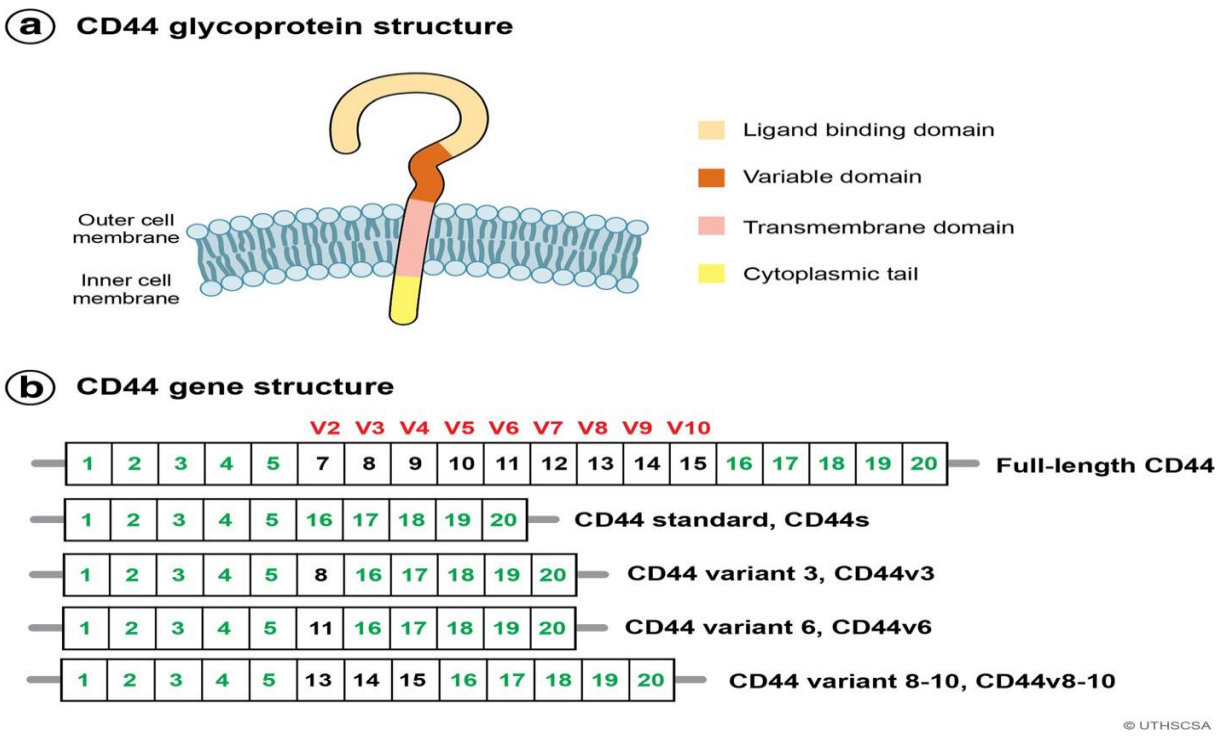

Figure 5 : CD44 protein and gene formation. **a** The 4 areas of the CD44 glycoprotein—the cytoplasmic tail, transmembrane domain, variable domain, and ligand binding domain—are shown with corresponding colors. **b** Humans have 19 exons that encode CD44, whereas mice have 20 exons. Humans lack exon 6, which codes for CD44 variation 1 (CD44v1). Green color exons are always

expressed as a standard form of CD44 (CD44s), and up to nine exon variants can be inserted by alternative splicing. Whole extend CD44, CD44s, CD44v-3, CD44v-6, and CD44v8-10 are shown stepswise.

Cell adhesion, migration and proliferation are all improved by cell signaling that results from conformational changes brought about by HA binding to CD44 that promote adaptor molecule binding to the intracellular cytoplasmic tail of CD44. HA stimulation of CD44 in breast tumor cells results in the production of the anti-apoptotic gene Bcl and the multidrug resistance gene P-glycoprotein, which encourages the growth and survival of tumor cells.

Regulation of CD44

The expression of CD44 is controlled due to its role in cancer cell activity. According to new research, certain signalling networks can trigger the production of CD44. Certain transcriptional activators and repressors have been found to control the activity of the CD44 promoter. Furthermore, the regulation of CD44 expression is linked to miRNAs and epigenetic processes. The mechanisms that govern the expression of CD44 may yield molecular targets that might be employed to modify CD44 expression in order to mitigate the oncogenic role of CD44. Several representative regulatory mechanisms are shown below-

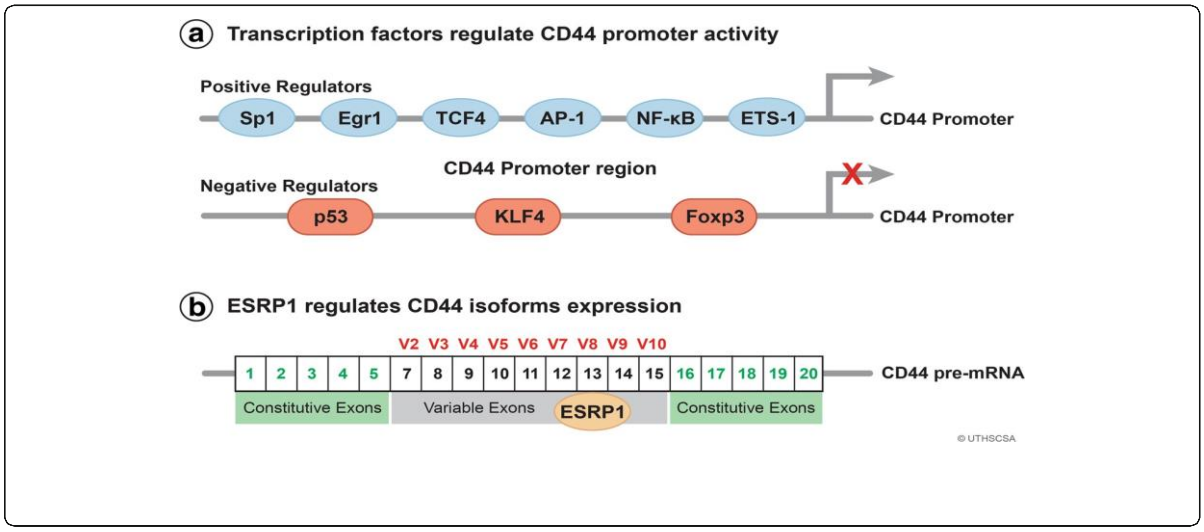

Figure 6: Molecules regulate promoter activity and expression. **a** The CD44 promoter region is bound by a number of transcription factors, including positive and negative regulators, which control its activity. **b** Alternative splicing, which produces distinct CD44 variant isoforms, requires epithelial splicing regulatory protein 1 (ESRP1).

Molecular targets of CD44 activation

Various signaling course, such as protein-kinases, cyto-skeletal alterations, intracellular pathways, proteinases, and transcriptional elements, are mediated by CD44 and contribute to the division, proliferation, invasion, and angiogenesis of cancer cells as well as metabolic shifts.

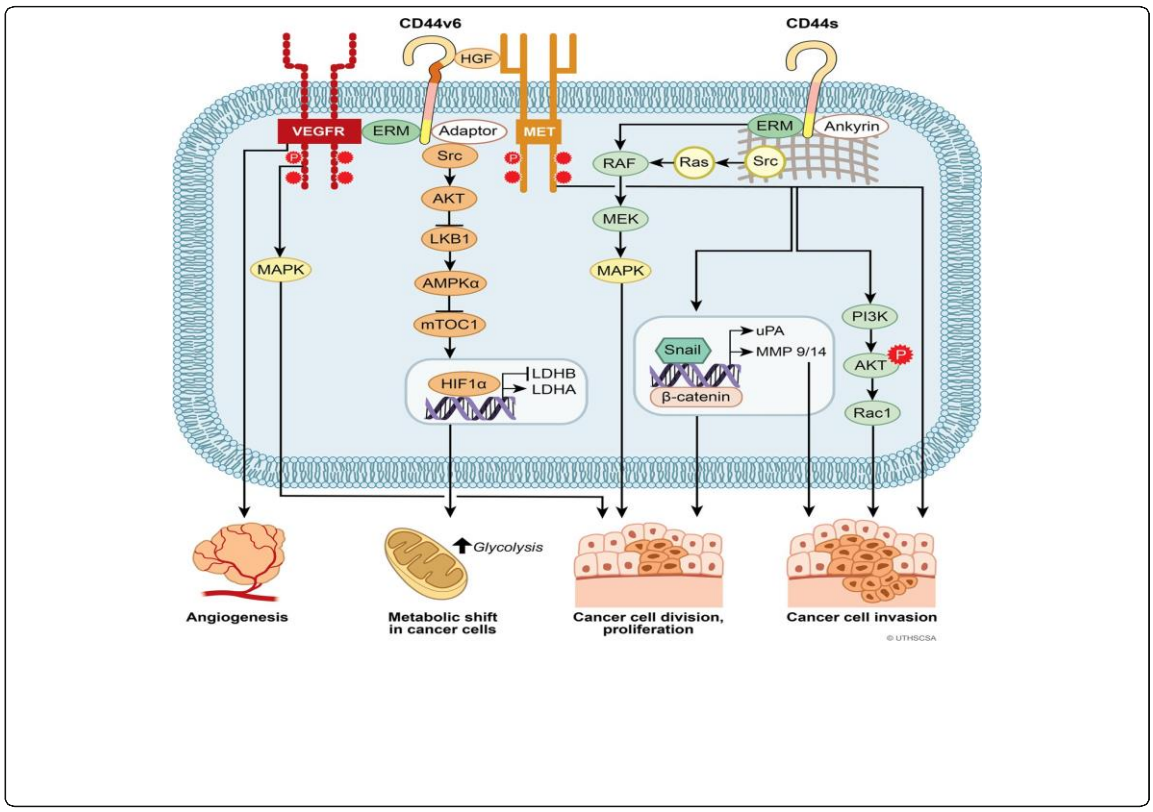

Figure 7: Downstream signalling pathways controlled by CD44. Representative CD44 isoforms that mediate particular downstream signalling pathways are demonstrated to be CD44s and CD44v6. Ezrin/radixin/moesin (ERM) proteins, which encourage cytoskeletal alterations and may interact with VEGFR to contribute to angiogenesis, division of cancer cell and proliferation, can be recruited by CD44v6. To encourage the invasion of cancer cells, CD44v can function as a co-receptor for the receptor tyrosine kinase c-Met. By altering the cytoskeleton and regulating the translocation of Snail/β-catenin

to the nucleus, CD44s can enhance the production of urokinase-type plasminogen activator (uPA) or transcription of the matrix metalloproteinases (MMP) family, which in turn increases the invasion of cancer cells. CD44s can also increase the initiation of the PI3K/AKT pathway, aiding to cancer cell infiltration and extension. Cancer cell division and proliferation are caused by CD44's modulation of the Src/MAPK signalling pathway. Additionally, CD44 induces a metabolic change in cancer cells by causing Hypoxia-inducible factor 1α (HIF1α) to bind to nuclear DNA, increasing glycolysis.

CD44 and microenvironment-

The growth factor interacts with CD44 to produce a malignant phenotype or to initiate tumor-promoting downstream signaling, which is one way that the tumor microenvironment actively promotes tumor progression.

Roles of CD44 in tumorigenicity

In order to mediate the tumorigenic qualities of tumor cells, which result in tumor development, metastasis, and chemoresistance, CD44 activates and modifies a variety of cell signaling networks.

Neovascularization, the formation of new blood vessels, is essential for tumor cells to metastasize and migrate to other organs. Easy extravasation through angiogenesis may result from the adherence of cancer cells to the blood vessels and increased expression of CD44 (CD44s and/or CD44v) by angiogenic agents (e.g., VEGF) generated by tumor cells. It implied that an increase in the number of microvessels and CD44 expression could be helpful indicators of breast cancer metastases. The ability of breast cancer cells to spread to the bone relies on their capability to arrest, stick to, and move through the bone marrow lining into the bone matrix beneath.

Similar studies in literature:

• In the study done by **D Korfias et al**. [15], the CD44 expression by IHC was studied in 104 cases of carcinoma breast. The study shows that CD44 expression is more in stage 3 (78%) when compared to stage 1 (23%) breast carcinoma. Among triple-negative breast carcinoma, the expression of CD44 is seen in 94% of cases. The CD44 expression is not significantly associated with the grade of the tumor, Estrogen receptor positive status, proliferation index, tumor size and patient's age. It was observed that there was a insignificant correlation between CD44 expression and Estrogen receptor negativity.

Sari Voutilainen et al, [16,] studied CD44 expression in 74 cases of breast carcinoma it was observed that CD44 expression is associated with Basal-like epithelial markers in the tumor cells, advanced T stage, and metaplastic variants.

According to **Weiyan Z et al.** [14,] of total 51 cases, positive CD44 was seen in 20 cases had no lymph node metastases and 30 cases showed lymph node metastases, showing increased expression of CD44 in carcinoma breast with metastasis to lymph nodes and triple-negative breast malignancy. It has not affected the overall survival as well as disease-free survival of patients.

Study conducted by **Yousef R et al** [17], It was observed that high CD44 expression is seen in a higher stage of the disease. There was no significant association between overall survival and disease-free survival and CD44 expression.

MATERIALS AND METHODS

Source of data:

Study setting: The study is conducted in the histopathology section, Pathology Department, BLDE (Deemed to be University), Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura.

Study period: 1st May 2023 to 31st December 2024.

Study design: Hospital-based cross-sectional study.

Study population: Clinically suspected cases of breast carcinoma presenting to surgery OPD of BLDE (Deemed to be University), Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura.

Inclusion criteria:

All mastectomy specimens of invasive breast cancer were collected in the histopathology section of the Department of Pathology in BLDE (Deemed to be University) Shri B.M. Patil Medical College, Hospital and Research Centre, Vijayapura.

Exclusion criteria:

Improperly fixed specimens were excluded from the study.

Sample size:

Using G*Power ver. 3.1.9.4 software for sample size calculation, the proportion of malignant breast carcinoma tumour cells staining for CD44 is 14.7%[4], the study would require a **sample size of 60** subjects with 95**%** level of confidence and 10% absolute precision**.**

Formula used

- $n=\frac{z^2 p^*}{d^2}$

Where Z means Z statistic at α level of significance

d^2 means Absolute error

P means the Proportion rate

q= 100-p

Statistical analysis

- Results are interpreted by staining pattern like partially and complete membrane staining were considered positive for CD44 stain.
- The data collected will be entered into an Excel sheet and statistical analysis will be conducted using a standard statistical package for the social sciences (Version 20).
- Results will be calculated and presented as Mean (Median) ± standard deviation, counts, percentages and diagrams.
- For not normally distributed variables, the Mann-Whitney U test is used. For Categorical variables between the two groups are compared using the Chi-square test/Fisher's exact test.
- P <0.05 will be considered statistically significant. All statistical tests will utilize a two-tailed approach.

Methods of Data Collection:

All breast specimens received in the department of Pathology which is diagnosed as breast carcinoma, will be studied from 1st May 2023 to 31st December 2024. The tissue will be preserved in 10% buffered formalin and processed. Two thin sections will be prepared from each tissue block. One thin piece will be stained by hematoxylin and eosin (H&E) for histopathological diagnosis. Another section will be mounted on poly L lysine-coated slide from paraffin-embedded tissue blocks, which will be subjected to CD44 immunohistochemical staining. The patient who has

undergone the IHC study of estrogen, progesterone and Her-2-Neu receptors will be taken for the study.

Outcome:

1. The stem cells are the precursors for the in situ/malignant lesions in the breast.
2. The present study will find out the presence of cancer stem cells in carcinoma breast which will be helpful in assessing the prognosis of the patient and overall survival.

IMMUNOHISTOCHEMICAL STAINING PROTOCOL [51]

Cut 4 micrometer sections on charged slides and incubate at 60-70 degree Celsius for 20 minutes

↓

Deparaffinization of tissue sections on poly-lysine-coated slides

↓

Hydrate through absolute alcohol two changes, each 5 minutes

↓

Rinse in distilled water, 2 changes, 2 minutes each

↓

Quenching of endogenous enzymes (which otherwise react with IHC reagents, giving false positive results)

↓

Antigen retrieval for 15 minutes

↓

Blocking of nonspecific binding sites

↓

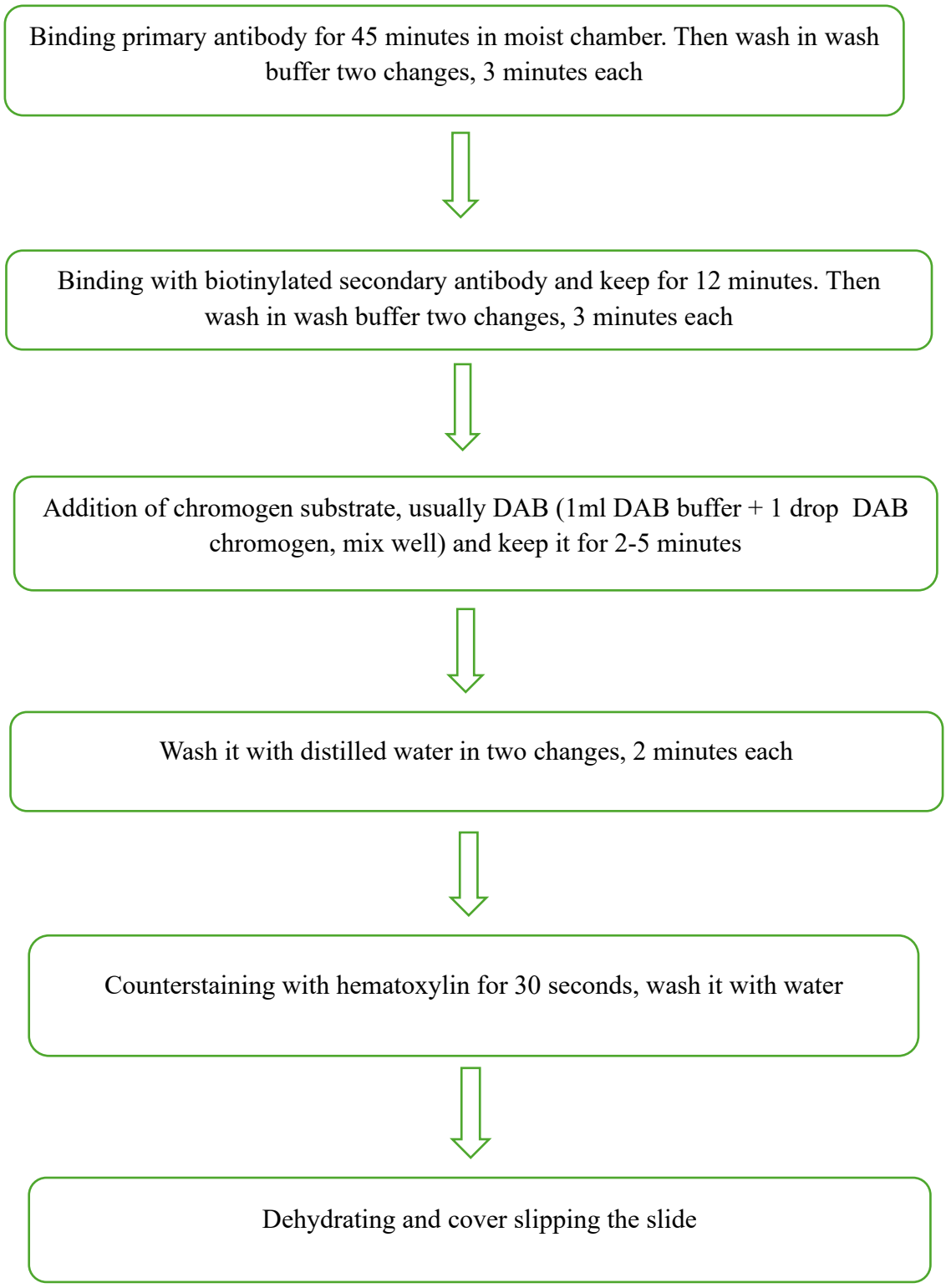
Binding primary antibody for 45 minutes in moist chamber. Then wash in wash buffer two changes, 3 minutes each
Binding with biotinylated secondary antibody and keep for 12 minutes. Then wash in wash buffer two changes, 3 minutes each
Addition of chromogen substrate, usually DAB (1ml DAB buffer + 1 drop DAB chromogen, mix well) and keep it for 2-5 minutes
Wash it with distilled water in two changes, 2 minutes each
Counterstaining with hematoxylin for 30 seconds, wash it with water
Dehydrating and cover slipping the slide

Table 5 - IHC INTERPRETATION OF ER, PR, HER2/ neu AND CD44 MARKER

IHC MARKER	Proportion score		Intensity score		Interpretation
	Ps	Range	IS	Type	
ER/PR	0 1 2 3 4 5	0 <1 1-10 11-33 34-66 67-100	0 1+ 2+ 3+	No staining Weak positive staining Moderate Positive staining Strong Positive staining	**Allred score=PS+IS** Negative= <2 Positive=>2 Maximum score=8
HER2/neu					**ASCO guideline** 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

IHC MARKER	INTERPRETATION
CD44	CD44 showing partial membrane positivity and complete membrane positivity were considered positive in the study.

RESULTS

Present study was done on 60 patients who were identified to have invasive breast carcinoma. Clinicopathological parameters like age of the patient, size of the tumor, histological variant, histological grade and lymph node status were correlated with ER, PR and HER2/neu receptors.

EXPRESSION OF CD44 IN THE STUDY POPULATION

Staining intensity and pattern of CD44 expression was done in malignant tumor cells. All positive cases exhibited positivity in the cytoplasm.

TABLE 6- DISTRIBUTION OF CASES ACCORDING TO CD44 POSITIVITY

CD44	No. of patients	Percentage
Positive	51	85%
Negative	09	15%
Total	60	100%

CD44 IHC score was either partial or complete membrane staining were considered positive. Here, CD44 positivity was seen in 51(85%) cases. CD44 was considered negative when it showed negative or no membrane staining. In this study, 9 (15%) cases were CD44 negative.

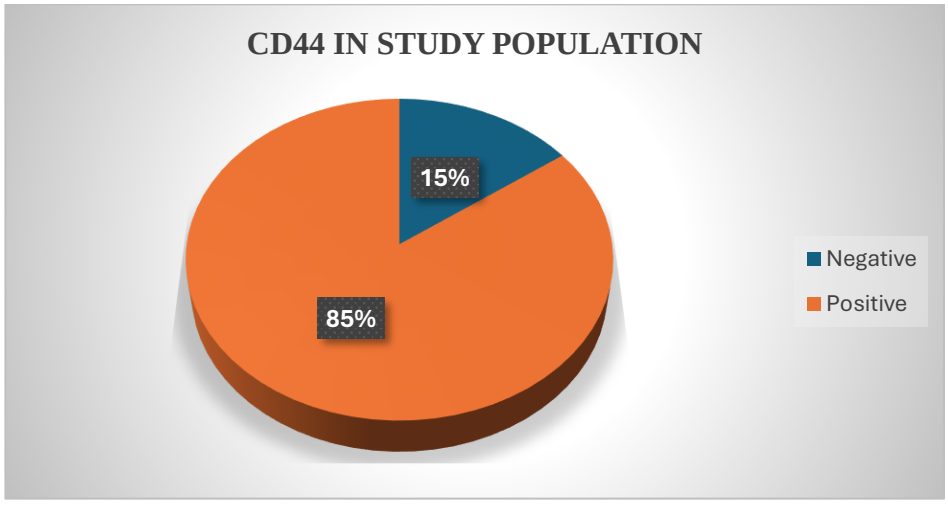

Figure8: Graphical representation of CD44 positivity in the study population

GENDER DISTRIBUTION IN STUDY POPULATION

Among 60 cases, 59 cases (98%) were females and 01 case (2%) was male.

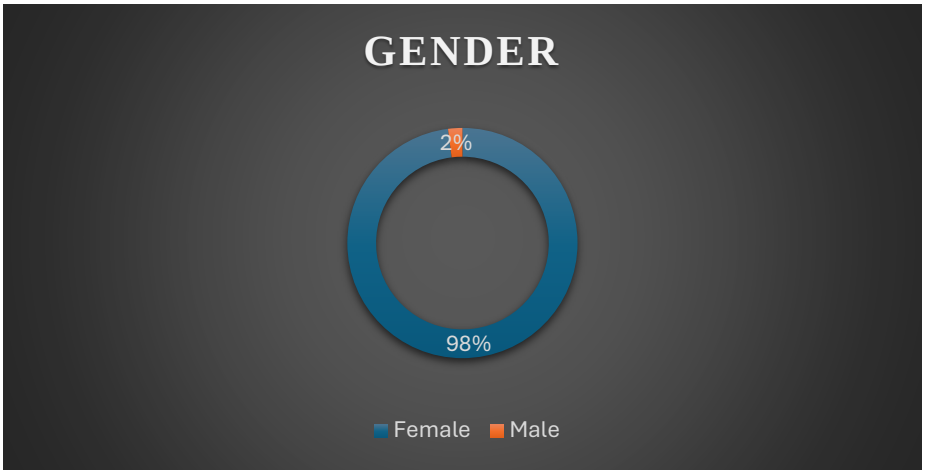

Figure 9: Numbers of females and males in the study

Correlation between CD44 expression and various clinicopathological parameters

1)AGE WISE DISTRIBUTION

Patients with invasive breast carcinoma are range between the ages of 30 and 80, with an average age of 54.5 years and a median age of 55 years.

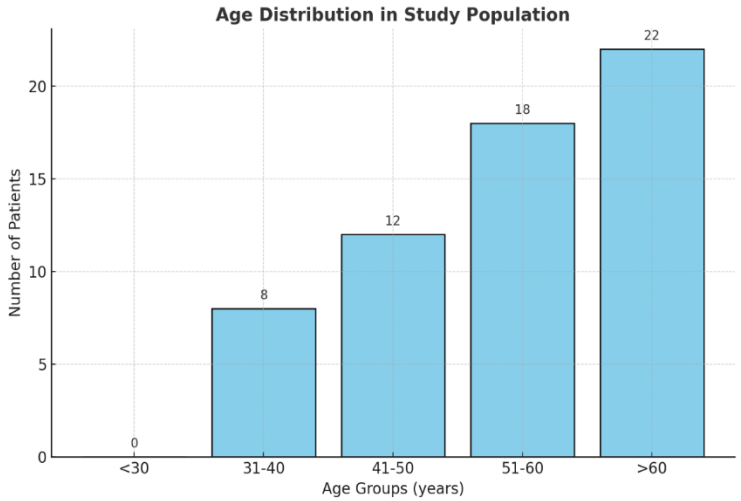

Figure 10: Age distribution in study populations

Table-7: CORRELATION OF CD44 EXPRESSION WITH THE AGE OF THE PATIENTS

AGE (years)	CD 44			Chi-square test	p value
	Negative	Positive	Total		
< 30	0 (0%)	0 (0%)	0 (0%)	6.99	**0.008**
31-40	2 (22.2%)	6 (12%)	8 (13.3%)		
41-50	4 (4.44%)	8 (15.6%)	12 (20%)		
51-60	0 (0%)	18 (35.2%)	18 (30%)		
>60	3 (33.3%)	19 (37.2%)	22 (36.6%)		
Total	9 (100%)	51 (100%)	60 (100%)		
Statistically Significant					

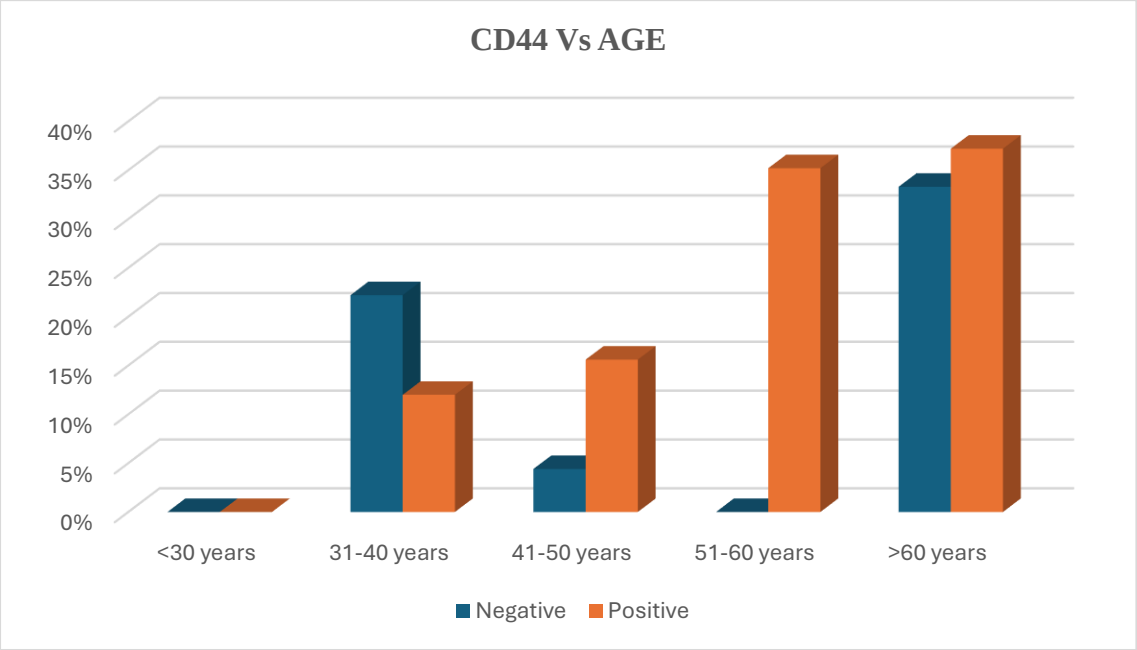

Figure 11: Graphical expression of CD44 with age

The highest number of CD44 positivity was seen in the study population more than 60 years of age, with 19 (37.2%) cases out of 22 cases followed by 18 cases (35.2%) in age group of 51-60 years, while the study group with age less than 50 years of age i.e, between 41-50 years show positivity in 8 cases (15.6) out of 12 cases and 6 cases (12%) positivity out of total 8 cases under age group of 31-40 years. **P value was 0.008, which shows statistically significant** correlation between CD44 expression and patient's age. (Table 7, Fig: 11)

2) <u>SIZE OF TUMOR</u>

Table-8: DISTRIBUTION OF CASES ACCORDING TO SIZE OF THE TUMOR

TUMOUR SIZE	No. of patients	Percentage
T0	1	1.6%
T1	15	25%
T2	28	46.6%
T3	9	15%
T4	7	11.6%
Total	60	100.0

Tumor size varied from 1 cm to 10 cm. In majority of the cases i.e in 28 (46.6%) cases, tumor size was between 2-5cm (T2), 14 (23.3%) cases had tumor size less than 2cm (T1), 9(15%) cases had tumor size >5cm (T3) and 7(11.6%) cases were of T4 showing direct extension to the the chest wall and/ skin. (Table 8)

Table-9: CORRELATION OF CD44 EXPRESSION AND TUMOR SIZE

TUMOR SIZE	CD 44			Student's t test	Mann Whitney U	p value
	Negative	Positive	Total			
T0	0 (0%)	1(2%)	1(1.6%)	-3.20	180	**0.002**
T1	2(22.2%)	13(25.4%)	15(25%)			
T2	4(44%)	24(47%)	28(46.6%)			
T3	1(11.1%)	8 (15.6%)	9 (15%)			
T4	2(22.2%)	5 (9.8%)	7 (11.6%)			
Total	9 (100%)	51 (100%)	60 (100%)			
Statistically Significant						

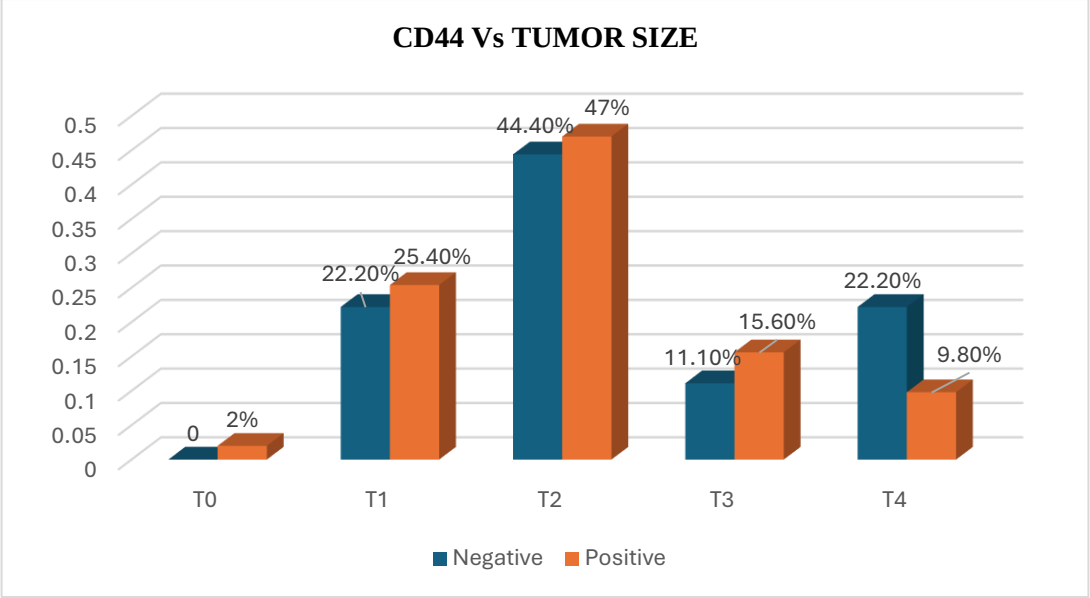

Figure 12: Graphical representation of CD44 with tumor size

The size of the tumor varied from 1 cm to 10 cm. 24 (47%) cases with CD44 positivity comes under group of tumor size T2, followed by 13 (25.4%) cases which belongs to tumor size T1, which is followed by 8 (15.6%) cases of tumor size T3. The least cases with CD44-positivity comes under tumor size of T4, i.e 5(9.8%) cases.

4 (44%) cases with CD44 negative expressions are associated with the group of tumor size T2, followed by 2 (22.2%) cases each belongs to T1 and T4, and only 1(11.1%) case with negative CD44 expression fall under tumor size T3.

The **p value was 0.002**, **which shows highly statistical significant** association between CD44 expression and the tumor size. (Table 9, Fig;12)

3)HISTOLOGICAL TYPE

Table-10: DISTRIBUTION OF CASES ACCORDING TO HISTOLOGICAL TYPE

HISTOLOGICAL TYPE	No. of patients	Percentage
Infiltrating ductal carcinoma NOS	54	90%
Invasive lobular carcinoma	2	3.3%
Encapsulated Papillary carcinoma	2	3.3%
Invasive Papillary carcinoma	1	1.6%
Mucinous carcinoma	1	1.6%
Total	60	100.0

Out of 60 cases studied, maximum number of cases were of Infiltrating ductal carcinoma NOS, i.e., 54 cases (90%), 2 (3.3%) cases was of Invasive lobular carcinoma, one case (1.6%) was of mucinous carcinoma and remaining 3 cases were papillary neoplasm, of which 2 (3.3%) cases were of Encapsulated papillary carcinoma and 1 case (1.6%) was of Invasive papillary carcinoma. (Table 10)

Table-11: COMPARISION OF CD44 WITH HISTOLOGICAL TYPE

HISTOLOGICAL TYPE	CD 44			Chi-square test	p value
	Negative	Positive	Total		
Infiltrating ductal carcinoma	9 (100%)	45(88.2%)	54 (90%)	1.17	0.27
Invasive lobular carcinoma	0 (0.0%)	2 (4%)	2 (3.3%)		
Encapsulated Papillary carcinoma	0 (0.0%)	2 (4%)	2 (3.3%)		
Invasive Papillary carcinoma	0 (0.0%)	1 (2%)	1 (1.6%)		
Mucinous carcinoma	0 (0.0%)	1 (2%)	1 (1.6%)		
Total	9 (100%)	51(100%)	60 (100%)		
Statistically Insignificant					

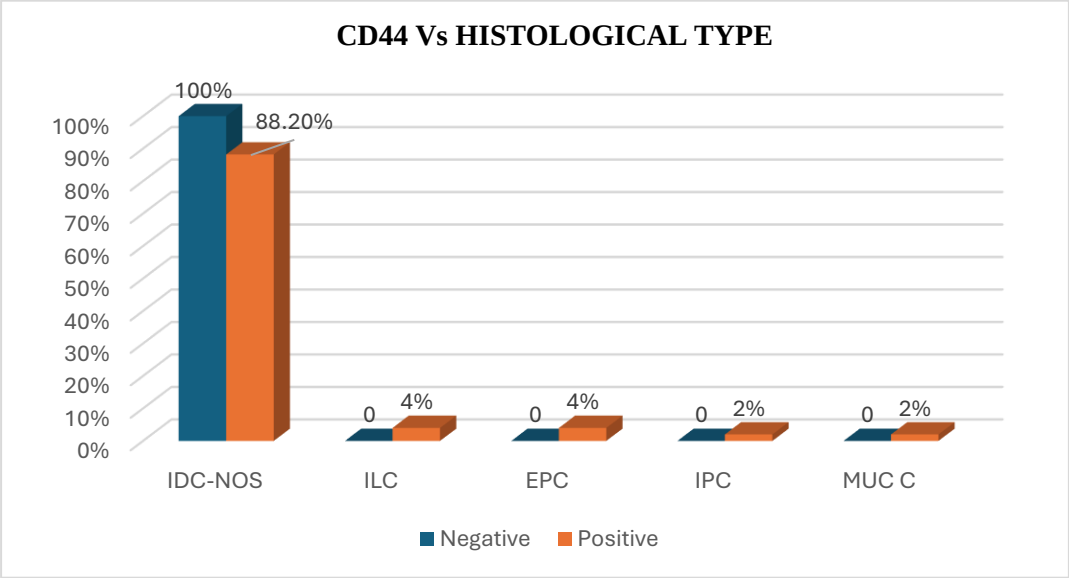

Figure 13: Graphical representation of CD44 with histological type

Among total 60 cases of infiltrating ductal carcinoma-NOS, 45 cases (88.2%) showed CD44 positivity and 9 cases (100%) showed CD44 negativity. 2 cases (4%) of invasive lobular carcinoma showed CD44 positivity. Among papillary neoplasm 2 cases (4%) of encapsulated papillary and 1 case (2%) of invasive papillary showed CD44 positivity. One case (2%) of mucinous carcinoma showed CD44 positivity. The p value was 0.27, showing no statistical significant correlation between CD44 and histological type. (Table 11, Fig:13)

4)HISTOLOGICAL GRADE

Table-12: DISTRIBUTION OF CASES ACCORDING TO HISTOLOGICAL GRADING

HISTOLOGICAL GRADE	No. of patients	Percentage
Grade 1	18	30%
Grade 2	32	53%
Grade 3	10	17%
Total	60	100.0

In the present study, 18 (30%) cases belonged to histological grade1, 32 (53%) cases belonged to grade 2 and 10 cases (17%) were of grade 3.(Table 12)

Table-13: COMPARISION OF CD44 WITH HISTOLOGICAL GRADING

HISTOLOGICAL GRADE	CD 44			Student's t test	Mann Whitney U	p Value
	Negative	Positive	Total			
Grade 1	2 (22.1%)	16 (31.3%)	18 (30%)	-2.27	248	**0.022**
Grade 2	6 (66.6%)	26 (51%)	32 (53.3%)			
Grade 3	1 (11.1%)	9 (17%)	10 (16.6%)			
Total	9 (100%)	51 (100%)	60 (100%)			
Statistically Significant						

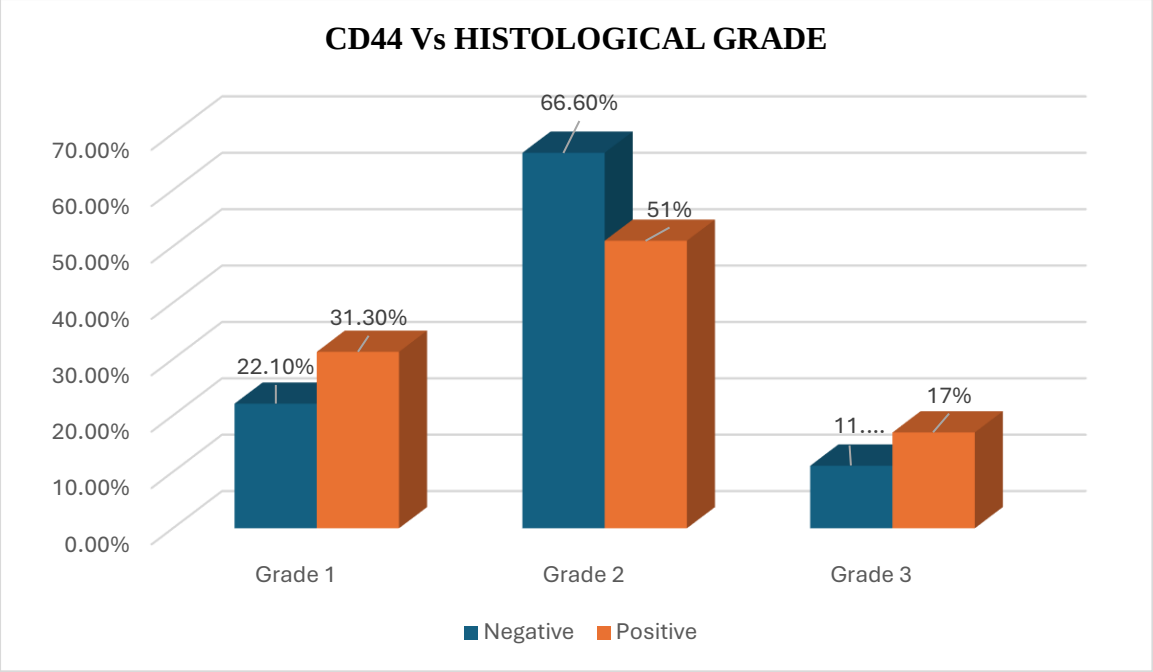

Figure 14: Graphical representation of CD44 with histological grade

Majority cases in present study having positive CD44 expression associated to Grade 2, i.e., 26 (51%) cases, followed by Grade 1 in which 16 (31.3%) cases showed CD44 positivity followed by 9 (17%) cases of Grade 3. 6 (66.6%) cases with negative CD44 expression comes under Grade 2, followed by 2 (22.1%) cases of Grade 1 and 1(11.1%) case of Grade 3.

The p value was 0.02, which shows statistical significant correlation between CD44 expression and the histological grade of the tumor. (Table13, Fig:14)

5)LYMPH NODE STATUS

Table-14: DISTRIBUTION OF CASES ACCORDING TO LYMPH NODE STATUS

LYMPH NODE STATUS	No. of patients	Percentage
Involved by tumor	40	66.6%
Not involved by tumor	20	33.3%
Total	60	100.0

Of total 60 cases, 40 cases (66.6%) showed lymph node involvement by tumor cells. (Table 14)

Table-15: CORRELATION OF CD44 EXPRESSION WITH LYMPH NODE STATUS

LYMPH NODE STATUS	CD 44			Chi-square test	p value
	Negative	Positive	Total		
Not Involved	2 (22.2%)	18 (35%)	20 (33%)	0.58	0.44
Involved	7 (77.7%)	33 (65%)	40 (66.6%)		
Total	9 (100%)	51 (100%)	60 (100%)		
Statistically Insignificant					

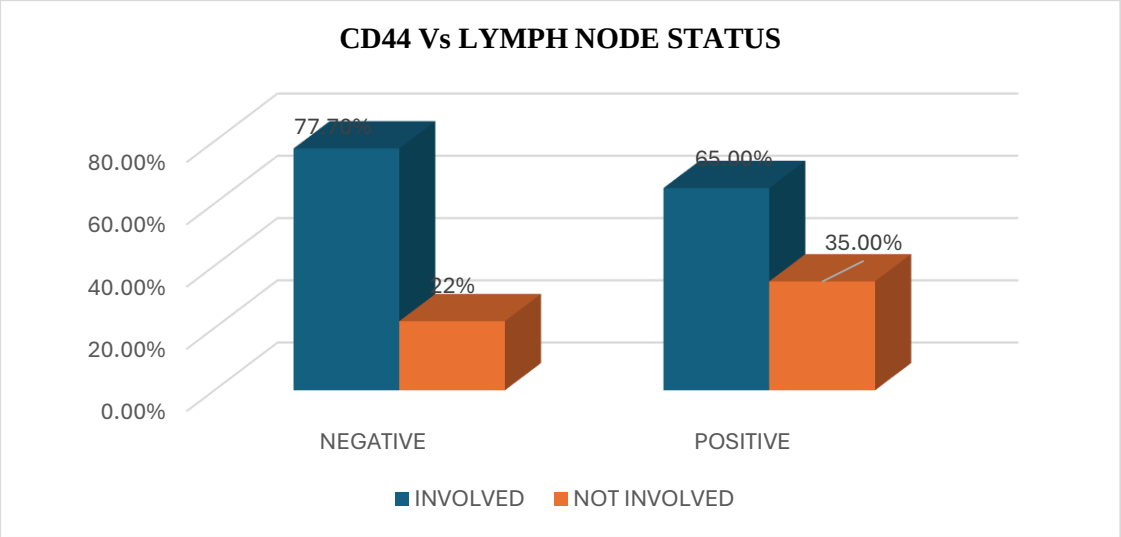

Figure 15: Graphical representation of CD44 with lymph node status

In the present study, of total 60 cases of invasive breast carcinoma, 40 (66.6%) cases shows definite nodal status; of these 33(65%) were CD44 positive, and 7(77.7%) cases were CD44 negative. 20 (33%) cases did not show lymph node metastases; 18(35%) cases shows CD44 expression.

P value was 0.44, which shows no statistically significant correlation between CD44 expression and lymph-node status. (Table 15, Fig:15)

6)ESTROGEN RECEPTOR STATUS

Table-16: ESTROGEN RECEPTOR EXPRESSION OF BREAST CARCINOMA CASES

ER STATUS	No. of patients	Percentage
Negative	22	36.6%
Positive	38	63.3%
Total	60	100.0

Among 60 cases of invasive breast carcinoma, 22 (36.6%) were ER-negative, and 38 cases (63.3%) were ER-positive. (Table 16)

Table-17: COMPARISION OF CD44 WITH ESTROGEN RECEPTORS STATUS

ER STATUS	CD 44			Chi-square test	p value
	Negative	Positive	Total		
Negative	5 (55.5%)	17 (33.3%)	22 (36.6%)	1.62	0.20
Positive	4 (44.4%)	34 (66.6%)	38 (63.3%)		
Total	9 (100%)	51 (100%)	60 (100%)		
Statistically Insignificant					

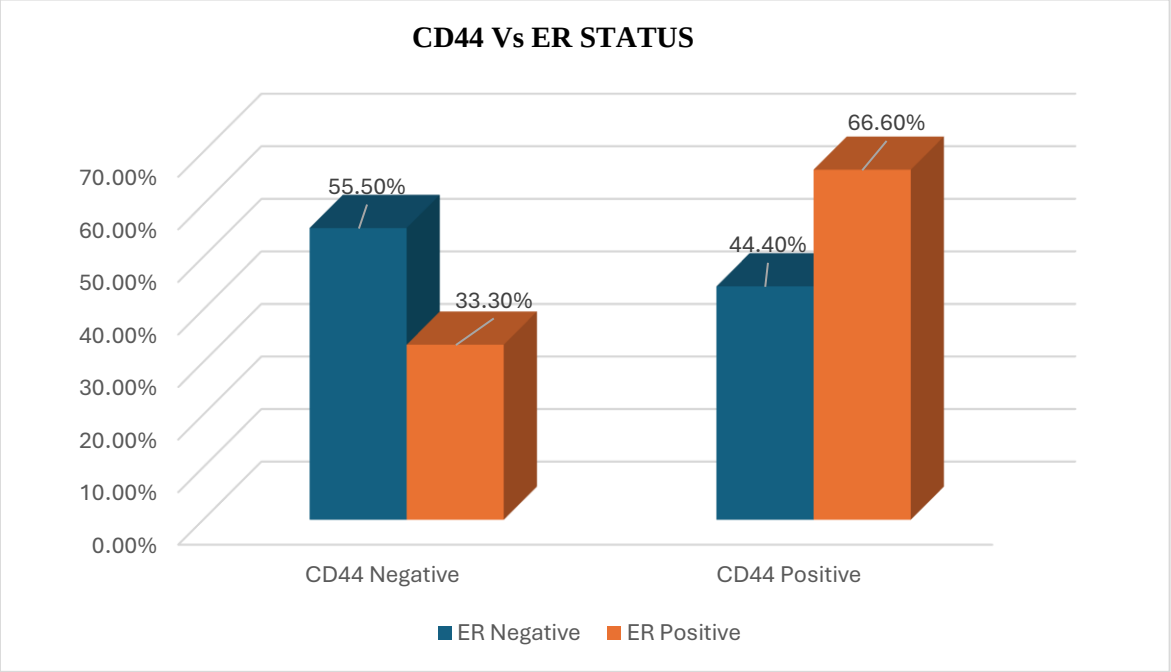

Figure 16: Graphical representation of CD44 with ER expression

Of 22 ER- negative cases, 17 (33.3%) cases showed CD44 positivity. Among 38 cases with positive ER expression, 34 (66.6%) cases showed CD44 expression. P value was 0.2 showing statistically insignificant correlation between CD44 and ER status of tumor. (Table17, Fig:16)

7)PROGESTERONE RECEPTOR STATUS

Table-18: PROGESTERONE RECEPTOR EXPRESSION OF BREAST CARCINOMA CASES

PR STATUS	No. of patients	Percentage
Negative	24	40%
Positive	36	60%
Total	60	100.0

Among 60 cases of invasive breast carcinoma, 24 (40%) were PR-negative, and 36 (60%) were PR-positive. (Table 18)

Table-19: COMPARISION OF CD44 WITH PROGESTERONE RECEPTORS STATUS

PR STATUS	CD 44			Chi-square test	p value
	Negative	Positive	Total		
Negative	5 (55.5%)	19 (37.2%)	24 (40%)	1.06	0.30
Positive	4 (44.4%)	32 (62.6%)	36 (60%)		
Total	9 (100%)	51 (100%)	60 (100%)		
				Statistically Insignificant	

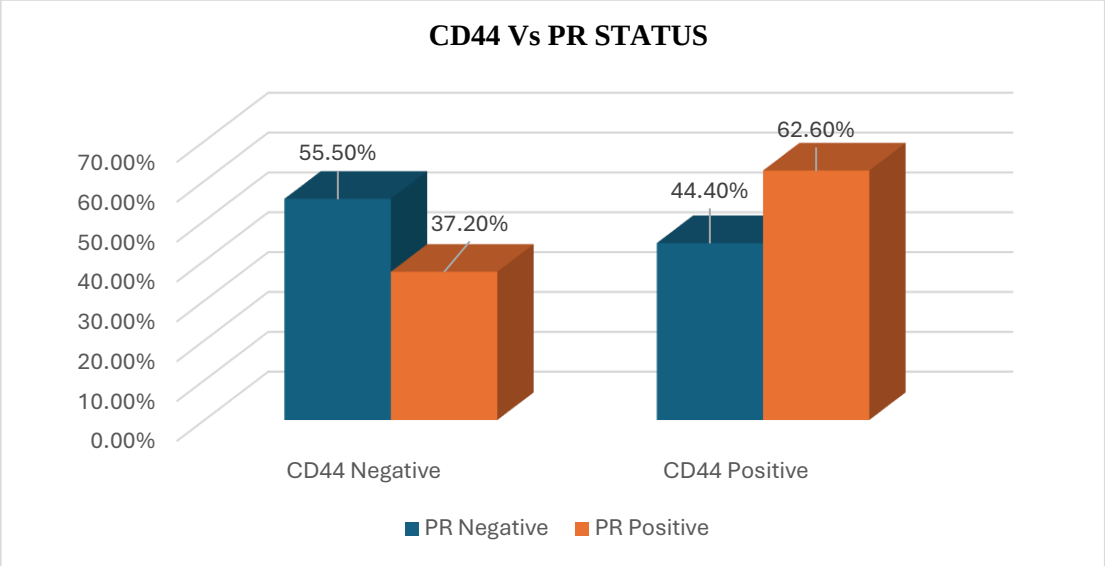

Figure 17: Graphical representation of CD44 with PR expression

Of 24 PR negative cases, 19 (37.2%) cases showed CD44 positivity. Of 36 cases with positive PR expression, 32 (62.6%) cases showed CD44 positivity. P value was 0.30, showing statistical insignificant correlation between CD44 expression and PR status of tumor. (Table 19, Fig:17)

8)HER2/neu STATUS

Table-20: HER2/neu RECEPTOR EXPRESSION OF BREAST CARCINOMA CASES

HER2/neu STATUS	No. of patients	Percentage
Equivocal	02	3.3%
Negative	30	50%
Positive	28	47%
Total	60	100.0

In the current study, out of 60 cases of invasive breast carcinoma, 30 cases (50%) were HER2/neu negative, 28 cases (47%) were HER2/neu positive and 2 cases (3.3%) showed equivocal expression. (Table 20)

Table-21: CORRELATION OF CD44 WITH HER2/neu RECEPTORS STATUS

HER2/ neu STATUS	CD 44			Chi-square test	p value
	Negative	Positive	Total		
Equivocal	0 (0.0%)	2 (4%)	2 (3.3%)	4.18	**0.04**
Negative	2 (22.2%)	28 (55%)	30 (50%)		
Positive	7 (78%)	21 (41%)	28 (47%)		
Total	9 (100%)	51 (100%)	60 (100%)		
Statistically Significant					

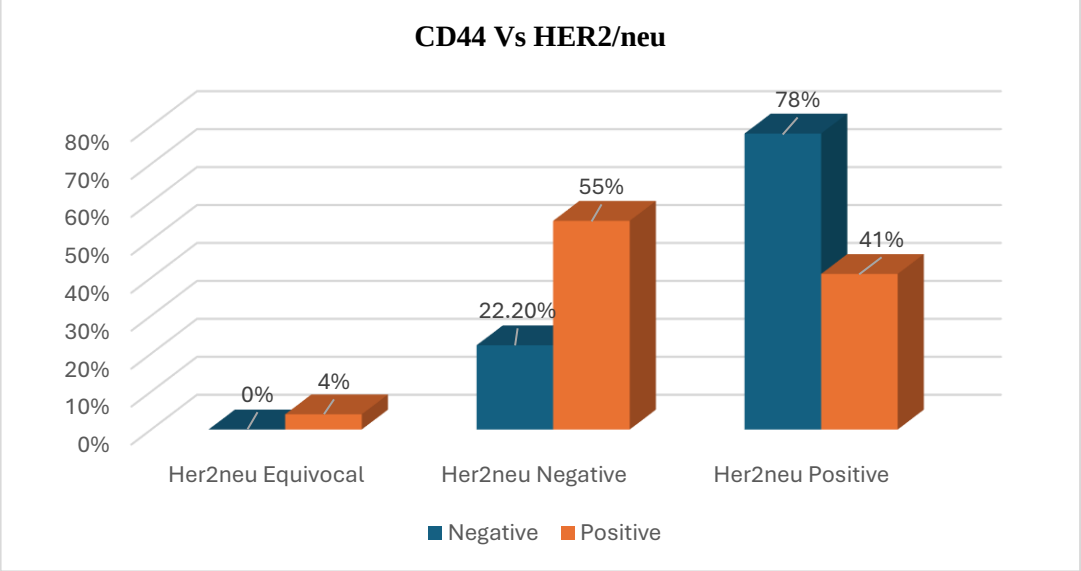

Figure 18: Graphical representation of CD44 with HER2/neu expression

Of 30 cases of HER2/neu negative cases, 28 (55%) cases present with CD44 positivity. Of 28 cases with positive HER2/neu expression, 21(41%) showed CD44 positivity.

P value was 0.04, showing statistically significant correlation between CD44 expression and HER2/ neu status of tumor. (Table 21, Fig:18)

TABLE 22- COMPARISON OF CD44 WITH VARIOUS CLINICOPATHOLOGICAL PARAMETERS

PARAMETERS	CD44		CHI-SQUARE TEST	P VALUE
	NEGATIVE	POSITIVE		
	NO. OF CASES(%)	NO. OF CASES(%)		
AGE				
<30	**0 (0%)**	**0 (0%)**	**6.99**	**0.008**
31-40	**2 (22.2%)**	**6 (12%)**		
41-50	**4 (4.44%)**	**8 (15.6%)**		
51-60	**0 (0%)**	**18 (35%)**		
>60	**3 (33%)**	**19 (37.2%)**		
HISTOLOGICAL TYPE				
IDC-NOS	**9 (100%)**	**45 (88.2%)**	**1.17**	**0.27**
ILC	**0 (0.0%)**	**2 (3.3%)**		
EPC	**0 (0.0%)**	**2 (3.3%)**		
IPC	**0 (0.0%)**	**1 (2%)**		
MUC C	**0 (0.0%)**	**1 (2%)**		
LYMPH NODE STATUS				
INVOLVED	**7 (77.7%)**	**33 (65%)**	**0.58**	**0.44**
NOT INVOLVED	**2 (22.2%)**	**18 (35%)**		

PARAMETERS	CD44		Student's t test	Mann Whitney U	P VALUE
	NEGATIVE	POSITIVE			
	NO. OF CASES (%)	NO. OF CASES (%)			
HISTOLOGICAL GRADE					
I	**2 (22.1%)**	**16 (31.3%)**	**-2.27**	**248**	**0.022**
II	**6 (66.6%)**	**26 (51%)**			
III	**1 (11%)**	**9 (17%)**			
TUMOR SIZE					
T0	**0 (0%)**	**1 (2%)**	**-3.20**	**180**	**0.002**
T1	**2 (22.2%)**	**13 (25.4%)**			
T2	**4 (44%)**	**24 (47%)**			
T3	**1 (11%)**	**9 (15%)**			
T4	**2 (22%)**	**5 (9.8%)**			

PARAMETERS	CD44		CHI-SQUARE TEST	P VALUE
	NEGATIVE	POSITIVE		
	NO.OF CASES(%)	NO.OF CASES(%)		
ER STATUS				
NEGATIVE	**5 (55.5%)**	**17 (33.3%)**	**1.62**	**0.20**
POSITIVE	**4 (44.4%)**	**34 (66.6%)**		
PR STATUS				
NEGATIVE	**5 (55.5%)**	**19 (37.2%)**	**1.06**	**0.30**
POSITIVE	**4 (44.4%)**	**32 (62.6%)**		
HER2 NEU				
EQUIVOCAL	**0 (0.0%)**	**2 (4%)**	**4.18**	**0.04**
NEGATIVE	**2 (22.2%)**	**28 (55%)**		
POSITIVE	**7 (78%)**	**21 (41%)**		

A statistically significant correlation was found between the expression of CD44 with patient's age, tumor size, histological grade and HER 2neu hormonal marker.

Statistically insignificant correlation was seen between the expression of CD44 and other clinicopathological parameters like histological type, lymph node status and ER and PR expression.

PHOTOMICROGRAPHS

GROSS- PICTURES

Figure19 -Macrophotograph of modified radical mastectomy specimen

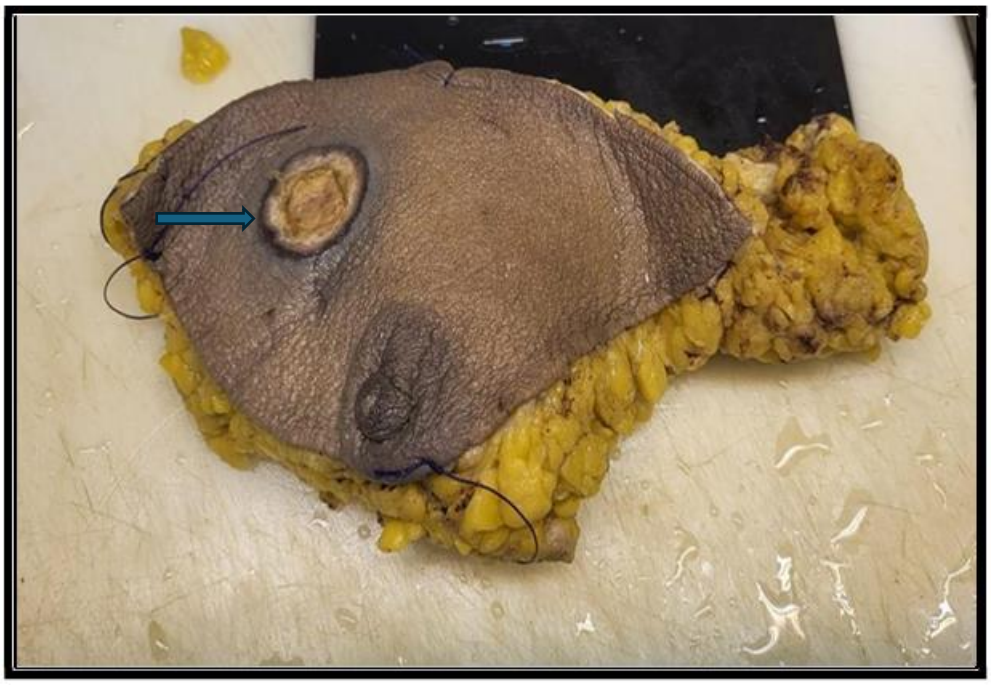

Figure20 - Gross Photograph of Invasive Breast Carcinoma showing skin ulceration measuring 4.2x3x1.5cm

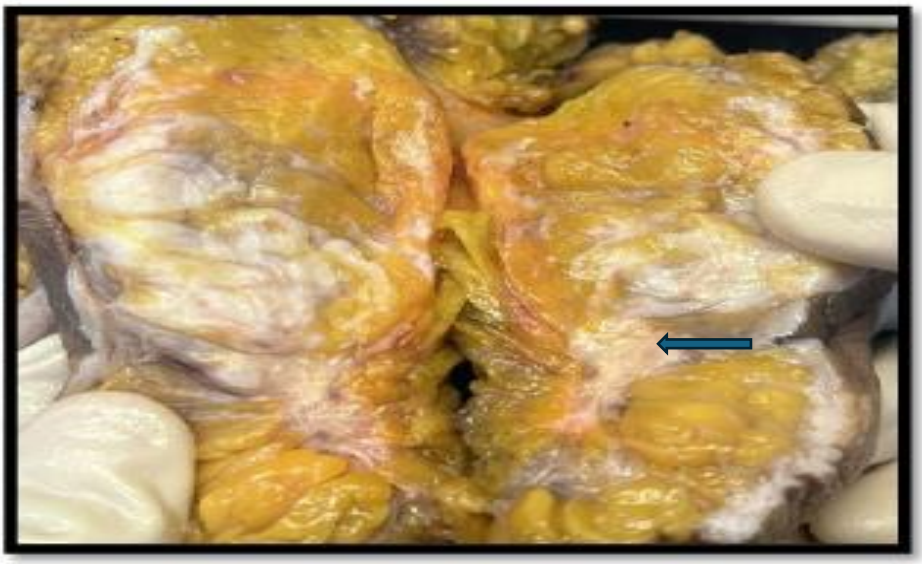

Figure 21-Macrophotograph showing cut section of Invasive breast carcinoma with tumor size <2 cm (pT1)

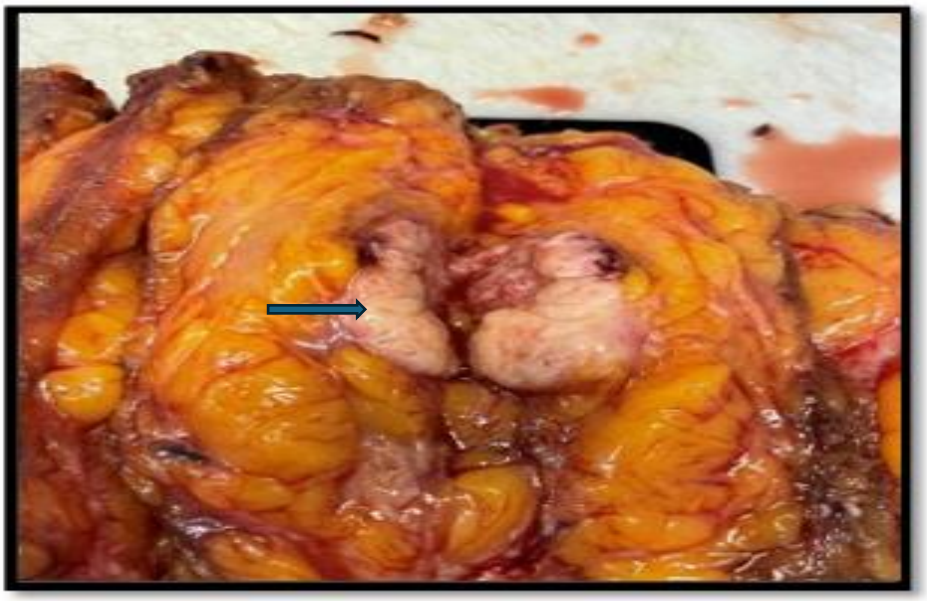

Figure 22-Macrophotograph showing cut section of Invasive breast carcinoma showing pale white growth with irregular border, tumor size 2-5cm (pT2).

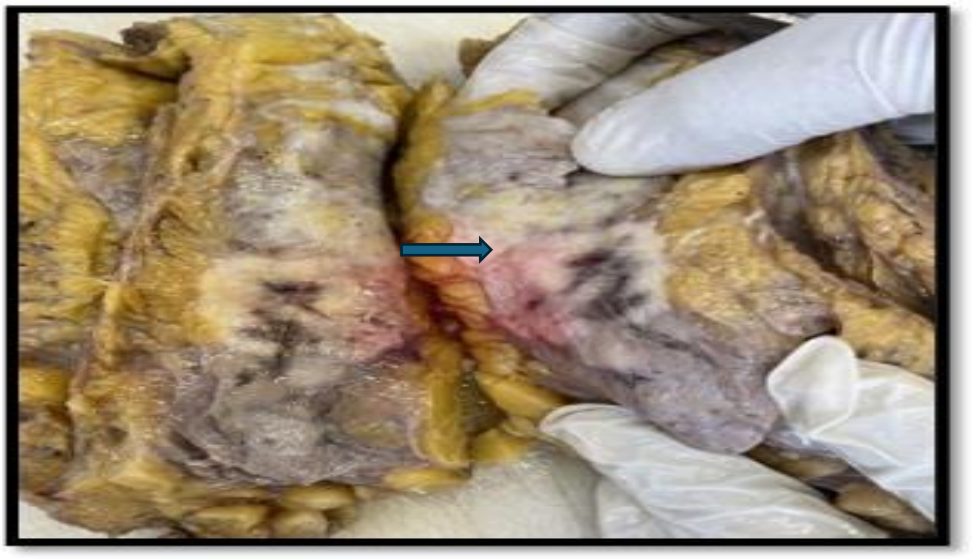

Figure23-Macrophotograph showing cut section of Invasive breast carcinoma with tumor size >5cm (pT3)

MICROSCOPIC- PICTURE

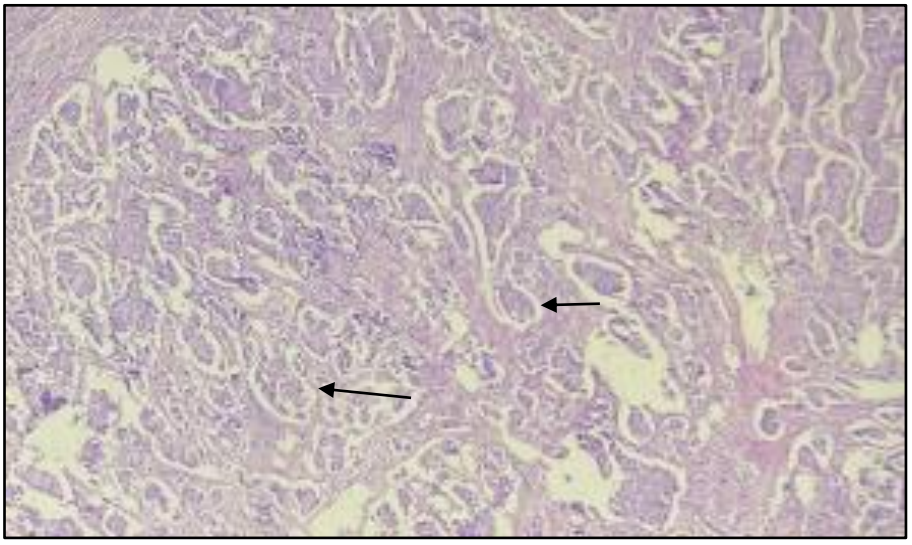

Figure 24: Microphotograph showing tumor tissue arranged in nests and lobules (H&E) (400x)

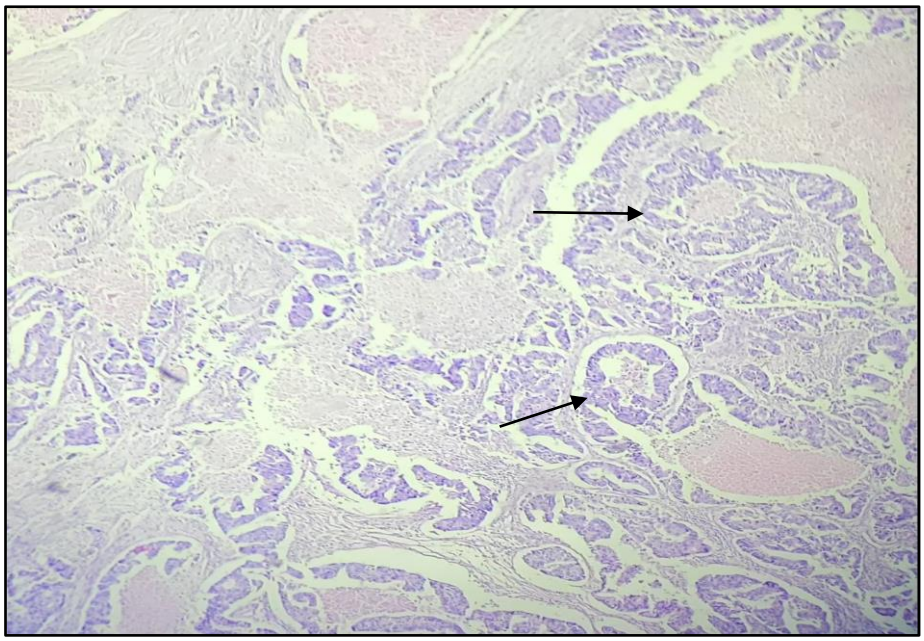

Figure 25: Microphotograph showing tumor tissue arranged in tubular pattern (H&E) (200x)

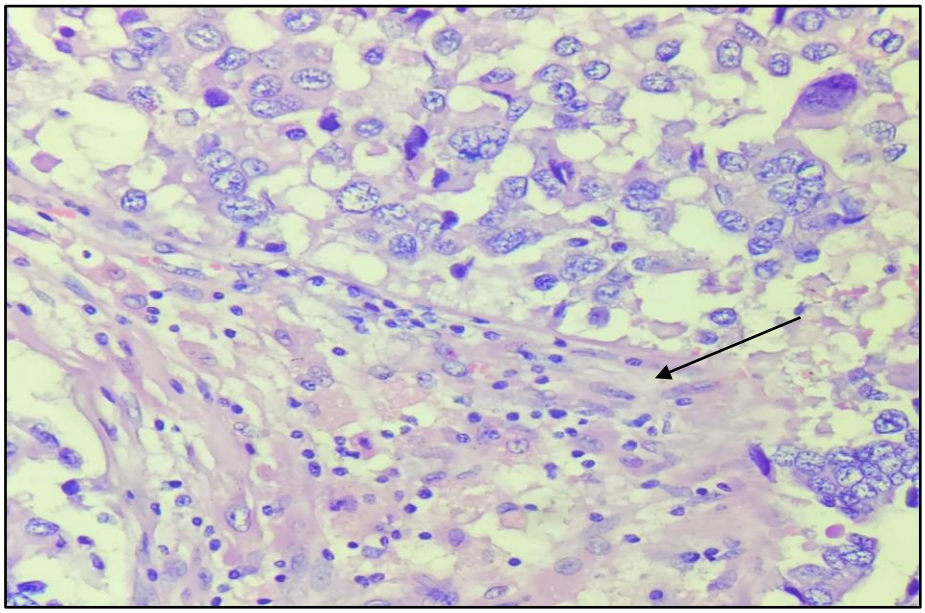

Figure 26: Microphotograph of invasive breast carcinoma NOS showing lymphoplasmacytic infiltrates in the intervening areas (H&E) (400x)

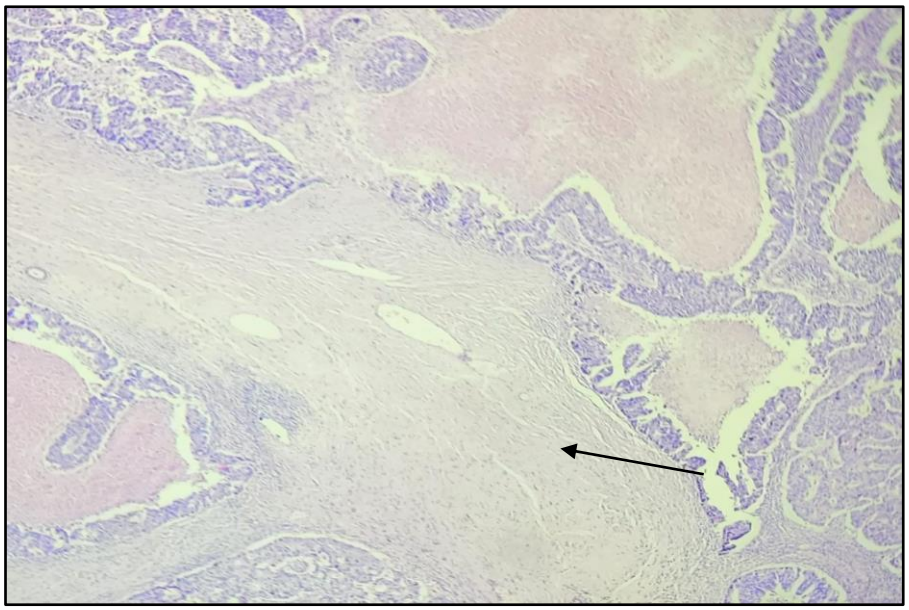

Figure 27: Microphotograph of invasive breast carcinoma NOS showing desmoplastic stroma(H&E) (100x)

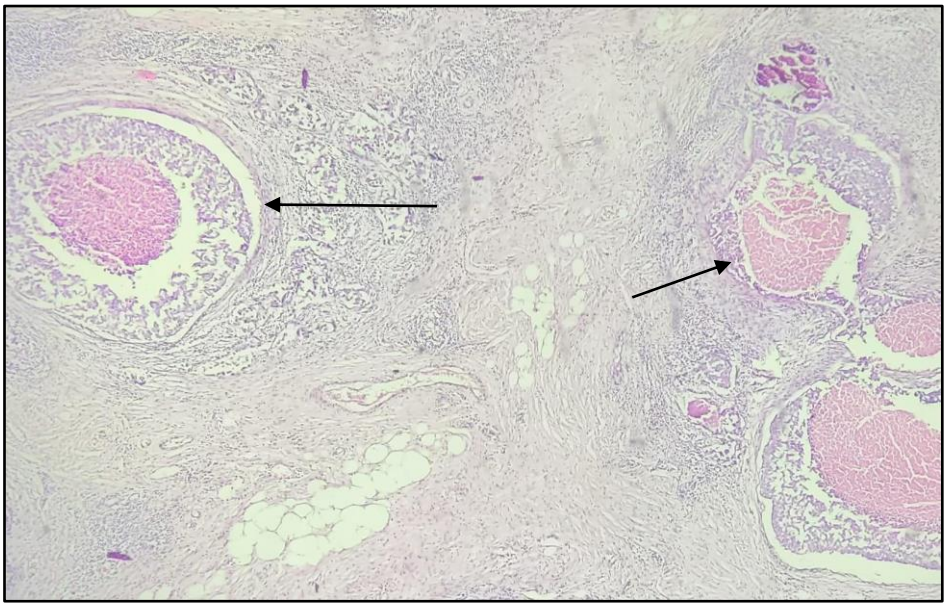

Figure 28: Microphotograph of invasive breast carcinoma NOS showing comedo necrosis (H&E) (200x)

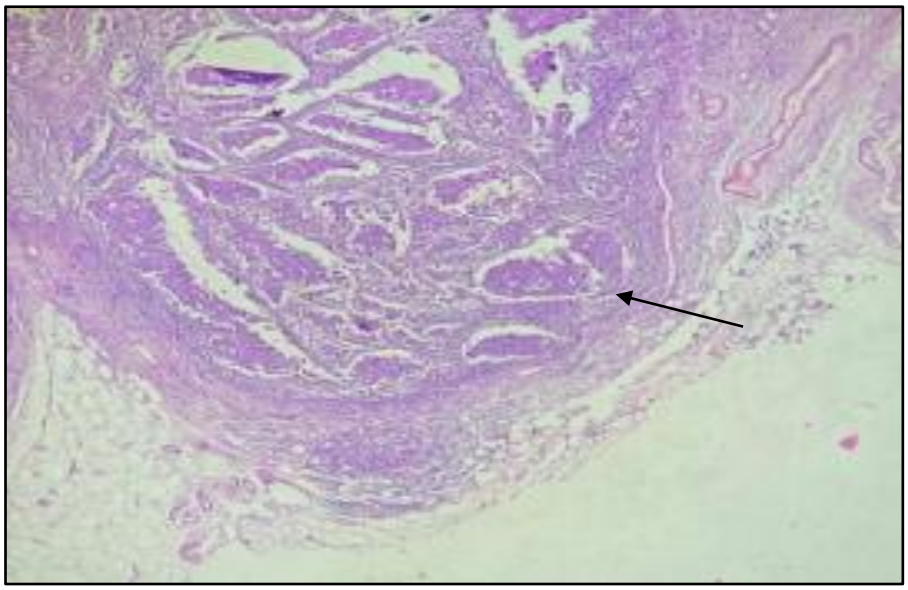

Figure 29 - Microphotograph of invasive breast carcinoma NOS showing tumor deposits in lymph node (H&E) (200x)

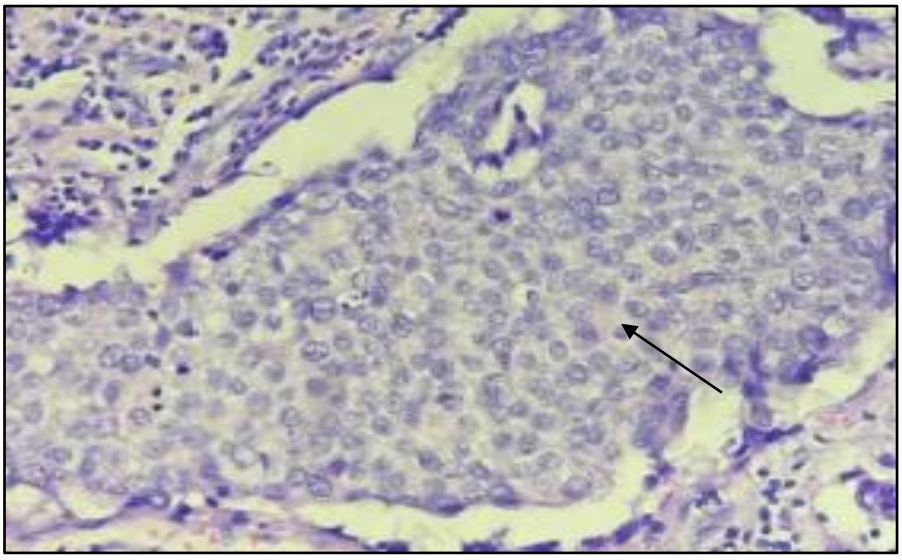

Figure 30 - Microphotograph of invasive breast carcinoma NOS showing marked nuclear pleomorphism (H&E) 400x

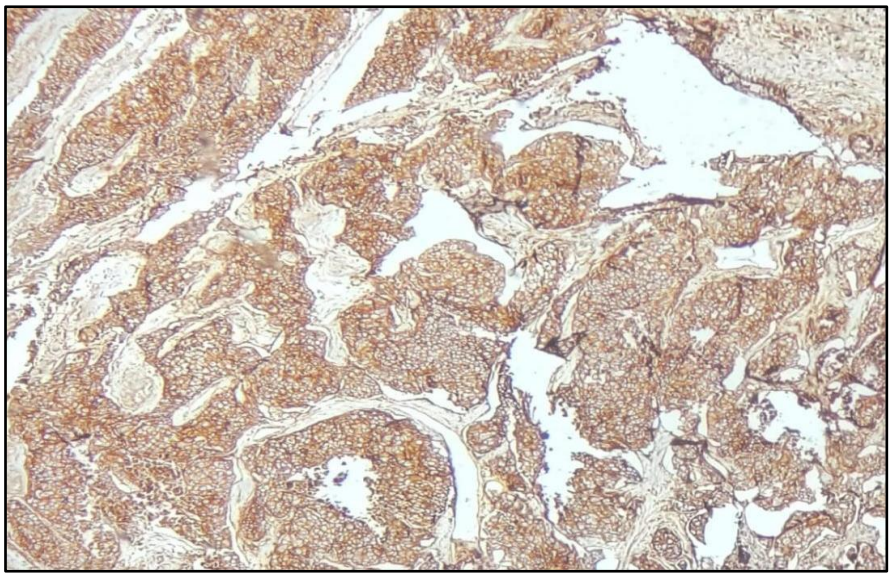

Figure 31: Microphotograph of IHC marker CD44 showing cytoplasmic staining in invasive breast carcinoma NOS (100x)

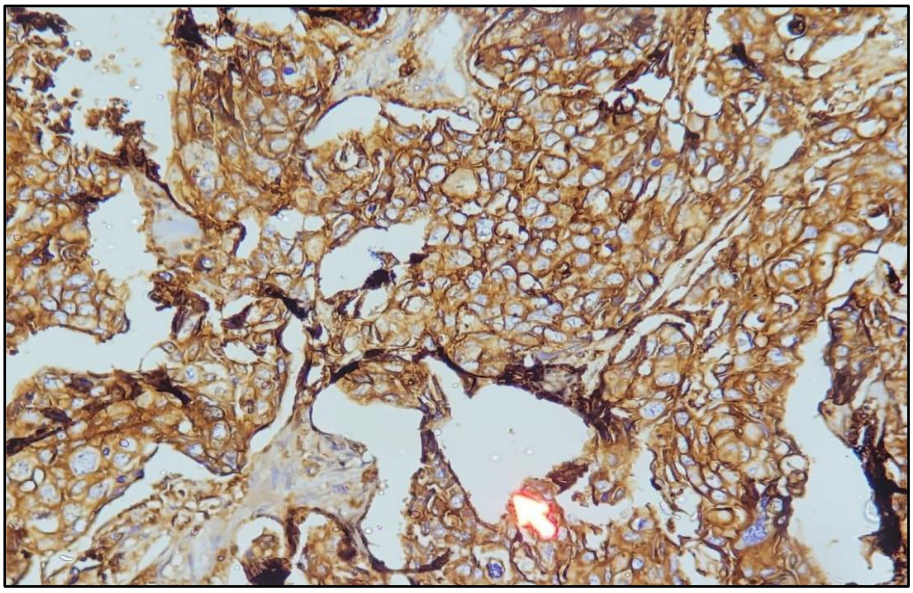

Figure 32: Microphotograph of IHC marker CD44 showing complete cytoplasmic staining in invasive breast carcinoma NOS (200x)

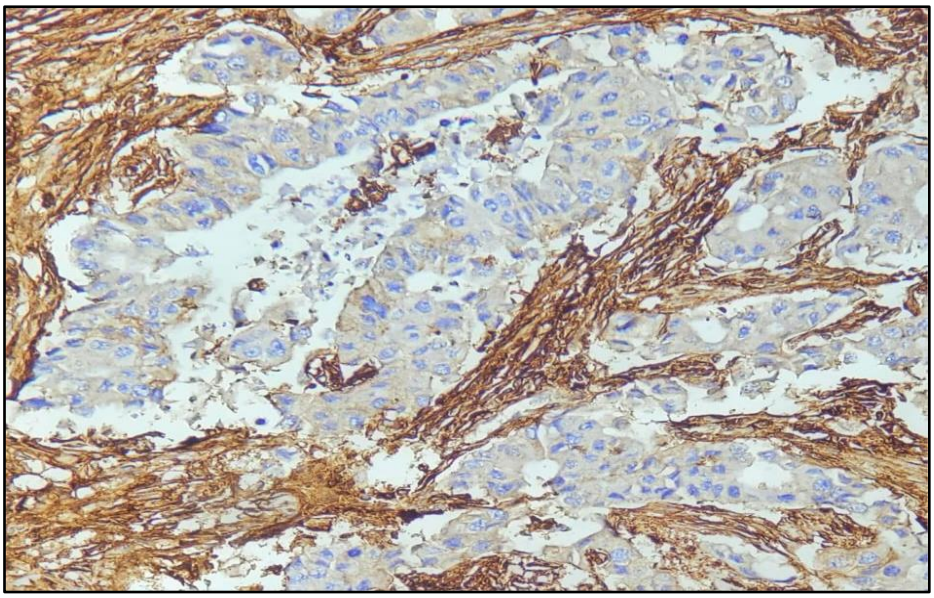

Figure 33: Microphotograph of IHC marker CD44 showing focally cytoplasmic staining in invasive breast carcinoma NOS (400X)

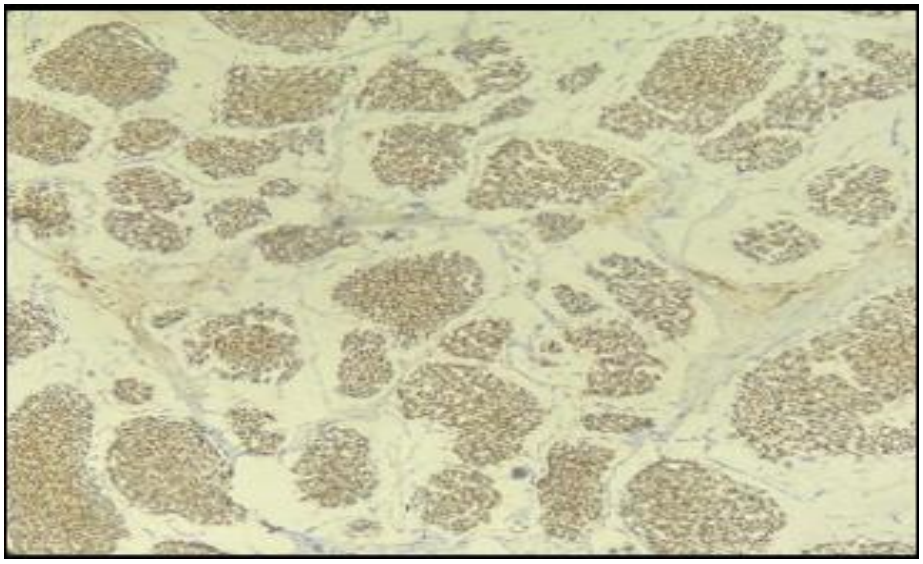

Figure 34: Microphotograph of IHC marker ER showing nuclear positivity in invasive breast carcinoma NOS (100x)

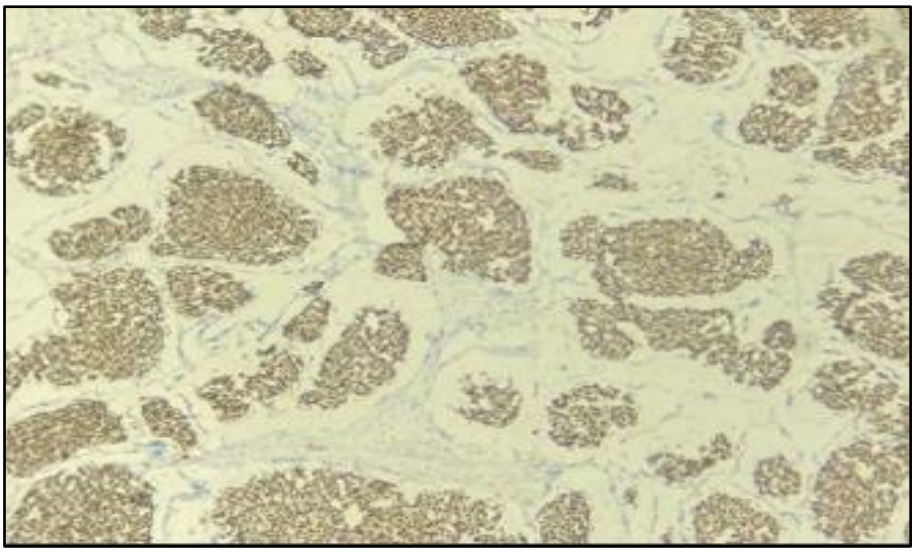

Figure 35: Microphotograph of IHC marker PR showing nuclear positivity in invasive breast carcinoma NOS (100x)

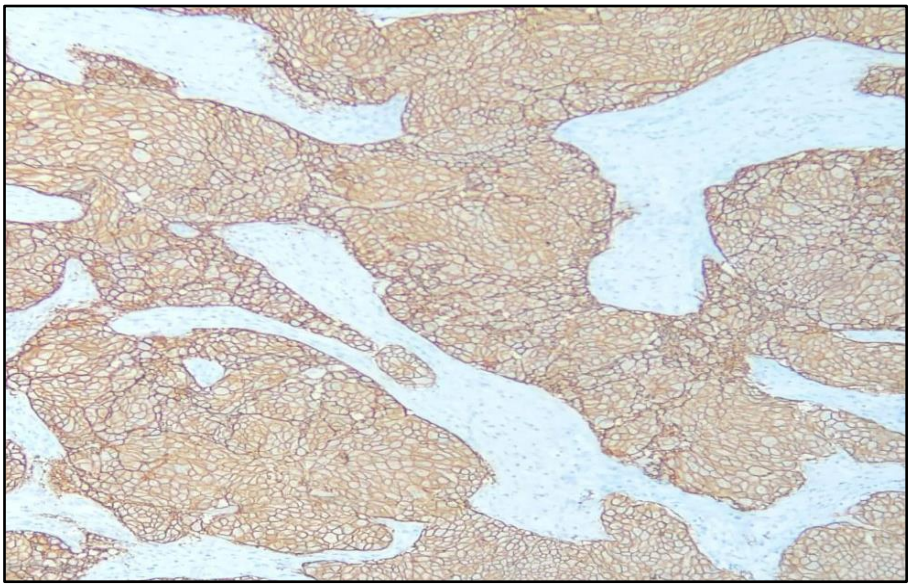

Figure 36: Microphotograph of IHC marker HER2/neu showing membranous positivity in invasive breast carcinoma NOS (200x)

DISCUSSION

Breast cancer is the most widespread form of carcinoma among women in both high- and low-resource environments, accounting for about one million of the estimated 10 million tumor found annually in both sexes globally[3,10]. Since metastatic disease is the cause of death associated with breast cancer, a deeper understanding of the molecular origins of spreading disease would be useful in the medical fields of detection, therapy and prognosis[52]. Although the awareness campaigns have begun to move the needle toward a younger diagnostic age, the frequency of aggressive tumors, such as "ER-ve," "PR-ve," "HER2/neu+ve," or "triple negative tumors," in this age range is more worrisome. There is an urgent need to concentrate on these aggressive tumors due to their increasing incidence[53].

A comprehensive search for prospective disease markers is necessary to develop tailored care, especially for those with therapeutic and prognosis implications[54]. In this instance, the marker in question provided strong proof that the evaluation and application of these markers for breast cancer will be crucial to the creation of more accurate, effective and patient-friendly treatments[54]. In order to ascertain whether CD44 is associated with a particular clinicopathological feature and hormonal state, as well as whether it is an independent prognostic marker, we investigated CD44 expression in instances of breast cancer.

Distribution of the study population based on Age and its correlation with CD44 expression-

Our study had included a total 60 cases of invasive breast carcinoma with age group ranging from thirty years to eighty years, with average age of distribution of patients having 54.5 years and median age was fifty-five years. The highest number of CD44 positivity was seen in the study population more than 60 years of age, with 19 (37.2%) cases out of 22 cases followed

by 18 cases (35.2%) in age group of 51-60 years, while the study group with age less than 50 years of age i.e, between 41-50 years show positivity in 8 (15.6) out of 12 cases and 6 cases (12%) positivity out of total 8 cases under age group of 31-40 years. P value was 0.008, which shows statistically significant correlation between CD44 expression and patient's age which means CD44 expression was seen more often in postmenopausal patients. Similar finding were found in study of M.A Elbaiomy et al.,[11] and Honeth G et al.,[56] found highest number of cases were in the age group of 50 to 70 years,i.e 43 (56.6%) cases and 30 to 50 years had 33 (43.4%) with a median age of 50 years. Honeth G et al,[56] and Weiyan Z et al.,[18] with median age was 64.1 years and 50 years respectively showed maximum number of cases were in age group of postmenopausal age which was equivalent to our study. In the study done by Yousef R et al.,[17] with mean age 47, maximum cases accounted were in the age group younger than 50 years of age, shows no stastistically significant correlation between CD44 and age of the patient.

Comparison of the size of the tumor with CD44 expression-

Women with tumor size < 1cm and negative lymph node status have 10- year survival rate of >90%, and if tumor size >2cm, 77% 10- year survival rate is noted. The risk of axillary lymph node metastasis also rises with the size of the primary tumor[27]. Tumor sizes in this study ranged from 1 to 10 cm. In current study, majority of the cases i.e in 28 (46.6%) cases, tumor size was between 2-5cm (T2) showed CD44 positivity and was statistically correlation with p value < 0.05. Whereas in the study conducted by, Weiyan Z et al.,[18] and Yousef R et al.,[17] the maximum number of cases belonged to size T2 (2-5 cm), i.e (32 out of 51 cases) and (59 out of 100 cases) and were not statistically significant with p value >0.05.

Comparison of CD44 expression with different histological types-

Histopathologically proved breast carcinoma cases were taken in this study. All cases were examined using a light microscope. The following types were included according to WHO

classification- Infiltrating ductal carcinoma- NOS, infiltrating lobular carcinoma, Encapsulated papillary carcinoma and Invasive papillary carcinoma. Majority cases were of Infiltrating ductal carcinoma- NOS, i.e., 54 out of 60 cases (90%). However, no histological types showed statistically significant differences in CD44 expression.. However in a study done by Honeth G et al.,[56] where majority of cases were infiltrating ductal carcinoma (176 out of 240 cases) shows statistical significant correlation with p value <0.05., whereas studied done by D Korfias et al.,[15] Sari Voutilainen et al.,[16] Weiyan Z et al.[18] didn't take histological type to correlate with CD44 expression.

Table 23-Correlation of CD44 expression with Histological Grade of the Tumor-

AUTHOR	SAMPLE SIZE	p VALUE FOR HISTOLOGICAL GRADE
Present study	60	**0.02**
Tomar R et al	58	>0.05
Yousef R et al	100	1.0
M.A Elbaiomy et al	32	**0.001**
D Korfias et al	104	**0.001**
Sari Voutilainen et al	75	1.0

Survival of breast carcinoma patients decreases with a higher grade.[1,27] Present study shows, Grade 2 tumors, were found to be in the highest percentage (53.3%) with statistical significant correlation having p value <0.05. The same findings were seen in a study conducted by Tomar R et al.[55] and by Yousef R et al.,[17] where 40 out of 58 cases (69%) and 82 out of 100 cases (82%) were of histological grade 2, respectively. They did not, find any statistically significant relationship between CD44 and histological grade, which was consistent with the research

carried out by these two authors. While in a study performed by M.A Elbaiomy et al.,[11] and D Korfias et al.,[15] Among the CD44 positive group, CD44 expression was found to be linked to a higher tumor grade, or Grade 3, with a p value of -0.001. They concluded that increased expression of CD44 in higher histologic grades suggests that tumor grade could have aggressive behaviour and have a poor prognosis. While the study conducted by Sari Voutilainen et al,[16] it was observed that more number of cases had tumor grade 3 but shows no statistical significant correlation.

Correlation of CD44 expression with Lymph Node Metastases-

The presence of nodal metastasis is correlated with the probability of distant metastasis. However, removing involved Lymph nodes doesn't lower the risk of future metastatic diseases. Ten to twenty percent of women who do not have axillary lymph node metastases develop distant metastases.[27,46] Recurrence after 10 to 25 years post-diagnosis has been associated with a higher initial involvement of lymph nodes, with rates varying from 12.7% to 24.6%.[57] In the current study, out of 40 cases of lymph node metastasis, 33 (65%) exhibited CD44 positivity. However, the analysis indicated that there is no statistically significant correlation among CD44 expression and lymph node metastasis, as evidenced by a p-value of 0.44. In the study performed by Honeth G et al.,[56] and Yousef R et al.,[17] same findings were noted. Honeth G et al.,[56] observed that, 45 cases (60%) out of 159 lymph node positive groups showed CD44 positivity. Yousef R et al.,[17] found that 45 cases (61%) out of 73 lymph node metastasis, showed CD44 positivity. However, no statistical correlation was found in these two studies. These findings were different with the different studies conducted by Weiyan Z et al.,[18] and D Korfias et al.,[15] where CD44 expression was significantly correlated with lymph node metastasis which may indicate that CD44 over-expression is strongly associated with aggressive features and

poor prognostic parameters in carcinoma breast. It has not affected the overall survival as well as disease-free survival of patients.

Table24 -Comparison of CD44 expression with ER, PR AND HER2/neu status-

AUTHOR	SAMPLE SIZE	p VALUE FOR HER2/neu STATUS
Present study	60	**0.04**
Honeth G et al	240	**0.002**
M.A Elbaiomy et al	32	0.006
Sari Voutilainen et al	62	0.41

According to the current work, there is a highly significant correlation between CD44 with HER2/neu expression and an insignificant association with positive ER and positive PR expression. In the present study, CD44 was statistically significant with HER2/neu expression showing (p value 0.04). Similar finding was seen in study done by Honeth G et al.,[56] where 38 out of 240 (16%) cases showed HER2/neu positivity having (p value 0.002). Our findings were inconsistent with the research conducted by M.A Elbaiomy et al.,[11] and Sari Voutilainen et al.,[16] where out of 32 cases 7 (22%) and out of 62 cases 2 (3%) shows HER2/ neu positivity but were statistical insignificant with p value of 0.006 and 0.417 respectively. However in the latter study PR expression shows statistical significant correlation with CD44 expression having p value (0.02). In a study done by Yousef R et al.,[17] CD44 was expressed more in ER positive and PR positive status than ER/PR negative status similar to our study, showing insignificant correlation with CD44 expression. They have also examined the relationship between CD44 and other markers such as Ki67 and p53, but the findings were inconclusive.

In our study CD44 expression was found statistically significant with tumor size, histological grade and HER2/neu status that specify the aggressive behaviour of the tumor and poor prognosis.

SUMMARY

- A hospital-based cross-sectional study was done. The study involved mastectomy specimens of primary breast cancer collected in the histopathology section of the Department of Pathology from 1st May 2023 to 31st December 2024.
- The histopathological diagnosis of all cases included in this study was based on routine microscopic examination of H&E stain.
- Data regarding the age of the patient and tumor size was collected, whereas slides were evaluated for histological type, histological grade of tumor and lymph-node status.
- The IHC markers- Oestrogen receptor, Progesterone receptor, HER2/neu and CD44 were studied in all the cases of invasive breast carcinoma.
- CD44 expression was then compared with various prognostic factors like patient's age, size of tumor, histological type, histological grade, lymph node status, ER, PR and HER2/neu status.
- The age group of the patients in the study ranged from thirty to eighty years, with the average age of patients being 54.5 years.
- CD44 positivity was seen in 51 (85%) cases among the 60 cases studied and 9 (15%) cases showed CD44 negative immune reactivity.
- CD44 expression was strongly associated with patient's age, tumor size, histological grade and HER2/neu status cases in this study with statistical significant correlation.
- No statistically significant expression was found between the histological type, lymph node status and ER, PR status.

CONCLUSION

In current study, CD44 expression is related with poor prognostic factors in breast cancer such as histological grade, tumor size and HER2/neu status. The study population showed an overall CD44 expression of 85%. Among CD44 positive groups, majority of cases (47%), had tumor size between 2-5cm shows grade 2 (51%) and HER2/neu negative cases were 55%. This suggests that CD44 expression is linked to a bad prognosis. Nonetheless, no meaningful statistical relationship was identified among other clinicopathological and prognostic factors such as histological type, lymph node metastasis and ER/PR status. Since CD44 expression maintain the stemness of tumor cells as well as good promotion of tumorigenesis it can be contemplated as a prognostic and therapeutic marker that helps in further treatment modalities.

Drawback of the current study: As there is diversity in the expression of CD44 with different parameters, a study with a larger sample size is needed.

Recommendations: Additional research is required to ascertain the precise prognostic significance of CD44 marker, effective therapeutic strategies that aim to eliminate CSCs by targeting these cells.

BIBLIOGRAPHY

1. Nassar MI, Bebars SM, Said RM, Mustafa TM. Immunohistochemical expression of cyclooxygenase- 2 (COX-2) in breast cancer. The Egyptian Journal of Hospital Medicine 2019 1st April;75(3):2397- 405.

2. Geršak K, Geršak BM, Gazić B, Klevišar Ivančič A, Drev P, Ružić Gorenjec N, Grašič Kuhar C. The possible role of anti-and protumor-infiltrating lymphocytes in pathologic complete response in early breast cancer patients treated with neoadjuvant systemic therapy. Cancers. 2023 Sep 29;15(19):4794.

3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-249.

4.Mehrotra R, Yadav K. Breast cancer in India: Present scenario and the challenges ahead. World Journal of Clinical Oncology 2022; 3(3): 209–218.

5. Masood S. Breast cancer subtypes: morphologic and biologic characterization. Womens Health (Lond). 2016 Jan;12(1):103-19.

6. Fan Y, He S. The characteristics of tumor microenvironment in triple negative breast cancer. Cancer Manag Res. 2022 Jan 3;(14):1-7.

7. Leyla Tekin, Serkan Yasar Celik. Immunohistochemical expression of COX 2 and its relationship with prognostic parameters in breast cancer. Cyprus journal of medical sciences 2021; 6(1): 39-43

8. Saha S, Ganguly S, Sarkar DK, Hazra A. Clinicopathological characteristics of patients of certain molecular subtypes and elevated postoperative cancer antigen 15.3 levels and its correlation with menopausal status. Indian J Pathol Microbiol 2016;(59):172-6.

9. Louhichi T, Saad H, Dhiab MB, Ziadi S, Trimeche M. Stromal CD10 expression in breast cancer correlates with tumor invasion and cancer stem cell phenotype. BMC cancer. 2018 Dec;(18):1-9.

10. Łukasiewicz S, Czeczelewski M, Forma A, Baj J, Sitarz R, Stanisławek A. Breast cancer—epidemiology, risk factors, classification, prognostic markers, and current treatment strategies—an updated review. Cancers. 2021 Aug 25;13(17):4287.

11. Elbaiomy MA, Akl T, Atwan N, Elsayed AA, Elzaafarany M, Shamaa S. Clinical Impact of Breast Cancer Stem Cells in Metastatic Breast Cancer Patients. Journal of Oncology 2020; Article ID 2561726:1- 8.

12. Dzobo K, Sinkala M. Cancer Stem Cell Marker CD44 Plays Multiple Key Roles in Human Cancers: Immune Suppression/Evasion, Drug Resistance, Epithelial-Mesenchymal Transition, and Metastasis. OMICS 2021 ;25(5):313-332.

13. Mesrati MH, Syafruddin SE, Mohtar MA, Syahil A. CD44: A Multifunctional Mediator of Cancer Progression. Biomolecules 2021, 11, 1850.

14. Li W, Ma H, Zhang J, Zhu L, Wang C, Yang Y. Unraveling the roles of CD44/ CD24 and ALDH1 as cancer stem cell markers in tumorigenesis and metastasis. Scientific reports 2017; 7(1):13856

15. Korfias D, Contis J, Frangou-Plemenou M, Gennatas K, Kondis A, Vlachodimitropoulos D. Stem cells in ductal breast cancer: immunohistochemical expression of CD44, CD24, CD133, and ALDH-1 markers in 104 cases. European Journal of Gynaecological Oncology 2020; 62(3): 2943.

16. Voutilainen S, Heikkila P, Sampo M, Nevanlinna H, Blomqvist C. Mattson J. Expression of markers of stem cell characteristics, epithelial-mesenchymal transition, basal-like phenotype, proliferation, and androgen receptor in metaplastic breast cancer and their prognostic impact. Acta Oncologica 2021;60(9):1233-1239.

17. Rousta Y, Sanaat Z, Naikanfar AR, Dolatkhah R, Fakhrjou A. Predictive Value of CD44 for Prognosis in Patients with Breast Cancer. Asian Pac J Cancer Prev 2020 ;21 (9): 2561-2567.

18. Zou W, Tang Y, Zheng R, Wang Z, Zeng H, Chen Z et al. Association of CD44 and CD24 phenotype with lymph node metastasis and survival in triple-negative breast cancer. Int J Clin Exp Pathol 2020;13(5):1008-1016.

19. Skandalakis JE. Embryology and anatomy of the breast. In: Spear SL, editor. Breast Augmentation: Principles and Practice. Berlin, Heidelberg: Springer Berlin Heidelberg; 2009; 3-24.

20. Hoda SA, Rosen PP, Brogi E, Koerner FC. Rosen's Breast Pathology. Philadelphia: Lippincott Williams & Wilkins; 2020.

21. Kopans DB: Breast Imaging, 3rd edn. Philadelphia, Lippincott Williams & Wilkins 2007, pp 7–43.

22. Haider R. Anatomy of the Breast. International Journal of Scientific Multidisciplinary Research. 2023 Jun 30;1(5):401-22.

23. Lindberg MR. Diagnostic Pathology: Normal Histology: Diagnostic Pathology: Normal Histology-E- Book. Elsevier Health Sciences; 2017 Aug 18.

24. World Health Organization. Global Health Estimates 2016: Disease Burden by Cause, Age, Sex, by Country and by Region, 2000–2016; World Health Organization: Geneva, Switzerland, 2018;1242-7

25. Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, Znaor A, Soerjomataram I, Bray F. Global cancer observatory: cancer today. Lyon, France: international agency for research on cancer. 2018 Nov 5;3(20):2019.

26. Sharma, R. Global, regional, national burden of breast cancer in 185 countries: Evidence from GLOBOCAN 2018. Breast Cancer Res. Treat. 2021, 187, 557–567.

27. Kumar V, Abbas AK, Fausto N, Aster JC. Robbins and Cotran pathologic basis of disease, professional edition e-book. Elsevier health sciences; 2014 Aug 27. 75

28. Key, T.J.; Appleby, P.N.; Reeves, G.K.; Travis, R.C.; Alberg, A.J.; Barricarte, A.; Berrino, F.; Krogh, V.; Sieri, S.; Brinton, L.A.; et al. Sex hormones and risk of breast cancer in premenopausal women: A collaborative reanalysis of individual participant data from seven prospective studies. Lancet Oncol. 2013;14:1009–1019.

29. Benz, C.C. Impact of aging on the biology of breast cancer. Crit. Rev. Oncol. 2008; 66: 65–74.

30. Shiovitz, S.; Korde, L.A. Genetics of breast cancer: A topic in evolution. Ann. Oncol. 2015; 26:1291– 1299.

31.Ng, J.; Shuryak, I. Minimizing second cancer risk following radiotherapy: Current perspectives. Cancer Manag. Res. 2014; 7: 1–11.

32. Tobon H, Salazar H: Ultrastructure of human mammary gland.I.Development of fetal gland throughout gestation. J Clin Endocrinol Metab 39:443-456, 1974.

33. Epstein JI, Lotan TL. The Breast In: Kumar V, Abbas AK, Aster JC. Pathologic Basis Of disease. 10th ed. Philadelphia; Elseiver; 2019:1037-99

34. Eroles, P.; Bosch, A.; Pérez-Fidalgo, J.A.; Lluch, A. Molecular biology in breast cancer: Intrinsic subtypes and signaling pathways. Cancer Treat. Rev. 2012; 38: 698–707.

35. Newman, L.A.; Reis-Filho, J.S.; Morrow, M.; Carey, L.A.; King, T.A. The 2014 Society of Surgical Oncology Susan G. Komen for the Cure Symposium: Triple-Negative Breast Cancer. Ann. Surg. Oncol. 2014; 22:874–882.

36. Prat, A.; Parker, J.S.; Karginova, O.; Fan, C.; Livasy, C.; Herschkowitz, J.I.; He, X.; Perou, C.M. Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast Cancer Res. 2010; 12: R68.

37. Shah H, et al. Clinical Significance of Notch Receptors In Triple Negative Breast Cancer; Research square: feb 2022;1-2

38. Nounou et al. Breast Cancer: Conventional Diagnosis and Treatment Modalities and Recent Patents and Technologies. Breast Cancer: Basic and Clinical Research 2015:9(S2) 17–34 doi:10.4137/BCBCR.S29420.

39. Kerlikowske K, Hubbard RA, Miglioretti DL, et al; Breast Cancer Surveillance Consortium. Comparative effectiveness of digital versus film-screen mammography in community practice in the United States: a cohort study. Ann Intern Med. 2011;155(8):493–502.

40. Van Goethem M, Tjalma W, Schelfout K, Verslegers I, Biltjes I, Parizel P. Magnetic resonance imaging in breast cancer. Eur J Surg Oncol. 2006;32(9): 901– 910.

41. Bray F, et al. The changing global patterns of female breast cancer incidence and mortality. Breast Cancer Res 2004;229

42. Mody VV, Nounou MI, Bikram M. Novel nanomedicine-based MRI contrast agents for gynecological malignancies. Adv Drug Deliv Rev. 2009;61(10): 795–807.

43. O'Connor M, Rhodes D, Hruska C. Molecular breast imaging. Expert Rev Anticancer Ther. 2009;9(8):1073–10.

44. WHO classification of tumours. breast tumours. Lyon: International Agency for Research on Cancer;5TH ed; 2019;1-161.

45. Norton KA, Wininger M, Bhanot G, Ganesan S, Barnard N, Shinbrot T. A 2D mechanistic model of breast ductal carcinoma in situ (DCIS) morphology and progression. J Theor Biol. 2010;263(4):393–406.

46. Rosai J. Rosai and Ackerman's surgical pathology. 11th ed. Edinburgh: Mosby; 2018. p. 1434-1512

47. Consensus Meeting. Oct 3 to 5, 1985, New York, Cancer Committee of the College of American Pathologists: Is 'fibrocystic disease' of the breast precancerous? Arch Pathol Lab Med 1986, 110: 171–173.

48. Tuffaha S.A. Et al. Immunohistochemistry in tumor diagnostics:Markers and Immunoprofile of breast tumors,Springer International publishing 2018:71-81

49. David J. Dabbas. Diagnostic Immunohistochemistry,Theranostic and Genomic Applications:Immunohistology of breast. Elsevier:5th edition,2019:718

50. Chen C, Zhao S, Karnad A, Freeman JW. The biology and role of CD44 in cancer progression: therapeutic implications. J Hematol Oncol. 2018 May 10;11(1):64. doi: 10.1186/s13045-018-0605-5. PMID: 29747682; PMCID: PMC5946470.

51. Kabiraj A, Gupta J, Khaitan T, Bhattacharya PT. Principle and Techniques of Immunohistochemistry – a Review. Int J Biol Med Res. 2015;6(3):5204–10.

52. Kesse-Adu R, Shousha S. Myoepithelial markers are expressed in atleast 29% of oestrogen receptor negative invasive breast carcinoma. Mod Pathol 2004;17:646-52.

53. Anders CK, Johnson R, Litton J, Phillips M, Bleyer A. Breast Cancer Before Age 40Years. Seminars in oncology. 2009;36(3):237-249.

54. Banin Hirata BK, Oda JM, Losi Guembarovski R, Ariza CB, Oliveira CE, Watanabe MA. Molecular markers for breast cancer: prediction on tumor behavior. Disease markers. 2014;(1):513158.

55. Tomar R, Rakheja G, Verma N, Thakur S, Khurana N, Ghuliani D. Role of CD44 as cancer stem cell marker in triple-negative breast cancer and its association with histological grade and angiogenesis. Indian Journal of Pathology and Microbiology. 2023 Apr 1;66(2):258-63.

56. Honeth, G., Bendahl, PO., Ringnér, M. *et al.* The $CD44^{+}/CD24^{-}$phenotype is enriched in basal-like breast tumors. *Breast Cancer Res* (2008). https://doi.org/10.1186/bcr2108.

57. Pedersen RN, Esen BÖ, Mellemkjær L, Christiansen P, Ejlertsen B, Lash TL, Nørgaard M, Cronin- Fenton D. The incidence of breast cancer recurrence 10-32 years after primary diagnosis. JNCI: Journal of the National Cancer Institute. 2022 Mar 1;114(3):391-9.

ANNEXURE I

Azadi Ka Amrit Mahotsav

BLDE

(DEEMED TO BE UNIVERSITY)

Declared as Deemed to be University u/s 3 of UGC Act, 1956

Accredited with 'A' Grade by NAAC (Cycle-2)

The Constituent College

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

BLDE (DU)/IEC/ 926/2023-24 10/4/2023

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this University met on **Saturday, 18th March, 2023 at 11.30 a.m. in the CAL Laboratory, Dept. of Pharmacology,** scrutinized the Synopsis/ Research Projects of Post Graduate Student / Under Graduate Student /Faculty members of this University /Ph.D. Student College from ethical clearance point of view. After scrutiny, the following original/ corrected and revised version synopsis of the thesis/ research projects has been accorded ethical clearance.

TITLE: "ASSESSMENT OF EXPRESSION OF IMMUNOHISTOCHEMICAL MARKER CD44 IN CARCINOMA BREAST".

NAME OF THE STUDENT/PRINCIPAL INVESTIGATOR: DR.RANU KUMARI

NAME OF THE GUIDE: DR.SATISH ARAKERI, ASSOCIATE PROFESSOR, DEPT. OF PATHOLOGY.

Dr. Santoshkumar Jeevangi
Chairperson
IEC, BLDE (DU),
VIJAYAPURA
Chairman,
Institutional Ethical Committee,
BLDE (Deemed to be University)
Vijayapura

Dr.Akram A. Naikwadi
Member Secretary
IEC, BLDE (DU),
VIJAYAPURA
MEMBER SECRETARY
Institutional Ethics Committee
BLDE (Deemed to be University)
Vijayapura-586103. Karnataka

Following documents were placed before Ethical Committee for Scrutinization.

- Copy of Synopsis/Research Projects
- Copy of inform consent form
- Any other relevant document

Smt. Bangaramma Sajjan Campus, B. M. Patil Road (Sholapur Road), Vijayapura - 586103, Karnataka, India.
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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 _____ of 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 "**ASSESSMENT OF EXPRESSION OF IMMUNOHISTOCHEMICAL MARKER CD44 IN CARCINOMA BREAST**" under the guidance of **Dr**. **SATISH ARAKERI**, requesting my participation in the study. Apart from routine treatment procedures, pre-transfusion, post-transfusion, and follow-up observations will be utilized for the study as reference data.

The Doctor has also informed me that during the conduct of this procedure may encounter adverse results. Most of the above complications are treatable but not anticipated; hence there is a chance of aggravating my condition. In rare circumstances, it may prove fatal despite anticipated diagnosis and the 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 a useful reference for the treatment of other similar cases soon. Also, I may benefit from getting relief from suffering or a cure for 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 anyone other than my legal hirer or me except for academic purposes.

The Doctor informed me that though my participation is purely voluntary, based on the information given, I can ask for any clarification during the course of treatment/study related to diagnosis, the procedure of treatment, result of treatment, or any 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 81 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 Doctor:

Signature of the patient:

Witness: 1.

2.

Date:

Place:

B.L.D.E (DEEMED TO BE UNIVERSITY)

ಶ್ರೀ ಬಿ.ಎಂ. ಪಾಟೀಲ್ ಮೆಡಿಕಲ್ ಕಾಲೇಜು, ಆಸ್ಪತ್ರೆ ಮತ್ತು ಸಂಶೋಧನಾ ಕೇಂದ್ರ, ವಿಜಯಪುರ- 586103

ಪ್ರಬಂಧ/ಸಂಶೋಧನೆಯಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ಮಾಹಿತಿ ಪಡೆದ ಸಮ್ಮತಿ

ನಾನು, ಕೆಳಗಿನವರು_____ ಸಹಿಯಿಟ್ಟವರು, ಮಗ/ಮಗಳು/ಪತ್ನಿಯ _____ ವಯಸ್ಸು _____ವರ್ಷಗಳು, ಸಾಮಾನ್ಯವಾಗಿ ನಿವಾಸಿಸುವ ಸ್ಥಳದ ಹೆಸರು_____, ಇಲ್ಲಿ ಹೇಳಿದ್ದೇನೆ/ಘೋಷಿಸುತ್ತೇನೆ ಡಾಕ್ಟರ್ ಹೆಸರು_____ ಅವರು ಆಸ್ಪತ್ರೆ ಹೆಸರು_____ ಅವರು ನನ್ನನ್ನು ಪೂರ್ಣವಾಗಿ ಪರೀಕ್ಷಿಸಿದರು ದಿನಾಂಕದಲ್ಲಿ_____ ಸ್ಥಳ ಹೆಸರು_____ ಮತ್ತು ನನಗೆ ನನ್ನ ಭಾಷೆಯಲ್ಲಿ ವಿವರಿಸಲಾಗಿದೆ ನಾನು ಒಂದು ರೋಗ (ಸ್ಥಿತಿ) ಅನುಭವಿಸುತ್ತಿದ್ದೇನೆ. ಮುಂದುವರಿದು ಡಾಕ್ಟರ್ ನನಗೆ ತಿಳಿಸಿದ್ದಾರೆ ಅವರು ಒಂದು ಪದ್ದತಿ/ಸಂಶೋಧನೆ ನಡೆಸುತ್ತಿದ್ದಾರೆ ಶೀರ್ಷಿಕೆಯುಳ್ಳ "**ASSESSMENT OF EXPRESSION OF IMMUNOHISTOCHEMICAL MARKER CD44 IN CARCINOMA BREAST**" ಡಾಕ್ಟರ್ **DR. SATISH ARAKERI**, ಮಾರ್ಗದರ್ಶನದಲ್ಲಿ ನನ್ನ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಯನ್ನು ಕೇಳಿದ್ದಾರೆ ಅಧ್ಯಯನದಲ್ಲಿ.

ಡಾಕ್ಟರ್ ನನಗೆ ಇದನ್ನು ಕೂಡಾ ತಿಳಿಸಿದ್ದಾರೆ ಈ ಕ್ರಮದ ನಡುವಲ್ಲಿ ಪ್ರತಿಕೂಲ ಫಲಿತಾಂಶಗಳನ್ನು ಎದುರಿಸಬಹುದು. ಮೇಲೆ ಹೇಳಿದ ಪ್ರಕಟಣೆಗಳಲ್ಲಿ, ಅಧಿಕಾಂಶವು ಚಿಕಿತ್ಸಿಸಬಹುದಾದರೂ ಅದನ್ನು ನಿರೀಕ್ಷಿಸಲಾಗುತ್ತಿಲ್ಲ ಆದ್ದರಿಂದ ನನ್ನ ಸ್ಥಿತಿಯ ಹಿರಿದಾಗುವ ಅವಕಾಶವಿದೆ ಮತ್ತು ಅಪರೂಪದ ಸಂದರ್ಭಗಳಲ್ಲಿ ಅದು ಮರಣಕಾರಕವಾಗಿ ಪರಿಣಮಿಸಬಹುದು ಹೊಂದಿದ ರೋಗನಿರ್ಧಾರ ಮತ್ತು ಯಥಾಶಕ್ತಿ ಚಿಕಿತ್ಸೆ ಮಾಡಲು ಹೊಂದಿದರೂ, ಮುಂದುವರಿದು ಡಾಕ್ಟರ್ ನನಗೆ ತಿಳಿಸಿದ್ದಾರೆ ನನ್ನ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆ ಈ ಅಧ್ಯಯನದ ಫಲಿತಾಂಶಗಳ ಮೌಲ್ಯಮಾಪನದಲ್ಲಿ ಸಹಾಯಕವಾಗುತ್ತದೆ ಇತರ ಸಮಾನ ಪ್ರಕರಣಗಳ ಚಿಕಿತ್ಸೆಗೆ ಉಪಯುಕ್ತ ಉಲ್ಲೇಖವಾಗಿದೆ, ಮತ್ತು ನಾನು ಅನುಭವಿಸುವ ರೋಗದಿಂದ ವಿಮುಕ್ತಿ ಅಥವಾ ಗುಣಮುಖಗೊಳ್ಳುವಲ್ಲಿ ನನಗೆ ಪ್ರಯೋಜನವಾಗಬಹುದು.

ಡಾಕ್ಟರ್ ನನಗೆ ಇದನ್ನು ಕೂಡಾ ತಿಳಿಸಿದ್ದಾರೆ ನನ್ನಿಂದ ನೀಡಿದ ಮಾಹಿತಿ, ಮಾಡಿದ ಪರಿಶೀಲನೆಗಳು / ಫೋಟೋಗ್ರಾಫ್‌ಗಳು / ವೀಡಿಯೋ ಗ್ರಾಫ್‌ಗಳು ನನ್ನ ಮೇಲೆ ತೆಗೆದುಕೊಳ್ಳಲಾಗುವ ಅನ್ವೇಷಕರು ರಹಸ್ಯವಾಗಿ

ಇಡುವರು ಮತ್ತು ನಾನು ಅಥವಾ ನನಗೆ ಕಾನೂನು ದೃಷ್ಟಿಯಲ್ಲಿ ಸಂಬಂಧಿತರನ್ನು ಹೊರತುಪಡಿಸಿ ಇತರ ವ್ಯಕ್ತಿಯಿಂದ ಮೌಲ್ಯಮಾಪನ ಮಾಡಲಾಗುವುದಿಲ್ಲ. ಡಾಕ್ಟರ್ ನನಗೆ ತಿಳಿಸಿದ್ದಾರೆ ನನ್ನ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆ ಶುದ್ಧವಾಗಿ ಸ್ವೇಚ್ಛಾಯಿತ, ನನ್ನಿಂದ ನೀಡಿದ ಮಾಹಿತಿಯ ಆಧಾರದ ಮೇಲೆ, ಚಿಕಿತ್ಸೆ / ಅಧ್ಯಯನದ ಸಂಬಂಧದಲ್ಲಿ ರೋಗನಿರ್ಧಾರ, ಚಿಕಿತ್ಸೆಯ ವಿಧಾನ, ಚಿಕಿತ್ಸೆಯ ಫಲಿತಾಂಶ ಅಥವ ಭವಿಷ್ಯದ ಪ್ರವೃತ್ತಿಗಳು ಬಗ್ಗೆ ಯಾವುದೇ ಸ್ಪಷ್ಟತೆ ಕೇಳಬಹುದು. ಅದೇ ಸಮಯದಲ್ಲಿ ನನಗೆ ತಿಳಿಸಲಾಗಿದೆ ನಾನು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನನ್ನ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಯನ್ನು ನಿಲ್ಲಿಸಬಹುದು ನಾನು ಬಯಸಿದರೆ ಅಥವಾ ಅನ್ವೇಷಕರು ಅಧ್ಯಯನದಿಂದ ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ನನ್ನನ್ನು ನಿಲ್ಲಿಸಬಹುದು.

ಪ್ರಬಂಧ ಅಥವಾ ಸಂಶೋಧನೆಯ ಸ್ವಭಾವ, ಮಾಡಿದ ರೋಗನಿರ್ಧಾರ ಮತ್ತು ಚಿಕಿತ್ಸೆಯ ವಿಧಾನವನ್ನು ಅರ್ಥಮಾಡಿಕೊಂಡು, ನಾನು ಕೆಳಗಿನ ಶ್ರೀ / ಶ್ರೀಮತಿ_____ ನನ್ನ ಪೂರ್ಣವಾದ ಪ್ರಜ್ಞೆಯ ಸ್ಥಿತಿಯಲ್ಲಿ ಹೇಳಿದ ಸಂಶೋಧನೆ / ಪ್ರಬಂಧದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ಒಪ್ಪುತ್ತೇನೆ.

ರೋಗಿಯ ಸಹಿ

ಡಾಕ್ಟರನ ಸಹಿ

ಸಾಕ್ಷಿಗಳು

1)

2)

ANNEXURE III

PROFORMA

- **NAME :**
- **AGE :** **OP/IP No.:**
- **SEX :**
- **RELIGION :**
- **OCCUPATION** **D.O.A:**
- **RESIDENCE :** **D.O.D:**
- **Presenting Complaints :**
- **Past history :**
- **Personal history :**
- **Family history :**
- **Treatment history :**
- **USG/Mammography :**
- **FNAC :**
- **Examination finding :**
- **Vitals :**
- **PR:**
- **BP:** **RR:**
- **Weight :** **TEMPERATURE:**
- **Procedure:**
- **Tumor type** (histologic type):
- **Tumor size:**
- **Lymph node status:**
- **HPR microscopy:**
- **MODIFIED SCRAFF BLOOM RICHARDSON GRADE:**
- **pTNM staging:**
- **IHC:**

ER, PR, HER2/neu expression:

CD44 expression:

KEY TO MASTER CHART

Sr. No. - Serial Number

Case number - Histopathology number

NST- No special type

NOS- Not otherwise specified

ER - Estrogen receptor immunostaining

PR - Progesterone receptor immunostaining

HER2 neu - Herceptin receptor immunostaining

CD44- Cluster of differentiation

MASTER CHART

SL NO.	Age/ Gender	HPR NO.	ER	PR	Her2/ neu	CD44	Diagnosis	Lymph node	Tumor size	Histological Grade
1	49/F	2811/21	Positive	Positive	Negative	Positive	IFILTRATING DUCTAL CARCINOMA NOS	Involved	T1	3
2	59/F	3900/21	Negative	Positive	Positive	Positive	INVASIVE DUCTAL CARCINOMA NST	Not Involved	T0	2
3	75/F	4003/21	Positive	Negative	Negative	Positive	INVASIVE DUCTAL CARCINOMA NST	Involved	T1	2
4	64/F	4238/21	Positive	Positive	Positive	Negative	INVASIVE DUCTAL CARCINOMA NST	Not Involved	T2	2
5	55/F	4340/21	Negative	Negative	Negative	Positive	INVASIVE DUCTAL CARCINOMA NST	Not Involved	T2	1
6	35/F	4560/21	Positive	Positive	Positive	Positive	INVASIVE BREAST CARCINOMA NST	Not Involved	T1	3
7	35/F	4452/21	Negative	Negative	Negative	Positive	IFILTRATING DUCTAL CARCINOMA NOS	Involved	T1	2
8	70/F	5823/21	Positive	Positive	Positive	Positive	IFILTRATING DUCTAL CARCINOMA NOS	Not Involved	T3	1
9	48/F	5907/21	Positive	Negative	Negative	Positive	IFILTRATING DUCTAL CARCINOMA NOS	Involved	T3	1
10	48/F	194/22	Positive	Positive	Positive	Negative	INVASIVE DUCTAL CARCINOMA NST	Involved	T2	2

11	54/F	1719/22	Negative	Negative	Negative	Positive	IFILTRATING DUCTAL CARCINOMA NOS	Involved	T1	1
12	50/F	1874/22	Positive	Positive	Negative	Positive	INVASIVE BREAST CARCINOMA NOS	Not Involved	T2	1
13	53/F	1890/22	Positive	Positive	Positive	Positive	IFILTRATING DUCTAL CARCINOMA NST	Involved	T1	2
14	33/F	2625/22	Positive	Positive	Positive	Positive	INVASIVE LOBULAR CARCINOMA	Involved	T2	1
15	35/F	3191/22	Negative	Negative	Positive	Negative	IFILTRATING DUCTAL CARCINOMA NST	Involved	T2	2
16	52/F	3458/22	Positive	Positive	Positive	Positive	IFILTRATING DUCTAL CARCINOMA NOS	Involved	T1	2
17	70/F	3518/22	Positive	Positive	Positive	Positive	IFILTRATING DUCTAL CARCINOMA NST	Involved	T2	2
18	35/F	3906/22	Positive	Positive	Negative	Positive	IFILTRATING DUCTAL CARCINOMA NST	Involved	T1	2
19	55/F	4228/22	Positive	Positive	Positive	Positive	IFILTRATING DUCTAL CARCINOMA NST	Involved	T2	1
20	45/F	4848/22	Positive	Positive	Negative	Positive	IFILTRATING DUCTAL CARCINOMA NOS	Involved	T3	1

21	66/F	4918/22	Negative	Negative	Positive	Positive	INVASIVE DUCTAL CARCINOMA NOS	Involved	T2	3
22	55/F	6024/22	Positive	Positive	Negative	Positive	INVASIVE BREAST CARCINOMA NST	Not Involved	T1	1
23	82/F	7243/22	Positive	Positive	Negative	Positive	INVASIVE SOLID PAPILLARY CARCINOMA	Involved	T3	3
24	54/F	836/23	Positive	Negative	Positive	Positive	INVASIVE BREAST CARCINOMA NST	Involved	T3	1
25	48/F	981/23	Negative	Negative	Positive	Negative	INVASIVE BREAST CARCINOMA NST	Involved	T3	2
26	39/F	942/23	Negative	Negative	Positive	Negative	INVASIVE BREAST CARCINOMA NST	Involved	T4	1
27	67/F	1434/23	Negative	Negative	Positive	Negative	INVASIVE DUCTAL CARCINOMA NOS	Not Involved	T4	3
28	45/F	2828/23	Positive	Positive	Negative	Positive	INVASIVE BREAST CARCINOMA NST	Involved	T2	2

29	65/F	2872/23	Positive	Positive	Negative	Positive	IFILTRATING DUCTAL CARCINOMA NOS	Involved	T2	2
30	80/F	2582/23	Positive	Positive	Negative	Positive	INVASIVE BREAST CARCINOMA NST	Involved	T4	2
31	63/F	3090/23	Negative	Negative	Negative	Negative	IFILTRATING DUCTAL CARCINOMA NOS	Involved	T2	2
32	77/F	3933/23	Positive	Positive	Negative	Positive	INVASIVE DUCTAL CARCINOMA NOS	Not Involved	T2	2
33	60/F	4341/23	Positive	Positive	Positive	Positive	INVASIVE BREAST CARCINOMA NOS	Involved	T4	1
34	42/F	4195/23	Negative	Negative	Negative	Positive	INFILTRATING DUCTAL CARCINOMA NOS	Not Involved	T1	2
35	58/F	240/23	Positive	Positive	Positive	Positive	INVASIVE DUCTAL CARCINOMA NOS	Involved	T1	2
36	50/F	273/23	Positive	Positive	Negative	Negative	INVASIVE DUCTAL CARCINOMA NST	Involved	T1	2

37	41/F	5298/23	Positive	Positive	Positive	Negative	INVASIVE BREAST CARCINOMA NOS	Involved	T1	2
38	65/F	5428/23	Negative	Negative	Negative	Positive	INVASIVE DUCTAL CARCINOMA NST	Involved	T4	2
39	60/F	5889/23	Positive	Positive	Negative	Positive	INVASIVE BREAST CARCINOMA NOS	Not Involved	T2	1
40	40/F	5904/23	Positive	Positive	Negative	Positive	INVASIVE BREAST CARCINOMA NOS	Involved	T3	2
41	58/F	6366/23	Positive	Positive	Negative	Positive	INVASIVE BREAST CARCINOMA NOS	Involved	T4	3
42	55/F	6534/23	Negative	Negative	Negative	Positive	INVASIVE DUCTAL CARCINOMA NOS	Involved	T2	2
43	58/F	466/23	Negative	Negative	Positive	Positive	INVASIVE DUCTAL CARCINOMA NOS	Not Involved	T1	1
44	62/F	7359/23	Positive	Positive	Negative	Positive	ENCAPSULATED PAPILLARY CARCINOMA	Not Involved	T2	2

45	54/F	19/24	Positive	Positive	Negative	Positive	MUCINOUS CARCINOMA NST	Not Involved	T2	1
46	60/F	178/24	Negative	Negative	Positive	Positive	INVASIVE BREAST CARCINOMA NST	Involved	T2	3
47	75/F	135/24	Negative	Negative	Positive	Positive	INVASIVE BREAST CARCINOMA NST	Involved	T2	2
48	71/F	130/24	Negative	Negative	Positive	Positive	INVASIVE DUCTAL CARCINOMA NOS	Not Involved	T2	2
49	40/F	226/24	Positive	Positive	Positive	Positive	INVASIVE DUCTAL CARCINOMA NOS	Involved	T2	2
50	70/F	14/24	Positive	Positive	Negative	Positive	INVASIVE DUCTAL CARCINOMA NOS	Not Involved	T2	2
51	75/F	2638/24	Negative	Negative	Positive	Positive	INVASIVE BREAST CARCINOMA NST	Involved	T4	3
52	58/F	2934/24	Negative	Negative	equivocal	Positive	INVASIVE BREAST CARCINOMA NST	Not Involved	T2	2

53	55/F	2972/23	Positive	Positive	equivocal	Positive	INVASIVE BREAST CARCINOMA NST	Involved	T2	1
54	37/F	2752/24	Positive	Positive	Positive	Positive	INVASIVE LOBULAR CARCINOMA NOS	Involved	T2	1
55	62/F	7359/24	Positive	Positive	Negative	Positive	ENCAPSULATED PAPILLARY CARCINOMA	Not Involved	T2	1
56	70/F	2767/24	Negative	Negative	Positive	Positive	INVASIVE BREAST CARCINOMA NOS	Involved	T2	3
57	45/F	3056/23	Negative	Negative	Negative	Positive	INVASIVE BREAST CARCINOMA NOS	Not Involved	T2	3
58	58/F	3700/24	Positive	Positive	Negative	Positive	INVASIVE BREAST CARCINOMA NOS	Not Involved	T1	2
59	70/F	6956/23	Negative	Negative	Positive	Positive	INVASIVE BREAST CARCINOMA NOS	Involved	T3	2
60	42/F	6824/23	Positive	Positive	Negative	Positive	INVASIVE BREAST CARCINOMA NOS	Involved	T3	2

RANU KUMARI

ASSESSMENT OF EXPRESSION OF IMMUNOHISTOCHEMICAL MARKER CD44 IN CARCINOMA BREAST”

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