"ASSESSMENT OF EXPRESSION OF NOVEL IMMUNOHISTOCHEMICAL MARKER PROGRAMMED DEATH LIGAND 1(PD-L1) AND ITS CORRELATION WITH CLINICOPATHOLOGICAL PARAMETERS IN BREAST CARCINOMA"

By

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Dissertation submitted to the

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

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In partial fulfillment 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 carcinoma (BC) is the leading cause of tumor burden, with an incidence of 11.8%. It will cross more than 20 lakhs by the year 2030. The incidence of breast carcinoma in India has increased by more than half in the last three decades. The PD-1/PD-L1 pathway represents an adaptive immune resistance mechanism that tumor cells exert. Despite numerous studies, there is a lack of literature on such studies in Indian patients. Moreover, the results obtained from these studies have not been uniform. Recent updates in the treatment modality of breast carcinoma indicate that the use of anti-PD-L1 monotherapy will help treat advanced breast carcinoma. As PD-L1 is a newly identified marker, studies are required to understand its immunoexpression and correlation with hormone receptor status and other prognostic factors, which helps in the therapeutic management of patients.

Hence, this study evaluated the expression of PD-L1 in breast carcinoma.

OBJECTIVES:

1. Evaluate the immunohistochemical expression of PD-L1 marker on carcinoma Breast.

2. To analyze the correlation between the expression of the PD-L1 marker with ER, PR, HER2/neu receptors, and with various clinicopathological factors, including Age of the patient, tumor size, histologic type, histologic grade, lymph node status and pTNM staging.

MATERIAL AND METHODS:

Study design: Hospital-based cross-sectional study.

Study Setting: The study will be held in the Histopathology section of the Department of Pathology, BLDE (Deemed to be University). Shri B.M Patil Medical College, Hospital, and Research Center, Vijayapura

Study population: All breast specimens were received in the Pathology BLDE department (Deemed to be University), Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura.

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

Sample size: Using G*Power ver. 3.1.9.4 software for sample size calculation, the proportion of malignant breast carcinoma tumor cells staining for PD-L1 is 14.7%; the study would require a sample size of 50 subjects with 95% confidence level and 10% absolute precision.

Data collection method: All breast specimens received in the Department of Pathology diagnosed with 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 sections will be prepared from each tissue block. For histopathological diagnosis, one tissue section will be stained by hematoxylin and eosin stain. Another tissue section will be mounted on poly L lysine-coated slide from paraffin-embedded tissue blocks, subjected to PD-L1 (programmed death ligand 1) immunohistochemical staining. The patient who has undergone the IHC study of estrogen, progesterone and Her-2-Neu receptors will be taken for the study.

RESULTS:

PD-L1 expression was seen in 14 out of 50 cases (28.0%). Expression of PD-L1 showed a statistically significant correlation with HER2/Neu.

The expression of PD-L1 is not statistically significant with clinicopathological parameters such as histologic grade, TNM staging, and ER/PR expression. Hence, it cannot be used as a prognostic tumor size and grade marker. It can be helpful to assess the indication for the use of anti-PD-L1 inhibitors in advanced breast disease.

Standardizing immunohistochemical (IHC) reporting for PD-L1 ensures reproducibility and reliability in evaluating breast carcinoma. Consistent and accurate PD-L1 assessment could significantly impact the application of novel targeted immunotherapies in treating breast carcinoma.

KEYWORDS: Breast carcinoma, PD-L1, Prognosis.

LIST OF ABBREVIATIONS USED

PD-L1: Programmed Death-Ligand 1

PD-1: Programmed cell death-1

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

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

PR: Progesterone Receptor

TNBC: Triple Negative Breast Cancer

TNM: Tumor Node Metastasis

BHGI: The Breast Health Global Initiative

NK: Natural killer cell

DC: Dendritic cell

CPS: Combined positive score

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INTRODUCTION

Breast carcinoma (BC) is the leading cause of tumor burden, with an incidence of 11.8%. It will cross more than 20 lakhs by the year 2030. The incidence of breast carcinoma in India has increased by more than half in the 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. [1]

According to WHO 2022 projections, breast cancer rates vary significantly based on global human development levels. In nations with a very high Human Development Index (HDI), about 1 in 12 women will receive a breast cancer diagnosis at some point in their lives, while approximately 1 in 71 will die from the disease. [2]

The incidence of breast carcinoma in women in India is 27%, which is the highest among all other types of carcinomas. The overall incidence and mortality of females diagnosed with breast cancer are highest in Asian countries like India and Pakistan. [3]

Breast cancer is an intricate, multidimensional disease that is caused by a confluence of environmental, hormonal, and hereditary variables. These etiological factors have an impact on breast cancer etiology. [4]

Approximately half of the breast cancers develop in women over the age of 40 years. There is no identifiable risk factor. The notifiable risk factors that increase the risk are the increasing age of the patient, obesity, consumption of alcohol, family history, radiation exposure, and postmenopausal hormone therapy. [5]

Breast cancer most commonly presents as a painless lump, dimpling, redness, retraction of the nipple and/or abnormal nipple discharge. Breast cancer is more commonly seen in females as it is the most decisive risk factor. Breast carcinoma rarely occurs in men**,** with an incidence of 0.5-1%. [5]

Tumor size, histologic type, histologic grade, age at diagnosis, pTNM staging, lymph node metastases, vascular invasion, response to chemotherapy, and molecular profile, HER2/neu, are some of the variables that affect the prognosis of breast cancer. [6]

Breast cancer is molecularly categorized into three subtypes: luminal (characterized by Estrogen receptor positivity (ER+)/Progesterone receptor positivity (PR+)), HER2-enriched, and triple-negative breast cancer. The expression of these markers influences the patient's treatment approach. However, in cases of advanced or metastatic breast cancer, there is a critical need to identify novel molecular targets to improve prognosis assessment and develop targeted therapies. [7]

Breast cancer patients often face a poor prognosis due to the high likelihood of local recurrence and metastasis, which significantly reduces treatment effectiveness.This failure can be linked to the biological traits and characteristics of the tumor-forming cells. [8] The intervening tumor microenvironment, which interacts with cancer cells to change different attributes of tumor formation such as tumor growth, vascularity, invasiveness and metastatic dissemination, adds to the complexity. Future cancer therapies would depend on anticancer treatment adaption to the heterogenicity of the tumor. [8] The Breast Health Global Initiative (BHGI) is creating suitable policies and practices to provide the best possible breast cancer prevention worldwide. [9] The molecular subtyping of breast cancer, which produces different expressions of biomarkers due to variations in the genetic makeup of DNA, substantially aids in the exact stratification of patients for the selection of the best treatment for individualized breast carcinoma care. This insight is needed to research the new biomarkers that can help target breast carcinoma treatment. [4] Molecular characterization also predicts the prognosis and aggressiveness of Breast carcinoma. [10]

Programmed Death-Ligand 1 (PD-L1) is an immunoregulatory protein that traverses the cell membrane. It binds to Programmed Cell Death-1 (PD-1) receptors present in diverse immune cells, including lymphocytes (T and B cells), natural killer (NK) cells, dendritic cells, and monocytes. In addition to causing T cell apoptosis, activation decreases T cell multiplication and T lymphocyte activity, decreases cytokine production, and induces Antigenic tolerance.[11] These deactivated T cells in the tumor microenvironment aid in tumor progression. [12]

PD-L1 is overexpressed in numerous malignancies like Lung, Urinary bladder, Colorectal, and renal malignancy. A worse prognosis is linked to increased PD-L1 expression. [13] One of the significant focuses of immune-oncology research is identifying the strategies to circumvent these tumor resistance pathways. [14] Several PD-1/PD-L1 inhibitors have been created in recent decades to treat various types of cancer. [15] A global clinical trial has demonstrated that adding an anti-PD-L1 drug to nab-paclitaxel significantly enhances progression-free survival in patients with metastatic triple-negative breast cancer compared to treatment with nab-paclitaxel monotherapy alone. [16]

In September 2014, the approval of Pembrolizumab for advanced melanoma paved the way for the broader clinical advancement of PD-1/PD-L1 inhibitors as anticancer therapies. More recently, the FDA has authorized the use of these inhibitors as anticancer therapies for nine additional cancer types. [17]

Recent years have seen the evolution of anti-PD-1/PD-L1 (Programmed Death-L1) medicines in breast cancer, mainly in the TNBC (triple negative subtype), with encouraging outcomes when administered either alone or in conjunction with conventional therapy. [18]

Although extensive research has been conducted, there is still a limited body of literature on PD-L1 expression in Indian breast carcinoma patients, with existing studies yielding inconsistent results. Given that PD-L1 serves as a potential prognostic biomarker in various solid tumors, including breast cancer, its expression must be examined to assess its significance in targeted immunotherapy. Therefore, this study seeks to assess PD-L1 expression in Indian breast carcinoma patients and explore its correlation with various prognostic parameters. [19]

The present study examines PD-L1 expression in breast carcinoma and its association with patient prognosis.

AIMS AND OBJECTIVES OF THE STUDY

1. Evaluate the immunohistochemical expression of PD-L1 marker on carcinoma Breast.

2. To analyze the correlation between the expression of the PD-L1 marker with ER, PR, HER2/neu receptors, and with various clinicopathological factors, including Age of the patient, tumor size, histologic type, histologic grade, lymph node status and pTNM staging.

REVIEW OF LITERATURE

EMBRYOLOGICAL DEVELOPMENT OF THE BREAST

Between the fourth and sixth week of pregnancy, bilateral ectodermal thickenings along the ventral body wall, called milk lines or mammary ridges, begin the breast's embryological development. Except in the pectoral area, where they give rise to the primitive mammary bud, these ridges typically regress from the axilla to the groin.

By the eighth week, the mammary bud invaginates into the underlying mesenchyme, proliferating to form secondary buds that later develop into solid epithelial cords. These cords canalize during the third trimester, forming the lactiferous ducts under hormonal influences such as estrogen and progesterone. [20]

By the fifteenth week, mesenchymal condensation occurs around the breast bud, and the mesenchyme surrounding the ducts differentiates into stromal tissue, which contributes to the formation of adipose tissue, connective tissue, and the smooth muscle of the nipple and areola.[21]

At puberty, estrogen influences ductal growth, while progesterone promotes alveolar development. Insufficient estrogen prevents further male development, leaving the ducts rudimentary. [22] During pregnancy, the combined action of estrogen, progesterone, and prolactin leads to further alveolar maturation, preparing the breast for lactation. [23]

The differentiation of breast structures is regulated by multiple molecular pathways, including Wnt/β-catenin and Hedgehog signaling, which are essential for proper mammary gland morphogenesis. Disruptions in these pathways may lead to congenital breast anomalies, such as polymastia (extra breast tissue) or amastia (absence of breast tissue). [24] The neonatal breast

can exhibit transient enlargement due to circulating maternal estrogen, a phenomenon known as “witch’s milk,” which resolves spontaneously. [25]

ANATOMY AND HISTOLOGY OF ADULT BREAST

The breast is enveloped by skin and subcutaneous tissue and positioned over the pectoralis muscle. Its primary morphofunctional unit is a complex branching structure organized into lobes, consisting of two key components: the terminal duct-lobular unit (TDLU) and the more extensive ductal system. The TDLU contains lobules made up of multiple acini and a terminal ductule, which functions as the gland’s secretory region. This unit connects to the subsegmental duct, which transitions into the segmental duct and ultimately leads to the collecting/lactiferous duct that drains into the nipple. Beneath the nipple, a fusiform expansion known as the lactiferous sinus is situated between the collecting and segmental ducts. [26]

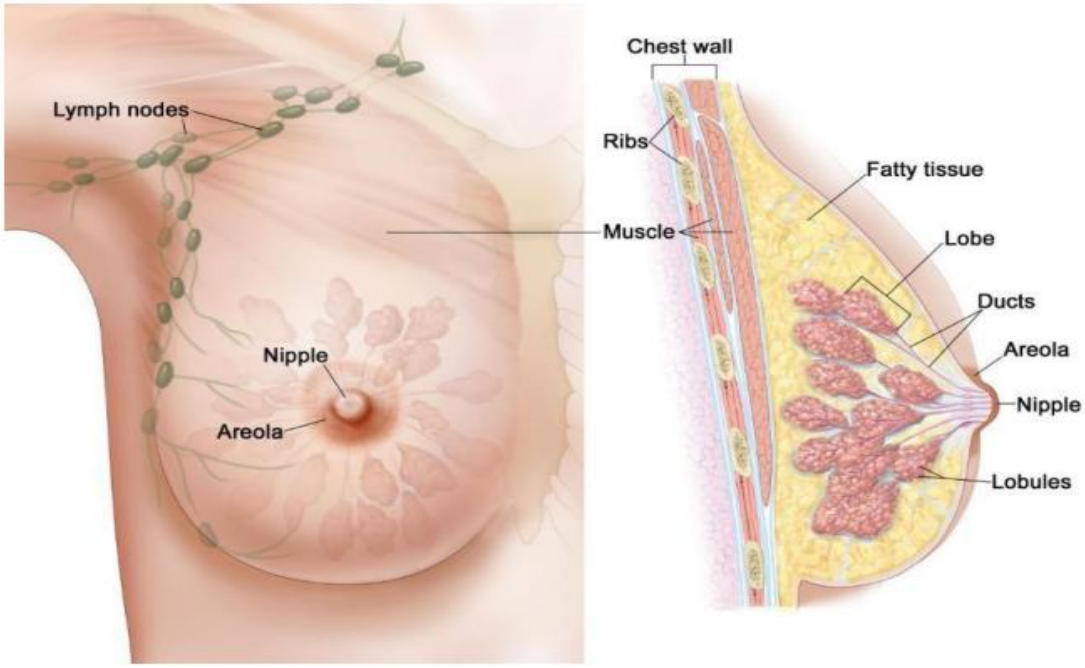

Figure 1: Anatomic structure of breast

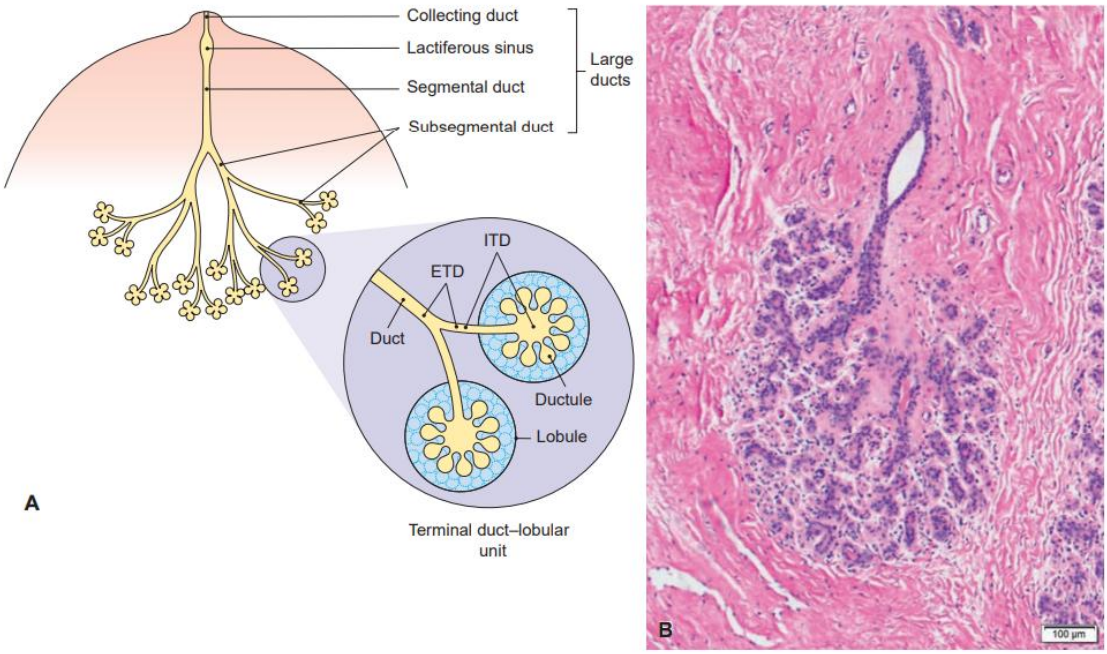

Figure 2: A. Diagrammatic and B. Photomicrographic representation of TDLU

Histologically, breast parenchyma is comprised of ducts, lobules and stroma. The ducts are lined by two-layered epithelium comprised of outer myoepithelial cells, which are flat and contractile in function, and the inner ductal cell layer. The ductal cells lie above the myoepithelial cell layer. Between the ducts lies a stroma composed of fibroblast, collagen fibers, arterioles, venules and adipose tissue. [27]

BREAST CARCINOMA

Breast carcinoma is the most frequently diagnosed cancer in women globally, recently overtaking lung cancer as the leading cause of cancer incidence globally. [2] In 2020, breast cancer was responsible for approximately 685,000 deaths among an estimated 2.3 million newly diagnosed cases, representing around 11.7% of all new cancer cases. Incidence rates vary widely, ranging from fewer than 40 per 100,000 in many regions of Asia

and Africa to over 80 per 100,000 in high-income areas such as North America and Western Europe. [28]

With an age-adjusted incidence rate reaching 25.8 per 100,000 women and a mortality rate of 12.7 per 100,000, breast carcinoma is the most common malignancy among Indian women. [29]

After China and the US, India is currently one of the top three nations in the world for the number of annual cases of breast cancer. An estimated 192,000 new cases were anticipated in India by 2022, and this burden is expected to increase during the ensuing years. [30]

The incidence and Death rates due to breast cancer have accelerated during the last three decades. Current projections indicate that by 2030, 2.7 million new cases will be identified globally each year, compared to 0.87 million fatalities. [31]

According to WHO estimations, malignant neoplasms are responsible for 107.8 million "Disability-Adjusted Life Years (DALYs)." Breast cancer is linked to 19.6 million cases. The incidence and mortality rates of breast carcinoma have increased within the last three decades. Between 1990 and 2016, the death rate from breast cancer doubled in 43 out of 102 countries, while the incidence doubled in 60 out of 102 cases. It is anticipated that the prevalence of breast cancer will rise even more in low- and middle-income nations due to Westernized lifestyles, better cancer screening, and improved cancer registration. [32]

The prevalence of breast cancer has started to rise after staying steady for a long time. This is because more cases were discovered with the advent of mammographic screening. Because of screening, stage I cancers and small lymph node-negative carcinomas have become more common. [33]

BREAST CARCINOMA RISK FACTORS

Several well-established risk factors contribute to the development of breast cancer, while many others are still being explored and debated. [9]

Table 1- Non-Modifiable and Modifiable Risk Factors

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

1. Female Sex

Female sex is a significant risk factor for developing breast cancer, mainly due to heightened hormonal stimulation.

Male breast cancer is uncommon, accounting for less than 1% of all cases. However, certain factors can increase a man's risk, including advancing age, BRCA1 or BRCA2 mutations, elevated estrogen levels, Klinefelter syndrome, a family history of breast cancer, and prior radiationexposure.

2. Older Age

People over 50 years make up around 80% of breast cancer patients nowadays, although over 40% of those over 65 are also affected.

3. Family history

Women with a first-degree relative diagnosed with breast cancer have a 2 to 3 times higher risk of developing the disease compared to the general population.This risk is further raised if the relative has a bilateral illness or was afflicted at a young age. [9]

4. Reproductive and Menstruation history

Early menarche, nulliparity, late menopause, and advanced age at first childbirth are all linked to a higher risk of breast cancer. Conversely, the disease is rare in women who have undergone bilateral oophorectomy.

5. Exogenous estrogens

Exogenous hormones have a complicated effect on breast cancer risk that changes depending on the length of treatment and the combination of drugs employed. [26]

6. Genetic Mutations

The critical genes BRCA1 (on chromosome 17) and BRCA2 (on chromosome 13) are highly penetrant. TP53, CDH1, PTEN, and STK11 are other highly penetrant genes in breast cancer.[34]

7. Contraceptive agents

Extensive epidemiologic studies have found no significant increase, or at most a minimal rise, in breast cancer risk among young, long-term users.

8. Ionizing radiation

Exposure to ionizing radiation has been associated with an increased risk of breast cancer, especially when it occurs during breast development.

9. Breast augmentation

Compared to the general population, breast implants do not raise the risk of breast cancer. [26]

RELATIVE RISK FACTORS FOR DEVELOPING BREAST CANCER [35]

The likelihood of developing breast cancer varies depending on several contributing factors:

Table 2: Relative risk factors for developing breast cancer

Risk Factors	Relative Risk
• Female • Advancing age • High-penetrance germline mutations • High penetrance Germline mutations • Family history (multiple first-degree relatives, early-onset cases, or multiple cancers in the family) • Personal history of breast cancer • High breast tissue density	>4.0
• Moderate penetrance Germline mutations • Higher-dose radiation to the chest at a early age • Family history- Having a close first degree relative with breast cancer	2.1–4.0
• Early menarche (Onset of menses before 12 years of age) • Late menopause (which is after age 55 years) • Late first pregnancy (after 35 years) • Nulliparity. (never having given birth) • Not breastfeeding • Exogenous hormone therapy • Lifestyle Factors- Obesity after menopause Lack of physical activity, Excessive alcohol consumption	1.1–2.0

PATHOGENESIS OF BREAST CARCINOMA

The majority of breast cancer patients are sporadic (90–95%), and only 5–10% of patients have a detectable genetic mutation.

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

GENETIC FACTORS

Genetic mutations involved in the development of breast cancers can be classified as familial or sporadic; most BRCA2 mutations are associated with ER-positive malignancies, while most BRCA1 mutations are associated with triple-negative tumors. The typical tumor suppressor properties of BRCA1 and BRCA2 mean that cancer can only develop when both alleles are inactive or dysfunctional. BRCA1 and BRCA2 encode proteins to repair specific types of DNA damage.

The BRCA1-encoded protein involves several processes, including DNA decatenation, ubiquitylation, chromatin remodeling, cell cycle checkpoint regulation, and the homologous recombination mechanism of DNA damage repair. BRCA2 encodes a protein involved in meiosis, cytokinesis, and DNA repair. Stated differently, BRCA1 and BRCA2 are necessary for precise homologous recombination-mediated repair of DNA double-strand tears. [36]

PTEN and TP53 are two other genes that have mutations linked to familial breast cancer. Mutations that upregulate PI3KAKT signaling are common in sporadic ER-positive and HER2-positive breast cancers, while somatic TP53 mutations are frequently observed in triple-negative and HER2-positive breast tumors. [33]

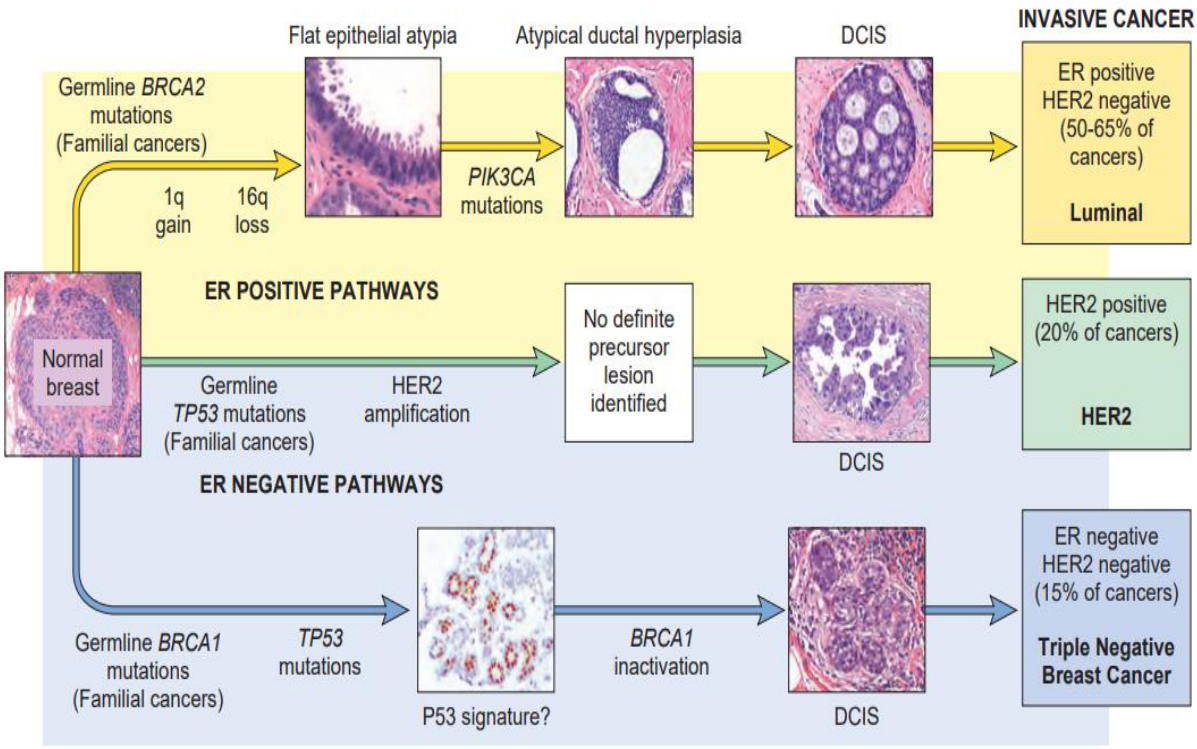

Figure 3- Significant pathways of breast cancer development

HORMONAL INFLUENCES

Estrogen promotes the production of various growth factors (GFs), including transforming growth factor (TGF), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF), among others. These factors can drive tumor growth through both paracrine and autocrine signaling pathways.

Numerous more genes, some of which are crucial for the growth or development of tumors, are also regulated by estrogen receptors in an estrogen-dependent fashion, likely to be involved in development and growth. [33]

ENVIRONMENTAL FACTORS

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

CLINICAL FEATURES

In the non-screened population, a palpable mass is the most common clinical presentation of invasive breast cancer (IBC). However, other symptoms may also occur, including skin retraction, nipple inversion, nipple discharge, and, less commonly, changes in breast size or shape, as well as alterations in skin color or texture, sometimes accompanied by ulceration. [3]

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. [38]

(i) **Luminal A**- Luminal A breast cancer is defined by the expression of estrogen (ER) and progesterone (PR) receptors while lacking HER2/neu. In this subtype, ER-associated transcription factors regulate the expression of genes specific to the luminal epithelium lining the ducts, while genes related to cell growth show reduced expression. Clinically, luminal A tumors are typically low-grade, slow-growing, and associated with the most favorable prognosis. [39,9]

(ii) **Luminal B**- They are higher grade and have poor prognosis. In addition to being ER-positive, PR-negative, HER2, or both could be there. Additionally, it has increased the expression of proliferation-related genes. [39,40,9]

(iii) **HER2-enriched** breast carcinoma is defined by HER2 overexpression and the absence of estrogen (ER) and progesterone (PR) receptors. This subtype primarily expresses proteins and genes associated with cellular proliferation, such as GRB7 and ERBB2/HER2. [39,40,9]

(iv) **Basal-like/Triple-Negative** Breast Cancer- These are a broad category of breast tumors with the characteristics of being ER,PR and HER2-negative. About 20% of all breast cancers are of this type. TNBC is more common in African-American women and women under 40 years of age. 11% and 16% of all TNBCs have BRCA1 or BRCA2 germline mutations. About 80% of breast tumors that develop in carriers of BRCA1 germline mutations are TNBCs. TNBC accounts for 11–16% of all TNBCs, although BRCA1 or BRCA2 germline mutations are present in about 80% of breast cancers resulting from BRCA1 germline mutations. TNBCs. They are biologically aggressive and are linked to a poorer prognosis. [41,42]

(v) **Claudin-Low** Breast Cancer- ER/PR negative and HER2-negative. are generally associated with a poor prognosis. They account for about 7–14% of invasive breast cancer patients. The Claudin-low type is defined by the reduced expression of genes associated with cell-cell adhesion, including occludin, E-cadherin, and claudins 3, 4, and 7. [43]

DIAGNOSIS OF BREAST CANCER

1. <u>Mammography</u>- X-ray images of the breast are used in mammograms. In particular, digital mammography has been replaced by conventional mammography for breast screening services. However, repeated mammography calls for careful consideration of any radiation risk. Additionally, false-positive calls result in extra imaging or histological evaluation, primarily percutaneous breast biopsy. [44]After examining the mammograms, radiologists group their findings into a final assessment category using the BI-RADS diagnostic system. The American College of Radiology (ACR) developed the Breast Imaging Reporting and Data System (BI-RADS) to standardize mammographic reporting by providing a structured

framework for assessment, classification, and conclusions. Recommendations for further management are based on the final evaluation category.

BIRADS Category 4-6 suggests malignancy on mammographs, BIRADS-4 indicates a suspicious finding for malignancy, BIRADS-5 indicates a very suggestive finding for malignancy, and BIRADS-6 indicates a biopsy-proven diagnosis for malignancy.

2. Magnetic resonance imaging (MRI)- Is a very powerful imaging method that generates high-resolution images without using dangerous radiation. [45,46] Breast MRI results are impacted by the way intravenous contrast infusion intensifies lesions. Because of neovascularization, the tumor tissue is permeable, allowing the contrast agent to diffuse. [33] Due to their paramagnetic characteristics, several 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 known adverse effects and limitations, with transmetallization of gadolinium being a documented concern. [47,32] Recent research and patents have explored novel carrier systems and advanced targeting strategies to enhance the efficacy of MRI contrast agents while minimizing toxicity. [44]

3. Molecular breast imaging (MBI)- In MBI, breast cancer tissues are illuminated with a radioactive tracer so that a nuclear medicine scanner can view them. This process is also known as the Miraluma test, sestamibi test, scintimammography, or specialized gamma imaging. The principal component in MBI is Tc-99m sestamibi, which is authorized for imaging breast tumors. MBI has a higher specificity and comparable sensitivity to MRI in detecting small breast lesions. [48]

4. Breast biopsy- Two primary needle biopsy techniques are used for breast cancer detection: Core needle biopsy (CNB) and Fine needle aspiration cytology (FNAC).

Unlike FNAC, which extracts cells, CNB removes a small cylindrical tissue sample known as a core. Typically, three to five cores are obtained, though additional samples may be collected if needed. The pathologist then examines these tissue samples to identify the presence of cancer.[48]

WHO CLASSIFICATION OF BREAST TUMORS (2019) - 5TH EDITION [49]

1. Epithelial Tumors of the Breast

1.1 Benign Epithelial Proliferations and Precursors

- Usual ductal hyperplasia
- Columnar cell lesions, including flat epithelial atypia
- Atypical ductal hyperplasia

1.2 Adenosis and Benign Sclerosing Lesions

- Sclerosing adenosis
- Apocrine adenosis and adenoma
- Microglandular adenosis
- Radial scar/complex sclerosing lesion

1.3 Adenomas

- Tubular adenoma
- Lactating adenoma
- Ductal adenoma

1.4 Epithelial-Myoepithelial Tumors

- Pleomorphic adenoma
- Adenomyoepithelioma

- Malignant adenomyoepithelioma

1.5 Papillary Neoplasms

- Intraductal papilloma
- Papillary ductal carcinoma in situ (DCIS)
- Encapsulated papillary carcinoma
- Solid papillary carcinoma (in situ and invasive)

1.6 Non-Invasive Lobular Neoplasia

- Atypical lobular hyperplasia
- Lobular carcinoma in situ

1.7 Ductal Carcinoma In Situ (DCIS)

- Ductal carcinoma in situ

1.8 Invasive Breast Carcinoma

- General Overview
- Invasive breast carcinoma of no special type
- Microinvasive carcinoma
- Invasive lobular carcinoma
- Tubular carcinoma
- Cribriform carcinoma
- Mucinous carcinoma
- Mucinous cystadenocarcinoma
- Invasive micropapillary carcinoma
- Carcinoma with apocrine differentiation
- Metaplastic carcinoma

2. Fibroepithelial Tumors and Hamartomas of the Breast

- WHO Classification of Fibroepithelial Tumors and Hamartomas
    - Hamartoma
    - Fibroadenoma
    - Phyllodes tumor

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3. Tumors of the Nipple

- WHO Classification of Tumors of the Nipple
    - Epithelial tumors
    - Syringomatous tumor
    - Nipple adenoma
    - Paget disease of the breast

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4. Mesenchymal Tumors of the Breast

- WHO Classification of Mesenchymal Tumors

4.1 Vascular Tumors

- Hemangioma
- Angiomatosis
- Atypical vascular lesions
- Post-radiation angiosarcoma of the breast
- Primary angiosarcoma of the breast

4.2 Fibroblastic and Myofibroblastic Tumors

- Nodular fasciitis
- Myofibroblastoma

- Desmoid fibromatosis
- Inflammatory myofibroblastic tumor

4.3 Peripheral Nerve Sheath Tumors

- Schwannoma
- Neurofibroma
- Granular cell tumor

4.4 Smooth Muscle Tumors

- Leiomyoma
- Leiomyosarcoma

4.5 Adipocytic Tumors

- Lipoma
- Angiolipoma
- Liposarcoma

4.6 Other Mesenchymal Tumors and Tumor-Like Conditions

- Pseudoangiomatous stromal hyperplasia

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5. Hematolymphoid Tumors of the Breast

- WHO Classification of Hematolymphoid Tumors

5.1 Lymphomas

- Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
- Follicular lymphoma
- Diffuse large B-cell lymphoma
- Burkitt lymphoma

- Breast implant-associated anaplastic large-cell lymphoma

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6. Tumors of the Male Breast

- WHO Classification of Male Breast Tumors
    - Gynaecomastia
    - Carcinoma in situ
    - Invasive carcinoma

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7. Metastases to the Breast

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8. Genetic Tumor Syndromes Associated with Breast Cancer

- BRCA1/2-associated hereditary breast and ovarian cancer syndrome
- Cowden syndrome
- Ataxia-telangiectasia
- Li-Fraumeni syndrome (TP53-associated)
- CHEK2-associated breast cancer
- CDH1-associated breast cancer
- PALB2-associated cancers
- Peutz-Jeghers syndrome
- Neurofibromatosis type 1
- Polygenic component of breast cancer susceptibility

MORPHOLOGY

NONINVASIVE (IN SITU) CARCINOMA

Ductal carcinoma in situ and lobular carcinoma in situ represent the two main morphologic subtypes of noninvasive breast carcinoma; both are restricted to the basement membrane and do not invade the surrounding stroma. [33]

Ductal Carcinoma Insitu (DCIS)- While there are other histologic forms of ductal carcinoma in situ (DCIS), comedocarcinoma, or DCIS of the comedo type, is more aggressive than other forms of DCIS. The most prevalent subtypes of noncomedo kinds of DCIS are.

1. Solid DCIS: The afflicted breast ducts are filled with cancer cells.

2. Cribriform DCIS: Tumor cells partially fill the damaged breast ducts, but spaces separate these cells. [50]

3. Papillary and micropapillary DCIS: Micropapillary DCIS cells are smaller than papillary DCIS cells, and the cancer cells form a fern-like pattern without fibrovascular core inside the impacted breast ducts. [51]

Lobular Carcinoma Insitu (LCIS)- Usually has a consistent uniform pattern. The monomorphic cells are organized in weakly cohesive clusters with bland, spherical nuclei. Since these lesions do not exhibit calcifications, unlike DCIS, it is typically an accidental finding. It has been noted that invasive breast cancer develops in one-third of LCIS women. 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 contralateral breast. LCIS is a direct precursor to several cancers and a marker of an increased risk of bilateral breast cancer. [33]

INVASIVE (INFILTRATING) BREAST CARCINOMA

MACROSCOPY: Most Invasive Breast Carcinoma presents as a large, grossly perceptible mass having an uneven, stellate border or nodular appearance. The tumor is firm to hard on palpation, with the margins usually not well circumscribed. Few tumors may feel gritty when cutting the tissue with a knife. [49] The tumor usually appears stellate or stab-like. There may be visible areas of bleeding, necrosis, and cystic degeneration if the tumor is relatively large. [26] To establish the lesion's size, location, focality, and other details for proper tissue collection, the gross findings and radiographic reports should always be compared. [49,52]

MICROSCOPY: The usual development pattern in microscopy is sheets, nests, cords, or single scattered tumor cells. Glandular/tubular division may be well-defined and hardly perceptible. Although tumor cells may vary in size and shape, they tend to be larger and more pleomorphic compared to the typical form of invasive lobular carcinoma.They also feature more mitotic figures and more noticeable nuclei and nucleoli. Sometimes, necrosis may occur. In rare instances, calcification frequently linked to in situ components has been documented; it may appear as a coarse or fine deposit. There may be focal apocrine metaplasia, squamous metaplasia or clear cell change.

The stroma might be highly fibrotic or cellular (also known as desmoplastic) and can be sparse or abundant. Identifying the tumor cells could be challenging when there is a lot of stroma. Elastosis may occur in some areas, primarily affecting the walls of veins and ducts. Calcification is possible. The interphase between the surrounding stroma and tumor tissue is typically where chronic mononuclear inflammatory cell infiltration is observed. [26, 33, 49]

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

1. Distant metastasis (M): The most significant prognostic factor is metastasis outside the local lymphnode.

2. Regional lymph nodes (N): It includes the number of involved lymph nodes and is the second most significant prognostic marker in breast cancer.

3. Tumor (T): Important factors include size, skin involvement (such as ulceration or dermal metastases), penetration of the chest wall, and presentation as inflammatory carcinoma.

4. Histologic grade: As histologic grade increases, survival decreases. The tumor is histologically graded using a modified ScarffBloom-Richardson grading system incorporating tubule formation, degree of nuclear pleomorphism, and mitotic count. The prognosis for well-differentiated carcinomas is favorable, whereas poorly-differentiated carcinomas are unfavorable.

Table 3: Modified scarff- bloom Richardson grading of invasive breast carcinoma

Tubule Formation

Score	Description
1	>75% of tumors show tubules
2	10–75% of tumors show tubules
3	<10% of tumors show tubules

Nuclear Pleomorphism

Score	Description
1	Uniform cells with small nuclei similar to normal breast epithelial cells.
2	Cells larger than usual, showing moderate pleomorphism with open vesicular nuclei and visible nucleoli.
3	Cells with vesicular nuclei and prominent nucleoli, showing marked pleomorphism.

Mitotic Count

Score	Description
1	0–8 mitoses / 10 HPF
2	9–16 mitoses / 10 HPF
3	>17 mitoses / 10 HPF

Final Tumor Grade

Total Score	Grade
3–5	Well-differentiated (Grade I)
6–7	Moderately differentiated (Grade II)
8–9	Poorly differentiated (Grade III)

5. ER, PR, and HER2 neu expression: Survival rates are lowest for the least favorable combination, characterized by the absence of ER, PR, and HER2, while the highest survival is observed in cases with the most favorable combination- positive ER and PR expression and absent HER2.

OTHER PROGNOSTIC FACTORS INCLUDE: [33]

• Lymphovascular invasion: Poor prognostic indicators include tumor cells in vascular spaces at the periphery of the tumor.

• Special histologic kinds: Tubular carcinoma and adenoid cystic carcinoma are two examples of histologic types of cancer that have a high correlation with extremely favorable survival.

• Gene expression profiling: The primary clinical utility of gene expression profiling is the identification of patients with antiestrogen-responsive tumors who do not require chemotherapy.

• The American Joint Committee of Cancer Staging System (AJCC) states that the combination of anatomic staging and breast cancer molecular characteristics is one of the most significant prognostic factors.

STAGING OF BREAST CANCER

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

Table 4 - TNM- Staging of Breast Cancer [49]

Category	Definition
T- Primary tumor	
TX	Primary tumors cannot be assessed.
T0	No evidence of a primary tumor
Tis	Carcinoma in situ
Tis (DCIS)	Ductal carcinoma in situ
Tis (LCIS)	Lobular carcinoma in situ
Tis (Paget)	Paget disease of the nipple is not associated with invasive carcinoma and/or carcinoma in situ (DCIS and/or LCIS) in the underlying breast parenchyma.
T1	Tumor 2 cm or less in the most significant dimension
T1mi	Microinvasion 0.1 cm or less in the most significant dimension
T1a	More than 0.1 cm but not more than 0.5 cm in the most significant dimension
T1b	More than 0.5 cm but not more than 1 cm in the most significant dimension
T1c	More than 1 cm but not more than 2 cm in the most significant dimension
T2	Tumor more than 2 cm but not more than 5 cm in the most significant dimension
T3	Tumors more than 5 cm in the most significant dimension
T4	Tumor of any size with direct extension to chest wall and/or skin (ulceration or skin nodules)
T4a	Extension to the chest wall (does not include pectoralis muscle invasion only)
T4b	Ulceration, ipsilateral satellite skin nodules, or skin edema (including peau d'orange)
T4c	Both 4a and 4b
T4d	Inflammatory carcinoma
Regional Lymph Nodes (N)	
NX	Regional lymph nodes cannot be assessed (e.g., previously removed)
N0	No regional lymph node metastasis
N1	Metastasis in movable ipsilateral level I and II axillary lymph nodes

N2	Metastasis in ipsilateral level I and II axillary lymph nodes that are clinically fixed or matted or in clinically detected ipsilateral internal mammary lymph nodes without clinically evident axillary node metastasis
N2a	Metastasis in axillary lymph nodes fixed to one another (matted) or other structures
N2b	Metastasis only in clinically detected internal mammary lymph nodes without axillary lymph node involvement
N3	Metastasis in ipsilateral infraclavicular (level III axillary) lymph nodes, with or without level I, II axillary lymph node involvement, or in clinically detected ipsilateral internal mammary lymph nodes with clinically evident axillary lymph node metastasis, or metastasis in ipsilateral supraclavicular lymph nodes with or without axillary or internal mammary lymph node involvement
N3a	Metastasis in infraclavicular lymph nodes
N3b	Metastasis in internal mammary and axillary lymph nodes
N3c	Metastasis in supraclavicular lymph nodes
Distant Metastasis (M)	
M0	No distant metastasis
M1	Distant metastasis detected

Table 5 - American Joint Commission on Cancer (AJCC) staging, 8th edition [53]

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

SIGNIFICANCE OF IHC IN CARCINOMA BREAST

The most popular use of immunostaining is in immunohistochemistry. It is a scientific method that uses antibodies to search for specific antigens in a tissue sample. The antibodies are typically attached to a fluorescent dye or an enzyme. An enzyme or dye is activated after the antibodies bind to the antigen in the tissue sample. A microscope can then be used to view the antigen.

IHC is crucial in identifying intracellular proteins and cell surface markers across all tissues. In breast carcinoma, the unique biological characteristics of the tumor help predict prognosis and guide systemic therapy. The use of immunohistochemical markers as prognostic and

predictive factors implicated in angiogenesis and apoptosis, along with molecular classification of breast cancer, thus aiding the management of the patients, are all common uses of these markers. Different subsets of proteins are expressed by the luminal, basal, and myoepithelial cell types that make up normal glandular breast tissue.

Normal glandular breast tissue consists of luminal, basal, and myoepithelial cell types expressing distinct proteins. Luminal cells produce cytokeratins (CK), estrogen receptors (ER), progesterone receptors (PR), milk fat globule membrane antigens (MFGM), epithelial membrane antigens (EMA), and β-lactalbumin. Myoepithelial cells express markers such as smooth muscle actin, calponin, S100, and p63, while basal cells show a variety of cytokeratins.

Key immunohistochemical markers for prognosis and treatment in breast carcinoma include p53, Ki-67, (HER2) and (ER/PR). IHC is also essential in distinguishing various breast lesions, such as in situ versus invasive carcinoma, benign proliferative changes versus malignancies, and pseudo-invasive lesions (e.g., adenosis, radial scar, and sclerosing) from actual invasive malignancies. Additionally, IHC helps characterize papillary lesions, microinvasive carcinomas (≤1 mm invasive foci), and atypical ductal hyperplasia (ADH). These lesions frequently lend themselves to IHC explanation. [54,55]

Myoepithelial Cells and Assessment of Stromal Invasion. In addition to being the most common lesions that the surgical pathologist encounters, epithelial lesions of the breast are also the most significant cause for concern when determining whether a lesion is benign or malignant. [54]

ESTROGEN RECEPTOR(ER):

Estrogen receptor (ER) belongs to the nuclear hormone receptor family of intracellular receptors. Once activated, ER binds to DNA and regulates the expression of numerous genes.

The ER gene is located on chromosomes 6q25.1 and 14q23.2. ERα receptors are predominantly found in the brain, ovarian stromal cells, breast cancer cells, and endometrium, while ERβ receptors are expressed in various tissues, including lungs, intestinal mucosa, prostate kidney, brain, bone, and endothelial cells. [54,55]

PROGESTERONE RECEPTOR(PR):

The progesterone receptor (PR), also referred to as nuclear receptor subfamily 3, group C, member 3 (NR3C3), is a cellular protein influenced by the steroid hormone progesterone. It is encoded by the PGR gene on chromosome 11q22 and exists in two isoforms, PR-A and PR-B, which differ in molecular weight. While PR-B enhances progesterone's effects, PR-A serves as a negative regulator of PR-B activity. [55,55]

HER2/neu RECEPTOR:

The HER2/neu (c-erbB-2) oncogene encodes p185, a transmembrane glycoprotein with tyrosine kinase activity. It is a member of the epidermal growth factor receptor (EGFR) family. Immunohistochemistry or FISH (or its chromogenic equivalent) can be used to measure its overexpression, and these techniques have a reasonable correlation. [55,54]

PD-L1- (PROGRAMMED DEATH - LIGAND 1)

The PD-1/PD-L1 pathway is crucial for maintaining immune tolerance within the tumor microenvironment. When PD-1 binds to its ligands, PD-L1 or PD-L2, it inhibits T cell activation, proliferation, and cytotoxic function, thereby diminishing the body's anti-tumor immune response.[57]

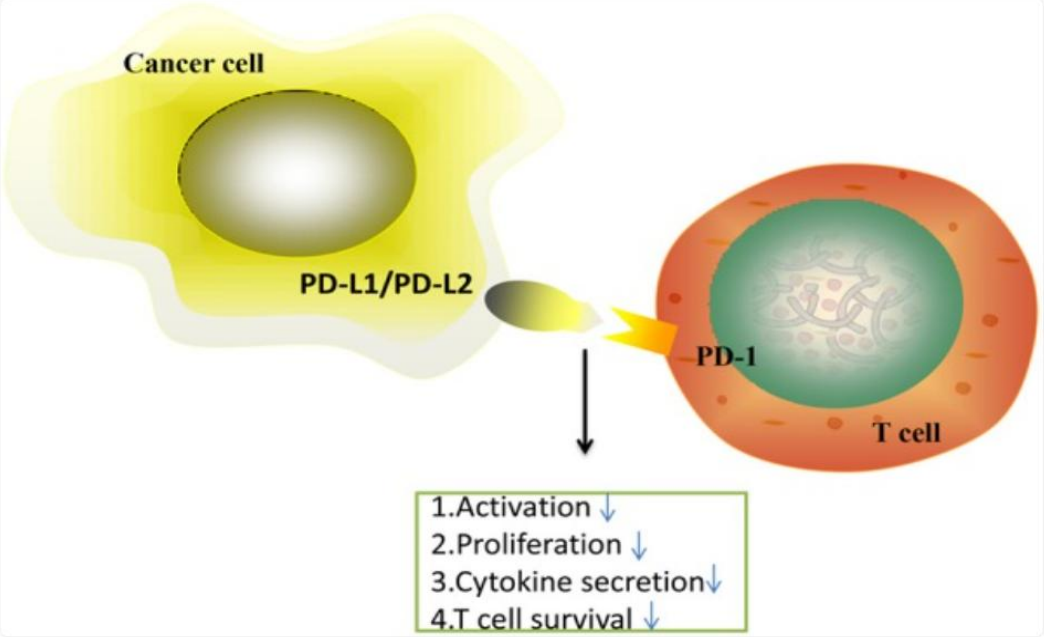

Figure 4- The PD-1/PD-L1 axis and its role

PD-1 (programmed Cell death-1 protein)

PD-1 (CD279) is a 288-amino acid type I transmembrane protein found on activated T cells, B cells, natural killer (NK) cells, macrophages, dendritic cells (DCs), and monocytes. It plays a crucial role in regulating both adaptive and innate immune responses. [58]

It serves a dual function, offering both protective and detrimental effects. While PD-1 is essential for maintaining immune tolerance and preventing overactive immune reactions, it also suppresses protective immune responses, allowing tumor cells to escape immune detection and hence promote tumor cell growth.

PD-L1

Programmed Death-Ligand 1 (PD-L1), also known as B7-H1 or CD274, is a 290-amino acid protein and a member of the B7 family of type I transmembrane protein receptors. It consists of two extracellular domains (IgV-like and IgC-like), a transmembrane domain, and a cytoplasmic domain [58]. PD-L1 is primarily expressed by macrophages, activated T and B cells,

dendritic cells (DCs), and certain epithelial cells, particularly under inflammatory conditions. Moreover, tumor cells exploit PD-L1 expression as an adaptive immune mechanism to evade anti-tumor immune responses. [57]

Research has demonstrated that blocking IFN-γ receptor 1 can suppress PD-L1 expression in acute myeloid leukemia mouse models via the MEK/ERK and MYD88/TRAF6 pathways. In contrast, IFN-γ enhances PD-L1 expression in ovarian cancer cells, promoting disease progression.

IFN-γ also stimulates the expression of protein kinase D isoform 2 (PKD2), a key regulator of PD-L1 expression. Inhibiting PKD2 activity reduces PD-L1 levels and enhances the antitumor immune response. Natural killer (NK) cells release IFN-γ via the Janus kinase (JAK)1, JAK2, and signal transducer and activator of transcription (STAT)1 pathway, leading to increased PD-L1 expression on tumor cells. Studies on melanoma cells further indicate that IFN-γ secreted by T cells regulates PD-L1 expression through the JAK1/JAK2-STAT1/STAT2/STAT3-IRF1 signaling cascade.

Both T cells and NK cells release IFN-γ, which induces PD-L1 expression in various target cells, including tumor cells.

PD-L1 acts as a pro-tumorigenic factor by binding to its receptors and triggering proliferative and survival signaling pathways, highlighting its role in advanced tumor progression. Beyond its immune-suppressive functions, PD-L1 also directly enhances tumor cell proliferation. For instance, in renal cancer, PD-L1 promotes stem cell-like properties and epithelial-to-mesenchymal transition (EMT), suggesting that its intrinsic mechanisms contribute to kidney cancerdevelopment. [57]

PD-L1 Testing in General

Immunohistochemistry (IHC) is routinely used to assess PD-L1 expression in clinical diagnostics. However, multiple commercially available assays, diverse scoring systems, varying cut-off values, and different antibody clones exist for PD-L1 detection. Since each IHC staining and scoring method has been validated in clinical trials to assess the efficacy of a specific immune checkpoint inhibitor (ICI),

(i) The approval of a specific immune checkpoint inhibitor (ICI) is often associated with a designated PD-L1 immunohistochemistry (IHC) assay, as PD-L1 status assessment is crucial for guiding treatment decisions.

(ii) Comparing the predictive value of various IHC antibody clones, scoring methods, and cut-off values remains challenging.

To address these inconsistencies, harmonization studies have been conducted to identify the most reliable assays and antibody clones. However, further research is required to assess different staining techniques, scoring approaches, and their predictive value for ICI response, particularly in breast cancer. [59]

Table 6: PD-L1 Immunohistochemistry, Detection Systems, Assays and Drugs [59]

Immune Checkpoint Inhibitor	Target	Pharmaceutical Manufacturer	IHC Detection System/Assay Producer	Detection Platform	Anti-PD-L1 Antibody Clone
Nivolumab (Opdivo®)	PD-1	Bristol-Myers Squibb	PD-L1 IHC 28-8 Agilent pharmDx/Dako,	EnVision Flex/Link48 autostainer	28-8 (monoclonal, rabbit)
Pembrolizumab (Keytruda®)	PD-1	MSD	PD-L1 IHC 22C3 pharmDx/Dako, Agilent	EnVision Flex/Link48 autostainer	22C3 (monoclonal, mouse)
Atezolizumab (Tecentriq®)	PD-L1	Roche/ Genentech	VENTANA PD-L1(SP142) assay/Ventana, Roche	OptiView DAB IHC detection kit +amplification kit/BenchMark ULTRA	SP142 (monoclonal, rabbit)
Durvalumab (Imfinzi®)	PD-L1	AstraZeneca	VENTANA PD-L1(SP263) assay/Ventana, Roche	OptiView DAB IHC detection kit/BenchMark ULTRA	SP263 (monoclonal, rabbit)

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: From 1st May 2023 to 31st December 2024.

Study design: It is a cross-sectional study conducted in the hospital.

Study population: Participants in the study were clinically suspected cases of breast cancer who arrived at the surgery outpatient department (OPD) of BLDE (DU), Shri B. M. Patil Medical College, Hospital and Research Center, Vijayapura, Karnataka.

Inclusion criteria: All primary breast cancer mastectomy specimens were received in the histopathology section of the Pathology department in BLDE (DU) Shri B.M. Patil Medical College, Hospital and Research Center, Vijayapura, Karnataka.

Exclusion criteria: Improperly fixed specimens.

Sample Size:

For sample size calculation G*Power version 3.1.9.4 tool was used. Based on previous studies, the proportion of malignant breast carcinoma tumor cells staining positive for PD-L1 is estimated at 14.7% (4). To achieve a 95% confidence level with 10% absolute precision, a total of 50 subjects was required for the study.

Formula Used:

$$n = \frac{Z^2 \times P \times q}{d^2}$$

Where:

- Z = Z statistic at the chosen significance level (α)
- P = Proportion rate
- q = 100−P
- d^2 = Absolute error

Statistical Analysis:

- Data collection and analysis were conducted using Microsoft Excel and SPSS version 20.
- Results were presented as Mean (Median) ± Standard Deviation, along with counts, percentages, and visual diagrams for interpretation.
- Chi-square test was applied to evaluate associations between categorical variables.
- A P-value < 0.05 was considered statistically significant.

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 sections will be prepared from each tissue block. One tissue section will be stained by hematoxylin and eosin stain for

histopathological diagnosis. An additional tissue section from paraffin-coated tissue blocks will be placed on a poly L lysine-coated slide and subjected to PD-L1 (programmed death ligand 1) immunohistochemistry.

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

Immunohistochemical staining protocol [56]

(i) On charged slides, place four micrometer-thick tissue sections. Then, incubate for 20 minutes at 60 to 70 degrees Celsius.

(ii) Deparaffinization of tissue sections on slides coated with polylysine.

(iii) Hydrate with two changes of absolute alcohol every five minutes.

(iv) Wash twice in distilled water for two minutes each.

(v) Endogenous enzymes can be quenched, which prevents them from reacting with IHC reagents and producing false-positive results.

(vi) Antigen retrieval for 15 minutes.

(vii) Blocking of nonspecific binding sites.

(viii) Primary antibody binding for forty-five minutes in a damp chamber. After that, wash for three minutes each in the wash buffer twice.

(ix) After binding with a secondary antibody that has been biotinylated, wait 12 minutes. After that, wash for three minutes each in the wash buffer twice.

(x) Add the chromogen substrate, often DAB (1 drop DAB chromogen + 1 milliliter DAB buffer, mix thoroughly), and let it sit for 2 to 5 minutes.

(xi) Wash it in distilled water for 2 minutes for each of the two changes.

(xii) Counterstaining with Hematoxylin for 30 seconds, wash it with water.

(xiii) Dehydrating and coverslipping the slide.

Table 7 - IHC Interpretation of ER, PR, HER2/ neu

IHC Marker	Proportion score (PS) Range(%)	Intensity score (IS) Type	Interpretation
ER/ PR	0 0% 1 <1% 2 1-10% 3 11-33% 4 34-66% 5 67-100%	0 No staining 1+ Weak positive 2+ Moderate positive 3+ Strong positive	**Allred Score = PS + IS** Negative: ≤2 Positive: ≥2 Maximum score: 8
HER-2-Neu	**ASCO guidelines** 0 = No or faint membrane staining observed in ≤10% of tumor cells. 1+ = Incomplete or faint membrane staining present in >10% of tumor cells. 2+ = Moderate or complete membrane staining detected in >10% of tumor cells. 3+ = Circumferential, complete, and intense membrane staining seen in >10% of tumor cells.		

PD-L1 Scoring system

Table 8 - IHC Interpretation of PD-L1

PD-L1	Combined Positive Score (CPS)
Positive	**Tumor cells**- Complete or partial membrane staining is present in >10% of tumor cells. **Immune cell**- Lymphocytes, macrophages exhibiting cytoplasmic or membrane staining.(Control)
Negative	**Tumor cells**- Complete or partial membrane staining is present in <10% of tumor cells. **Immune cell**- Lymphocytes, macrophages exhibiting cytoplasmic or membrane staining. (Control)

The specimens were analyzed using a microscope with an objective magnification range of 10–40x. PD-L1 scoring was conducted on all viable tumor cells across the entire slide.

A sample was deemed suitable for PD-L1 assessment if it contained at least 100 viable tumor cells. The scoring criteria for tumor cells included both partial and complete membrane staining, while cytoplasmic staining was excluded as it was considered nonspecific.

$$\text{PD-L1 Score (\%)} = \left(\frac{\text{Number of PD-L1-positive tumor cells} + \text{Number of PD-L1-positive immune cells}}{\text{Total number of vital tumor cells}} \right) \times 100$$

Additionally, the scoring system was extended to tumor-associated immune cells, including macrophages and infiltrating lymphocytes, that exhibited cytoplasmic or membrane staining.

Specimens were classified as positive for PD-L1 expression, Combined Positive Score (CPS) was ≥10%, meaning a minimum 10% of viable tumor cells displayed membrane staining at any intensity, along with immune cells showing membrane or cytoplasmic staining. Conversely, specimens were considered PD-L1 negative if the CPS was <10%, indicating that less than 10% of viable tumor cells exhibited membrane staining at any intensity. [60]

RESULTS

A total of 50 individuals diagnosed with invasive breast cancer were included in this study. Correlations were observed between ER/PR and HER2/neu status and various clinicopathological factors, such as tumor size, histological grade, histological type, lymph node metastasis, and patient age.

GENDER DISTRIBUTION IN STUDY POPULATION

Among 50 cases, 49 cases (98%) were females, and 01 case (2%) was male. (Table 9(Figure5)

Table 9: Distribution of cases according to gender

Gender	No. of patients	%
Females	49	98.0%
Males	1	2.0%
Total	50	100.0%

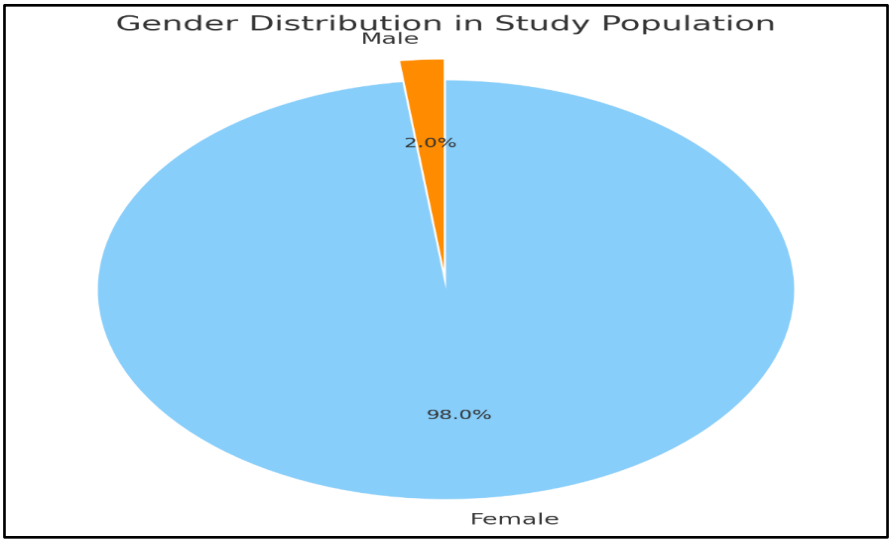

Figure 5: Number of females and males in this study

EXPRESSION OF PD-L1 IN THE STUDY POPULATION:

PD-L1 was identified by immunohistochemical (IHC) staining using a "Rabbit monoclonal antibody, Ventana SP263 Clone kit" with an FDA-approved automatic device (VENTANA BenchMark). PD-L1 expression was objectively evaluated by Combined positive score (CPS). PD-L1 positivity (CPS >10%) was seen in 14 (28%) cases. PD-L1 was considered negative when CPS <10%. In this study, 36 (72%) cases were PD-L1 negative. (Table 10) (Figure 6)

Table 10: Distribution of cases according to PD-L1 Expression

PD-L1	No. of patients	Percentage
Positive	14	28.0
Negative	36	72.0
Total	50	100.0

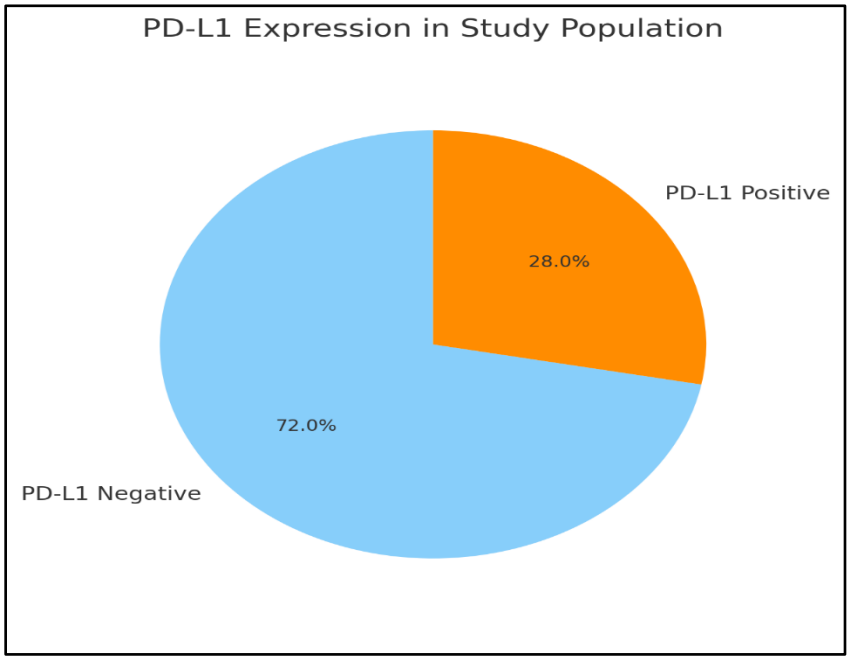

Figure 6: Graphical representation of PD-L1 positivity in the study population

Correlation between PD-L1 expression and the clinicopathological parameters

1. AGE

The patients with invasive breast cancer ranged in age from 30 to 80 years old; their median age was 55 years old, and their mean age was 55.7 years old. 68% of the cases were in the over-50 age group, while 32% of the cases were in the under-50 age group.

Table 11: Distribution of cases according to age

AGE(Patients)	No. of patients	Percentage
<30	00	(0%)
31-40	07	(14%)
41-50	09	(18%)
51-60	18	(36%)
>60	16	(32%)
Total	50	(100%)

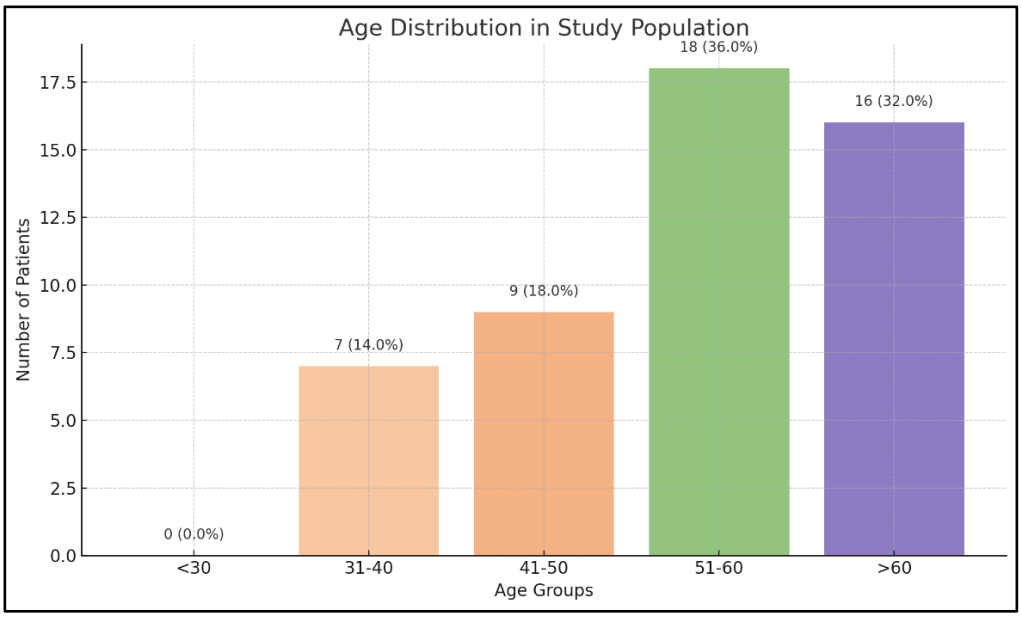

Figure 7: Age distribution in the study population

Table 12: Correlation of PD-L1 expression with the age of the patient

AGE(Years)	PD-L1			Chi-square test	P value
	Neg	Pos	Total		
<30	0(0%)	0(0%)	0(0%)	3.88	0.27
31-40	4(11.3%)	3(21.4%)	7(14%)		
41-50	7(19.4%)	2(14.2%)	9(18%)		
51-60	11(30.5%)	7(50%)	18(36%)		
>60	14(38.8%)	2(14.2%)	16(32%)		
Total	36(100%)	14(100%)	50(100%)		
Statistically insignificant					

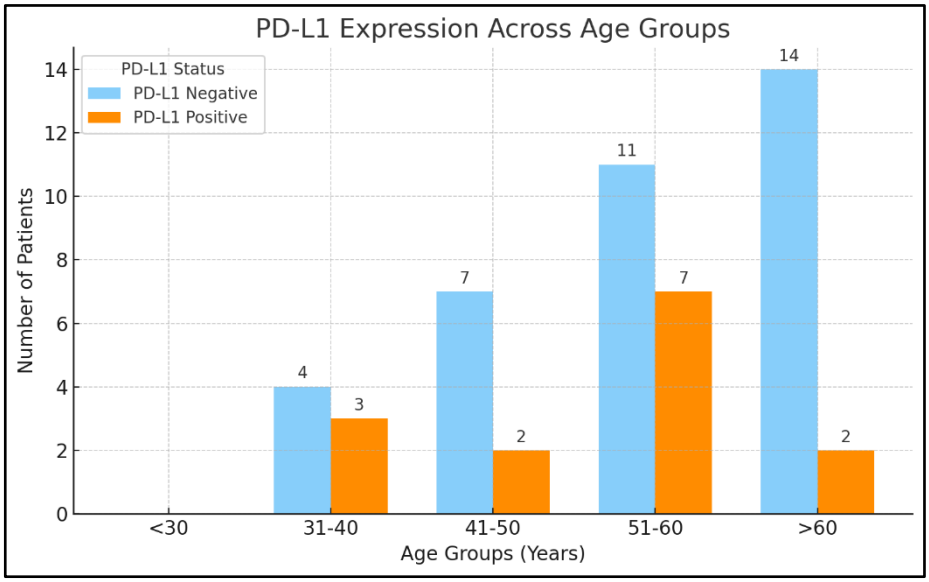

Figure 8: Graphical representation of PD-L1 with Age

The highest number of PD-L1 positivity was observed in the study population more than 50 years of age, with 9 (64.2%) cases out of 14. While the study group with less than 50 years of age showed PD-L1 positivity in 5 (35.6%) cases out of 14 cases. The p value is 0.27, which shows a statistically insignificant association between PD-L1 expression and the patient's age. (Table 12 & Figure 8).

2. SIZE OF THE TUMOR

Table 13- Distribution of cases according to the size of the tumor

TUMOR SIZE	No. of patients	Percentage(%)
T1	12	24.0
T2	24	48.0
T3	08	16.0
T4	06	12.0
Total	50	100.0

The size of the tumor varied between 1 cm to 16 cm. In the majority of the cases i.e., in 24 (48%) cases, tumor size was between 2-5cm (T2), 12(24%) cases had tumor size <2cm, 08 (16%) cases had tumor size of >5 cm (T3) and 6 (12%) cases were of T4 showing direct extension to the chest wall and skin.

Table 14: Correlation of PD-L1 expression with tumor size.

TUMOR SIZE	PD-L1			Chi square test	P value
	Neg	Pos	Total		
T1	07(19.4%)	05(35.7%)	12(24%)	3.50	0.321
T2	17(47.2%)	07(50.0%)	24(48.0%)		
T3	06(16.7%)	02(14.3%)	08(16%)		
T4	06(16.7%)	00(0.0%)	06(12%)		
Total	36(100%)	14(100%)	50(100%)		
Statistically insignificant					

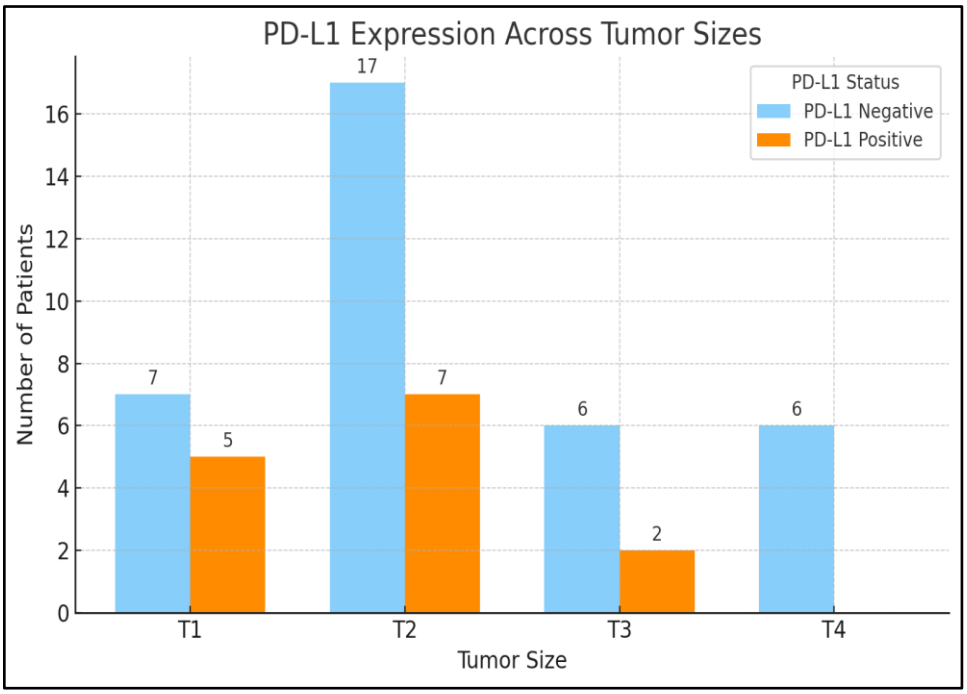

Figure 9: Graphical representation of PD-L1 with tumor size

The tumor ranged in size from 1 cm to 16 cm. Of the PD-L1 positive patients, 7 (50%) belong to the tumor size T2 group, 5 (35.7%) belong to the tumor size T1, and 02 (14.3%) belong to the tumor size T3. Tumor size T4 does not include any PD-L1 positive cases. Tumor size T2 accounted for 17 cases (47.2%) with PD-L1 negative expression, followed by tumor size T1 cases (70.4%) and tumor size T3 and T4 cases (66.7%). There is no statistically significant correlation between tumor growth and PD-L1 expression, as indicated by the p-value of 0.321.

3. HISTOLOGICAL TYPE

Table 15- Distribution of cases according to histologic type

Histologic type	No. of patients	Percentage
Infiltrating ductal carcinoma NOS	46	92%
Invasive lobular carcinoma	01	02%
Encapsulated papillary carcinoma	01	02%
Invasive papillary carcinoma	01	02%
Mucinous carcinoma	01	02%
Total	50	100%

Out of 50 cases studied, a maximum cases were of Infiltrating ductal carcinoma NOS, i.e., 46 cases (92%). One case (2%) was of Invasive lobular carcinoma, 1 case (2%) was invasive papillary carcinoma, 1 case (2%) was Encapsulated papillary carcinoma, and 1cases (2%) was Mucinous carcinoma.

Figure 10: Graphical representation of PD-L1 with histologic type

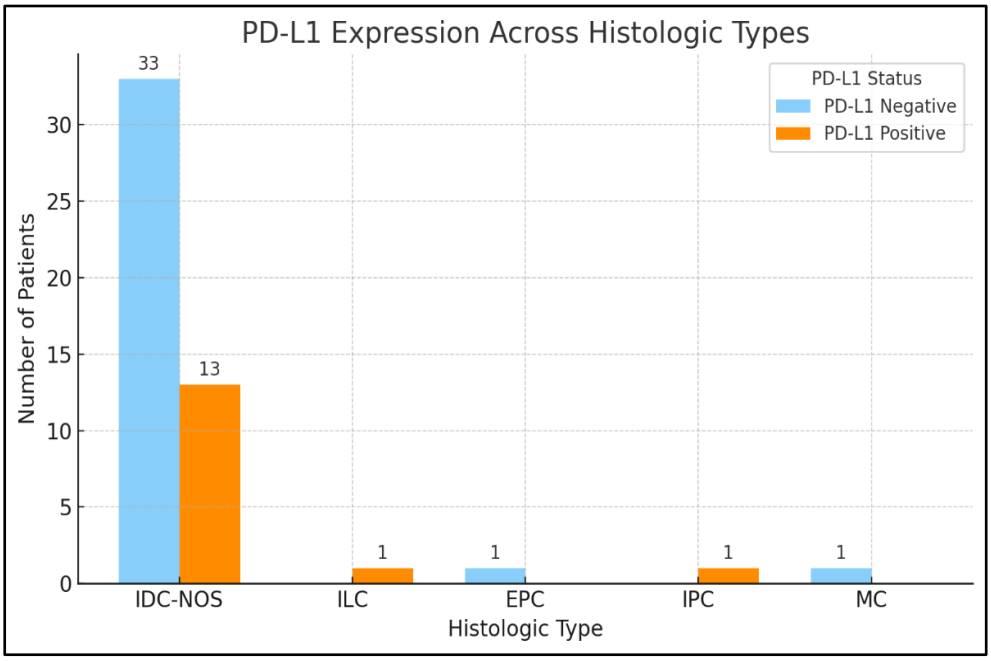

Table 16: **Correlation of PD-L1 expression with histologic type**

Histologic type	PD-L1			Chi-square test	p value
	Negative	Positive	Total		
Infiltrating ductal carcinoma-NOS	33(91.6%)	13(92.8%)	46(92%)	3.74	0.442
Invasive lobular carcinoma	0(0%))	1(7.14%)	1(2%)		
Encapsulated papillary carcinoma	1(2.7%)	0(0%)	1(2%)		
Invasive papillary carcinoma	1(2.7%)	0(0%)	1(2%)		
Mucinous carcinoma	1(2.7%)	0(0%)	1(2%)		
Total	36(100.0%)	14(100.0%)	50(100.0%)		
Statistically insignificant					

13 (92.8%) and 33 (91.6%) of the 46 infiltrating ductal carcinoma-NOS cases had PD-L1 negative and positive expression , respectively. PD-L1 positivity was observed in one case (7.14%) of invasive lobular cancer. PD-L1 negative was found in one case (2.7%) of encapsulated papillary carcinoma, one case (2.7%) of invasive papillary carcinoma, and one case (2.7%) of mucinous carcinoma.

The p-value was 0.44, showing no statistically significant association with PD-L1 and histological type.

4. HISTOLOGICAL GRADE

Table 17- Distribution of cases according to Bloom Richardson's histological grade

HISTOLOGIC GRADE	No. of patients	Percentage (%)
Grade I	20	40%
Grade II	23	46%
Grade III	07	14%
Total	50	100.0

In the present study, 20 (40%) cases belonged to histological grade I, 23 (46%) cases belonged to histologic grade II and 7(14%) cases were of grade III.

Table 18: **Correlation of PD-L1 expression with histologic grade**

HISTOLOGIC GRADE	PD-L1			Chi-square test	p value
	Negative	Positive	Total		
Grade 1	14(38.9%)	06(42.9%)	20(40%)	1.25	0.534
Grade 2	18(50%)	05(35.7%)	23(46%)		
Grade 3	4(11.1%)	3(21.4%)	7(14%)		
Total	36(100.0%)	14(100.0%)	50(100.0%)		
Statistically insignificant					

The majority of the cases in this study with positive PD-L1 expression were Grade I, comprising 06 (42.9%) cases. Next in line are Grade II, where 5 cases (35.7%) had PD-L1 positive, and Grade III, where 3 cases (21.4%) did. With negative PD-L1 expression, 18 cases (50%) fall into Grade II, 14 cases (38.9%) into Grade I, and 4 cases (11.1%) into Grade III. The p value was 0.534, which shows a statistically insignificant correlation between PD-L1 expression and the histological grade of the tumor.

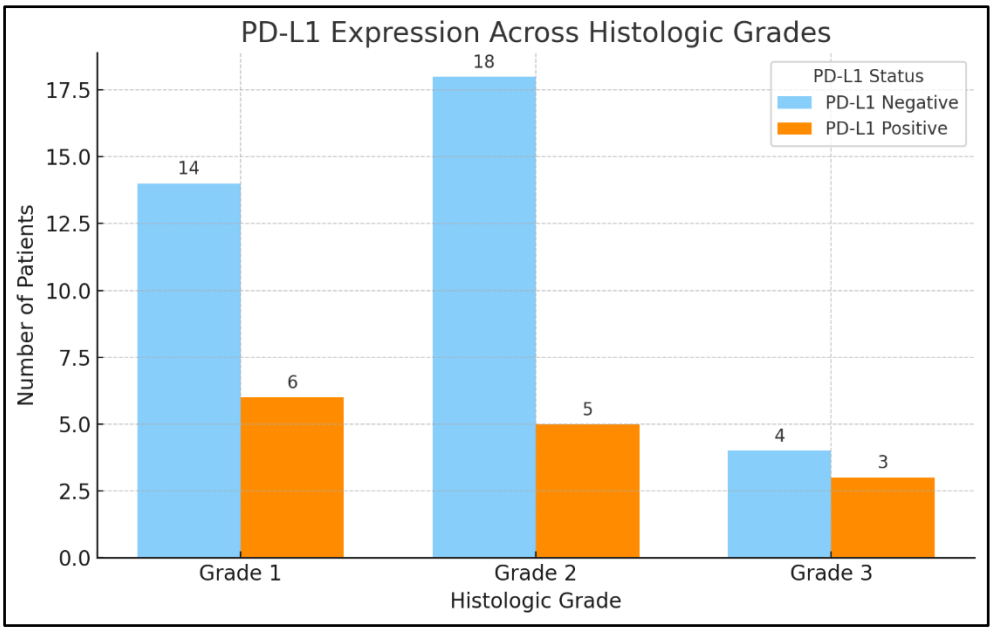

Figure 11: Graphical representation of PD-L1 with histologic grade

5. LYMPH NODE STATUS

Table 19- Distribution of cases according to lymph node status

Lymph node	No. of patients	Percentage (%)
Involved	30	60%
Not involved	20	40%
Total	50	100%

Lymph node metastasis was seen in 30(60%) cases.(Table 19)

Table 20: **Correlation of PD-L1 expression with lymph node status**

Lymph node status	PD-L1			Chi-square test	p value
	Negative	Positive	Total		
Involved	22(61.1%)	08(57.1%)	30(60%)	1.07	0.30
Not involved	14(38.9%)	06(42.9%)	20(40%)		
Total	36(100.0%)	14(100.0%)	50(100.0%)		
Statistically insignificant					

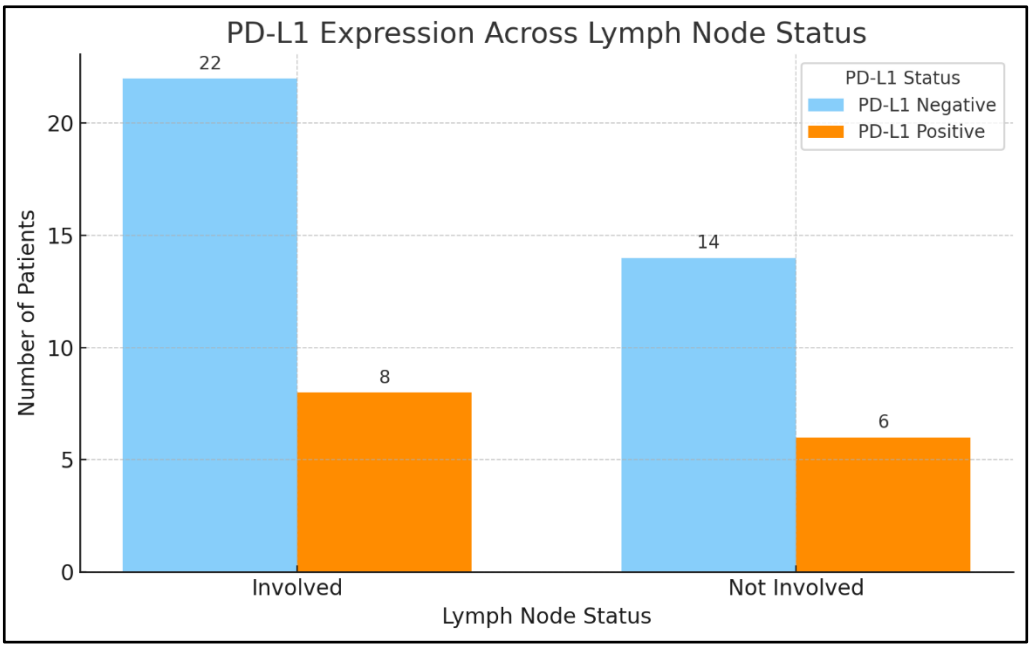

Figure 12: Graphical representation of PD-L1 with lymph node status

30 (60%) of the 50 invasive breast cancer cases in the current study showed positive nodal status; of them, 8 (57.1%) had PD-L1 positive and 22 (61.1%) had PD-L1 negative. Twenty cases (40%) had no lymph node metastases, while six cases (42.9%) expressed PD-L1.

The p value was 0.30, showing no statistical significance in the comparison of PD-L1 expression with lymph-node status.

6. ESTROGEN RECEPTOR STATUS

Table 21- Estrogen receptor expression of breast carcinoma cases

ER status	No. of patients	Percentage
Negative	18	36%
Positive	32	64%
Total	50	100%

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

Table 22: **Correlation of PD-L1 expression with Estrogen receptor status**

ER STATUS	PD-L1			Chi-square test	p value
	Negative	Positive	Total		
Negative	12(33.3%)	6(42.9%)	18(36.0%)	0.091	0.763
Positive	24(66.7%)	8(57.1%)	32(64.0%)		
Total	36(100.0%)	14(100.0%)	50(100.0%)		
Statistically insignificant					

Six (42.9%) of the 18 ER-negative cases had PD-L1 positive. Eight (57.1%) of the 32 patients with positive ER expression had PD-L1 expression. A statistically insignificant association between the tumor's ER status and PD-L1 expression was demonstrated by the p-value of 0.763.

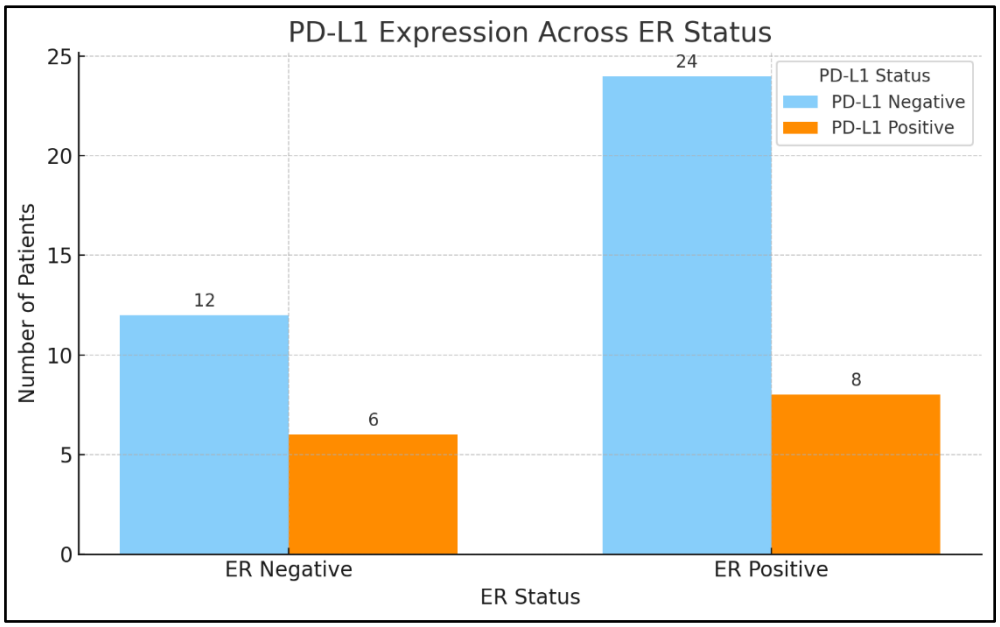

Figure 13: Graphical representation of PD-L1 with Estrogen receptor status

7. PROGESTERON RECEPTOR STATUS

Table 23- Progesterone receptor expression of breast carcinoma cases

PR status	No. of patients	Percentage
Negative	20	40%
Positive	30	60%
Total	50	100%

Among 50 cases of invasive breast carcinoma, 20 cases (40%) were PR-negative, and 30 cases (60%) were PR-positive

Table 24: Correlation of PD-L1 expression with Progesterone receptor status

PR STATUS	PD-L1			Chi-square test	p value
	Negative	Positive	Total		
Negative	13(36.1%)	7(50.0%)	20(40.0%)	0.335	0.563
Positive	23(63.9%)	7(50.0%)	30(60.0%)		
Total	36(100.0%)	14(100.0%)	50(100.0%)		
Statistically insignificant					

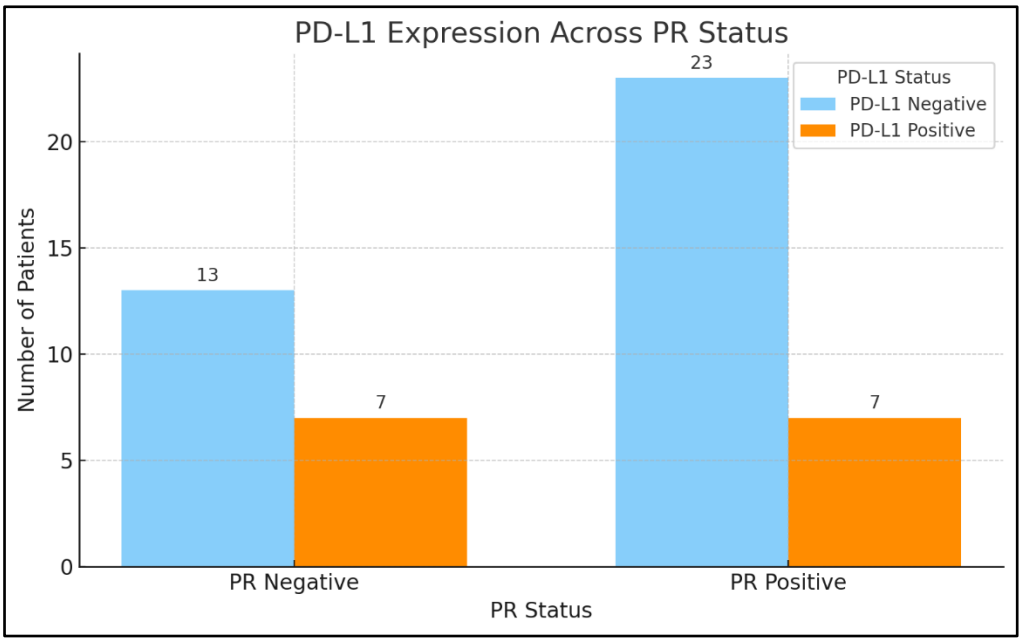

Figure 14: Graphical representation of PD-L1 with Progesterone receptor status

Seven (50%) of the 20 PR-negative cases had PD-L1 positive. Seven (50%) of the 30 individuals with positive PR expression also had PD-L1 positivity. A statistically insignificant association between the tumor's PR status and PD-L1 expression was indicated by the p value of 0.56.

8. HER2/neu STATUS

Table 25- HER2/neu receptor expression of breast carcinoma cases

HER2/neu	No. of patients	Percentage
Negative.	26	52.0%
Positive.	24	48.0%
Total.	50	100.0%

Of the 50 invasive breast cancer cases in the current study, 26 (52%) had HER2/neu negative results, and 24 (48%) had HER2/neu positive results.

Table 26: **Correlation of PD-L1 expression with HER2/neu receptor status**

HER2/neu STATUS	PD-L1			Chi-square test	P value
	Negative	Positive	Total		
Negative	22(61.1%)	4(28.6%)	26(52.0%)	3.07	**0.039**
Positive	14(38.9%)	10(71.4%)	24(48.0%)		
Total	36(100.0%)	14(100.0%)	50(100.0%)		
* **Statistically significant**					

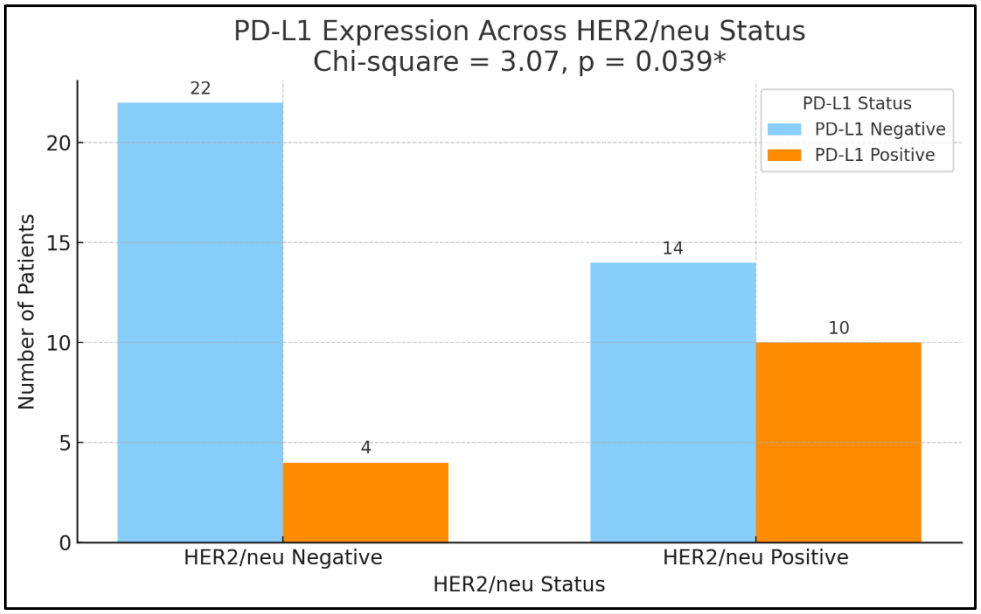

Figure 15: Graphical representation of PD-L1 with HER2/neu receptor status

Four (28.6%) of the 26 HER2/neu negative cases had PD-L1 positive. Ten (71.4%) of the 24 individuals with positive HER2/neu expression also had PD-L1 positivity. PD-L1 expression and the tumor's HER2/neu status were statistically significantly correlated, as indicated by the p-value of 0.039.

Table 27: Comparison of PD-L1 with various clinicopathological parameters

PARAMETERS	PD-L1 NEGATIVE NO OF CASES (%)	PD-L1 POSITIVE NO OF CASES (%)	CHI-SQUARE TEST	P VALUE
AGE				
<30	0 (0%)	0 (0%)	3.88	0.27
31-40	4 (11.3%)	3 (21.4%)		
41-50	7 (19.4%)	2 (14.2%)		
51-60	11 (30.5%)	7 (50%)		
>60	14 (38.8%)	2 (14.2%)		
TUMOR SIZE				
T1	07 (19.4%)	05 (35.7%)	3.50	0.321
T2	17 (47.2%)	07 (50%)		
T3	06 (16.7%)	02 (14.3%)		
T4	06 (16.7%)	00 (0%)		
HISTOLOGICAL TYPE				
IDC-NOS	33 (91.6%)	13 (92.8%)	3.74	0.442
ILC	00	1 (7.14%)		
EPC	01 (2.7%)	00 (0%)		
IPC	01 (2.7%)	00 (0%)		
MC	01 (2.7%)	00 (0%)		
HISTOLOGICAL GRADE				
I	14 (38.9%)	06 (42.9%)	1.25	0.534
II	18 (50%)	05 (35.7%)		
III	4 (11.1%)	03 (21.4%)		
LYMPH NODE STATUS				
Involved	22 (61.1%)	08 (57.1%)	1.07	0.30
Not involved	14 (38.9%)	06 (42.9%)		
ER STATUS				
Negative	12 (33.3%)	06 (42.9%)	0.091	0.763
Positive	24 (66.7%)	8 (57.1%)		
PR STATUS				
Negative	13 (36.1%)	07 (50%)	0.335	0.563
Positive	23 (63.9%)	07 (50%)		
HER 2 NEU				
Negative	22 (61.1%)	04 (28.6%)	3.07	**0.039***
Positive	14 (38.9%)	10 (71.4%)		

PHOTOMICROGRAPHS

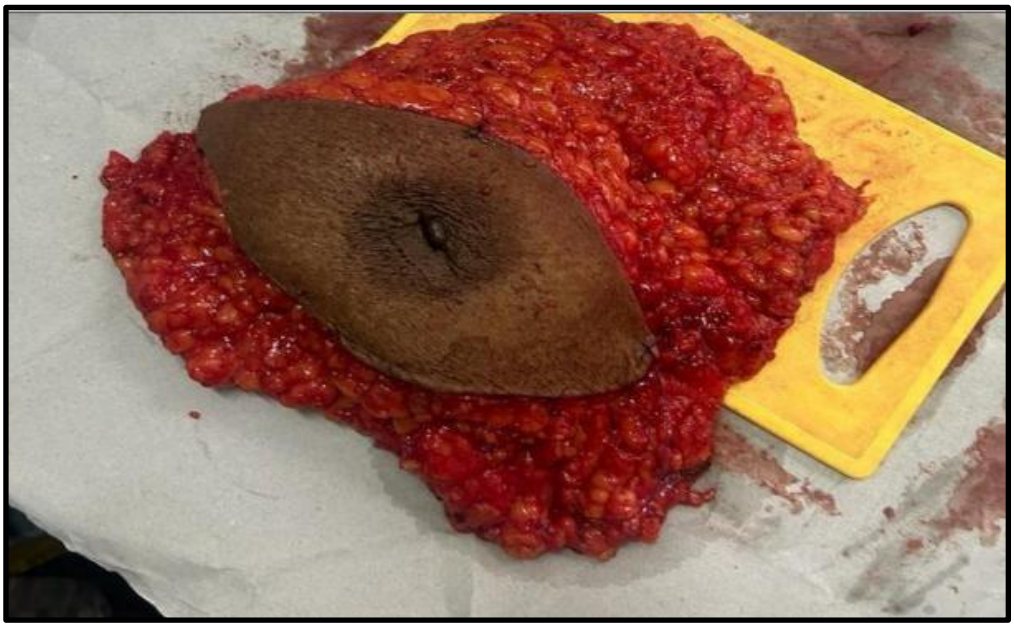

Figure 16: Gross Photograph of Modified radical mastectomy specimen measuring 16x14x5.5cm.

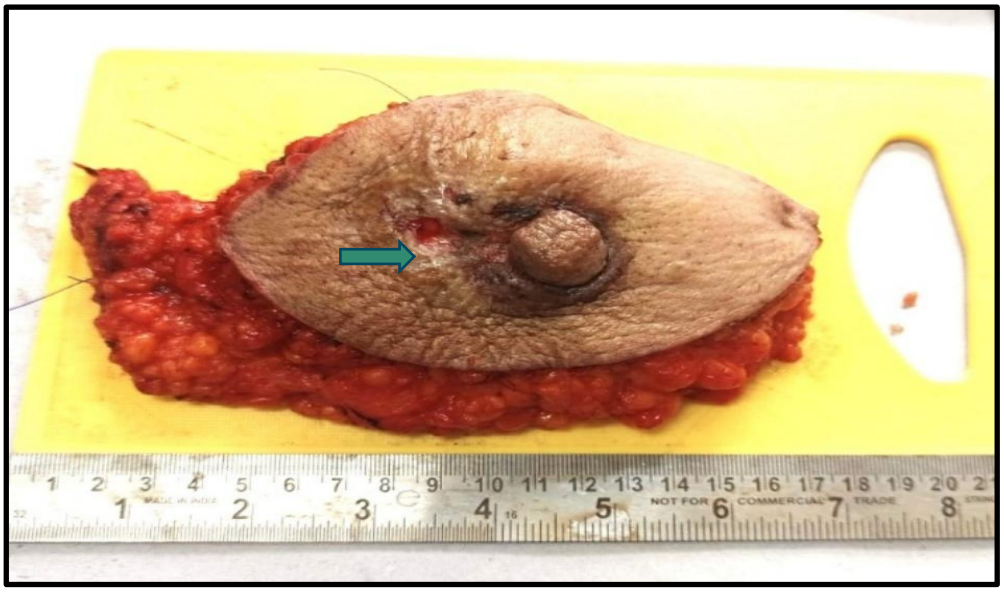

Figure 17: Gross Photograph of Modified radical mastectomy specimen measuring 16x11.5x6 cm. Skin ulceration noted measuring 0.7x0.7cm. 2cm lateral to nipple.

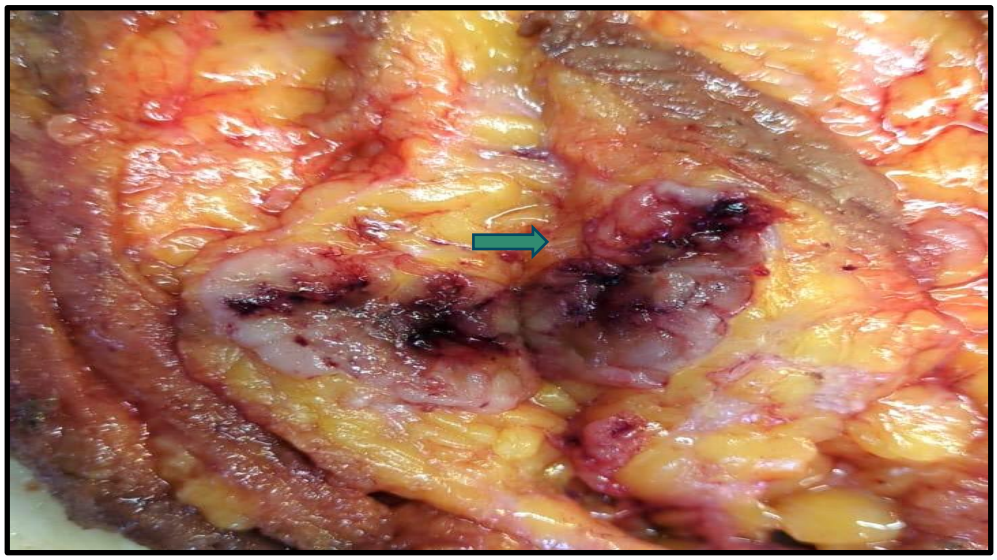

Figure 18: Macrophotograph showing cut section of Invasive breast carcinoma with tumor size 2x1.5 cm

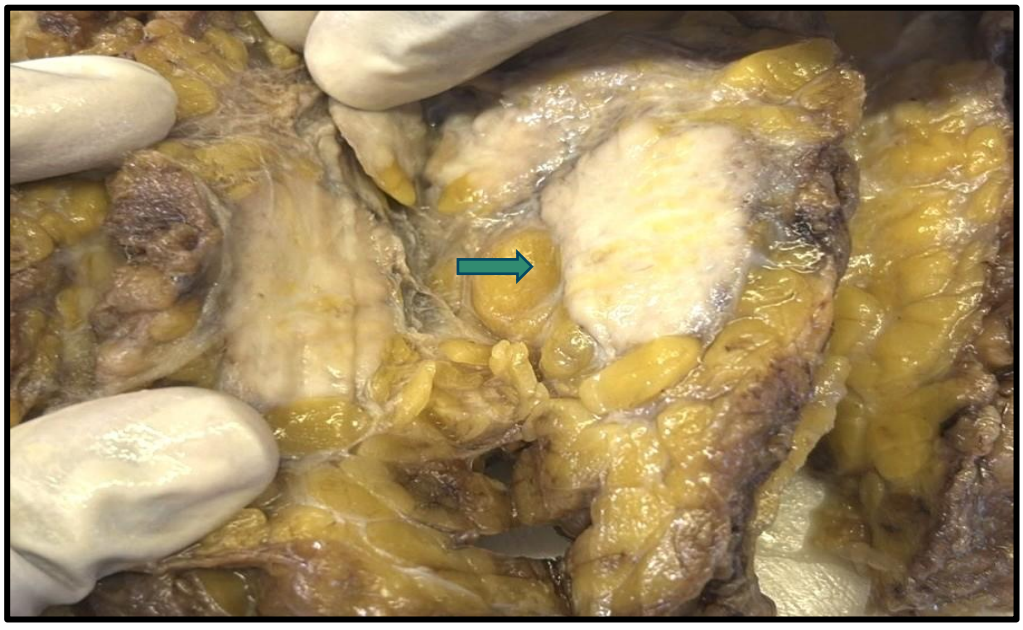

Figure 19 : Gross Photograph of Invasive Breast Carcinoma showing solid pale white growth measuring 4x3cm

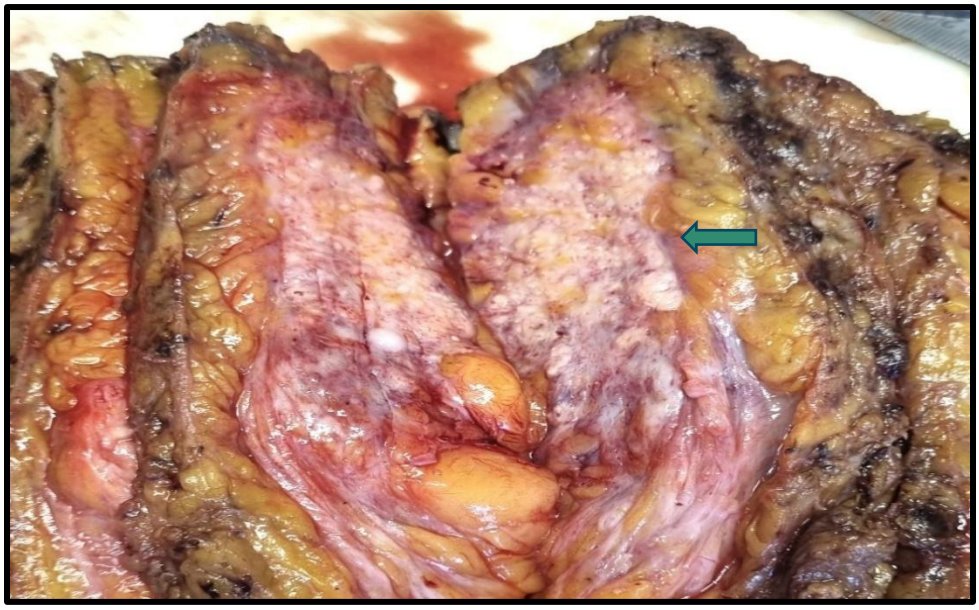

Figure 20: Macro photograph showing cut section of Invasive breast carcinoma with pale white irregular growth measuring 7.5x3.5cm

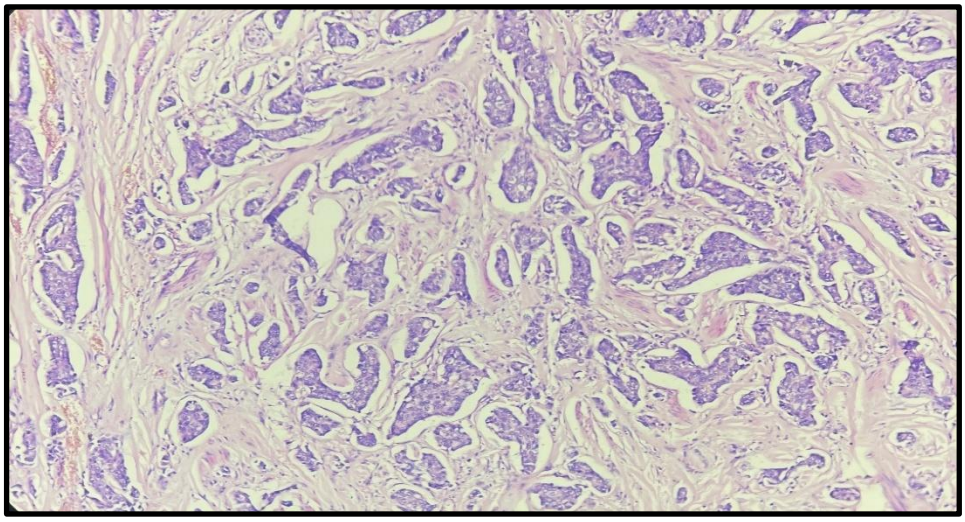

Figure 21: Microphotograph showing tumor tissue arranged in nests and lobules (H&E) (400x)

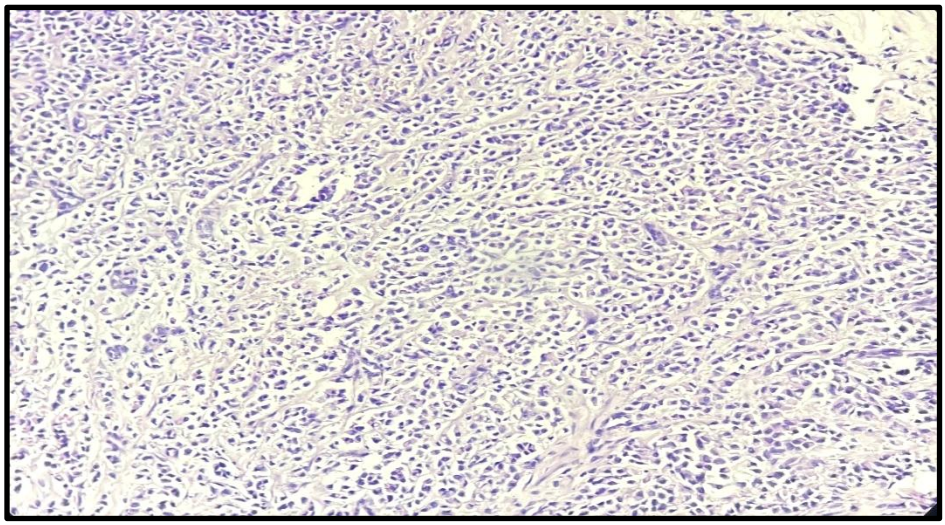

Figure 22: Microphotograph showing Invasive lobular carcinoma (H&E) (400x)

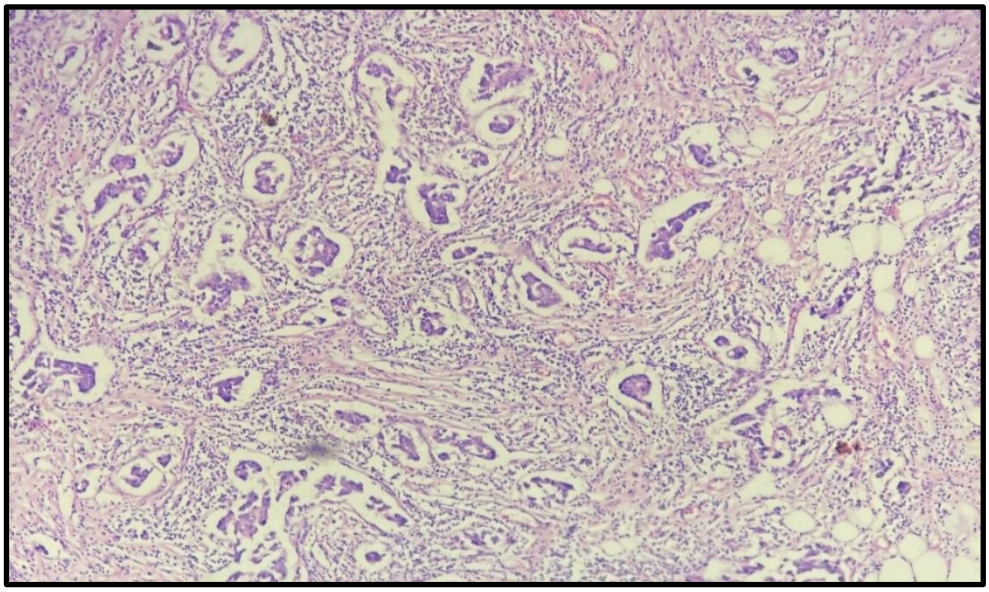

Figure 23: Microphotograph of invasive breast carcinoma NOS- Grade 1. Tumor cells showing mild nuclear Pleomorphism (H&E) (400x)

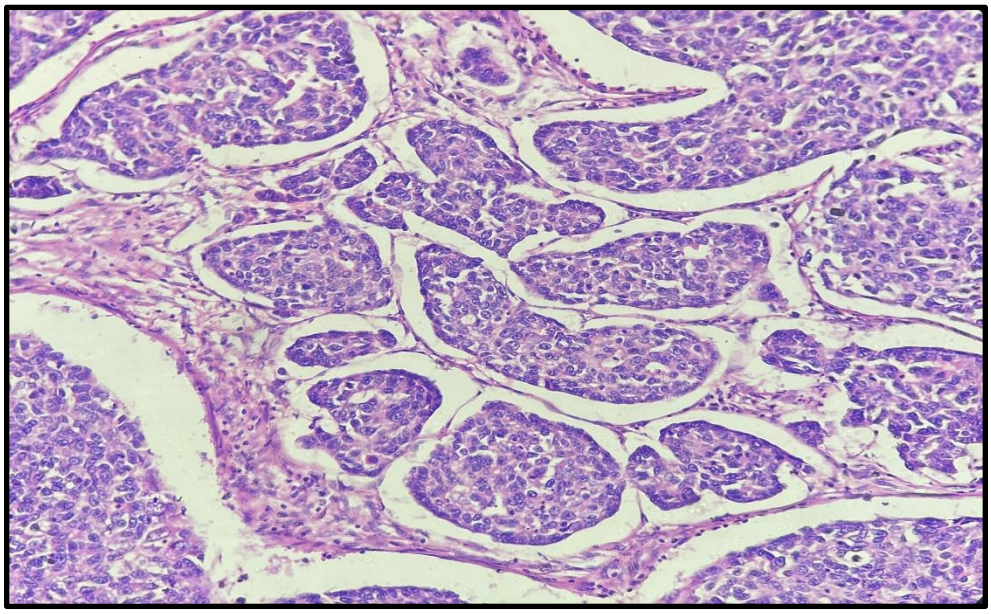

Figure 24: Microphotograph of invasive breast carcinoma NOS- Grade 2. Tumor cells showing moderate Nuclear pleomorphism (H&E) (400x)

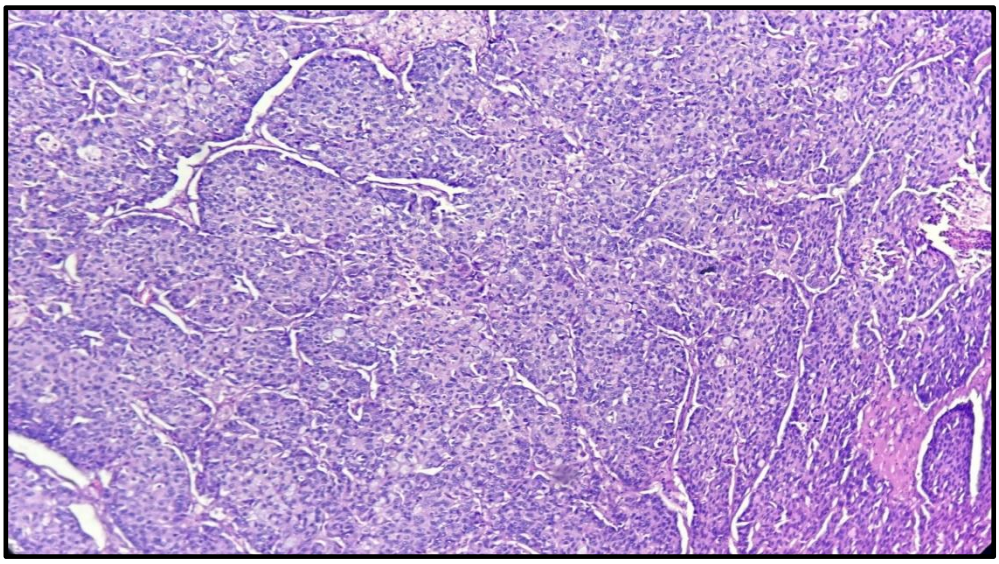

Figure 25: Microphotograph of invasive breast carcinoma NOS Grade 3- tumor cells showing marked pleomorphism (H&E) (400x)

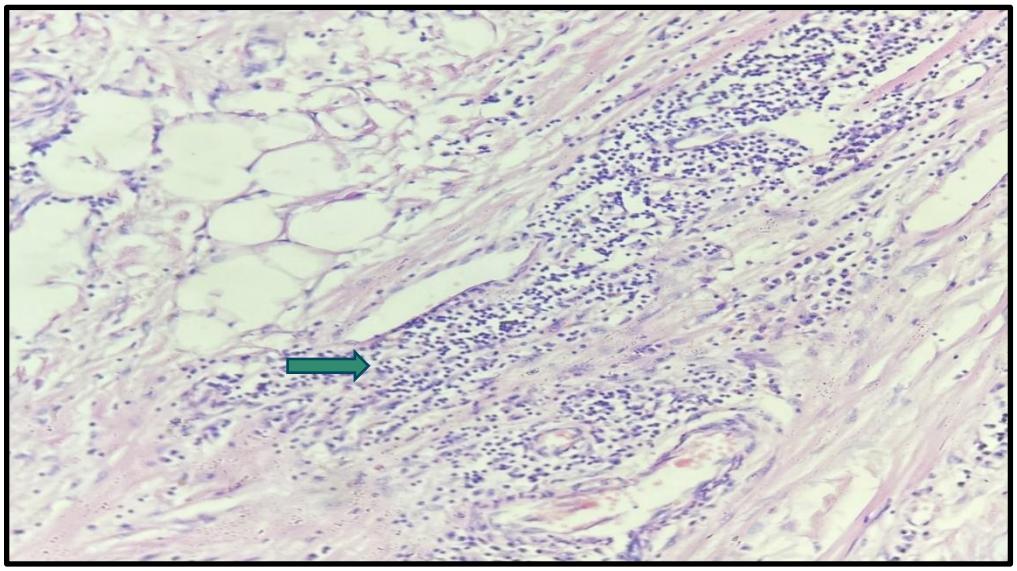

Figure 26: Microphotograph of invasive breast carcinoma showing lymphocytic infiltrate in the Intervening areas (H&E) (400x)

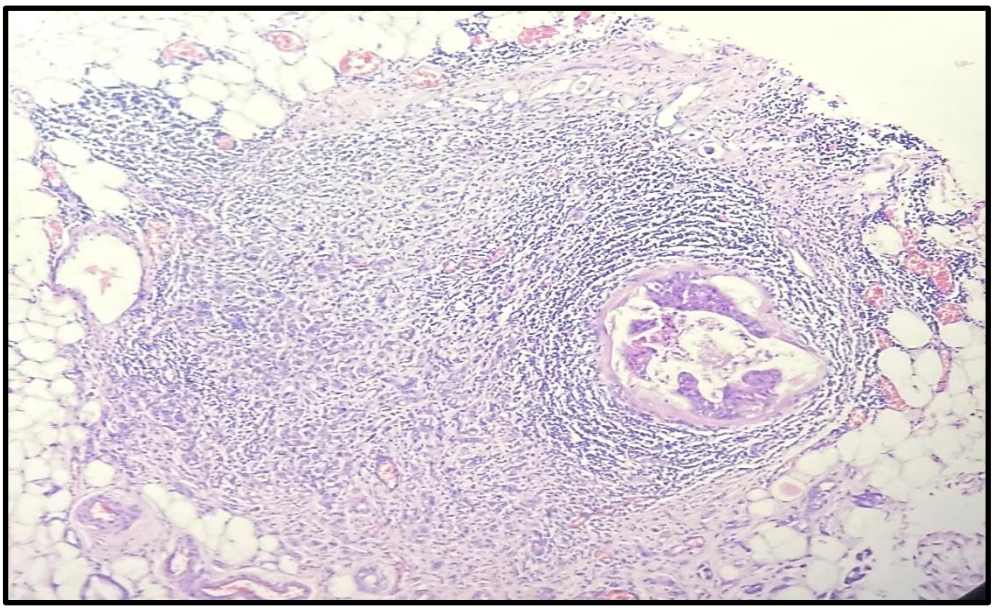

Figure 27: Microphotograph of invasive breast carcinoma showing tumor deposits in lymph node (H&E) (400x)

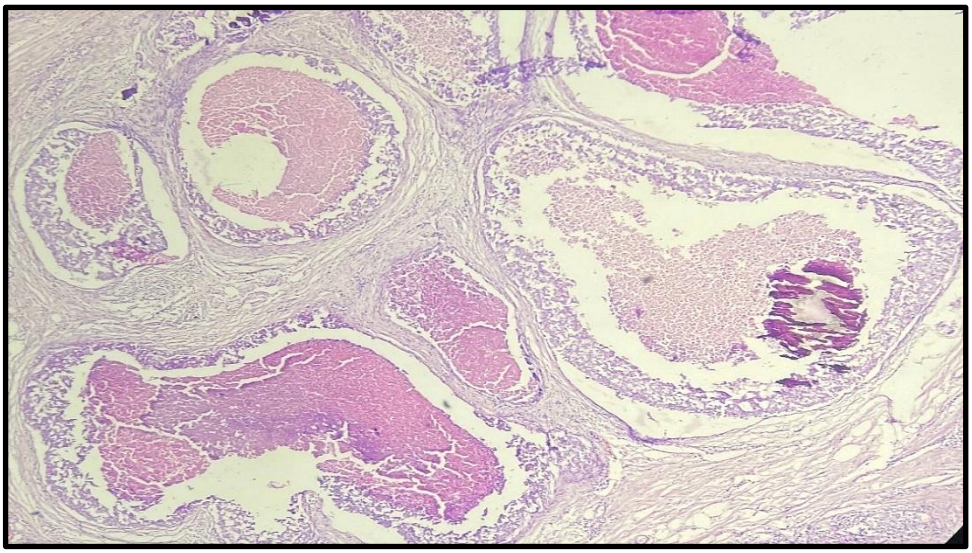

Figure 28: Microphotograph of invasive breast carcinoma showing comedo necrosis (H&E) (400x)

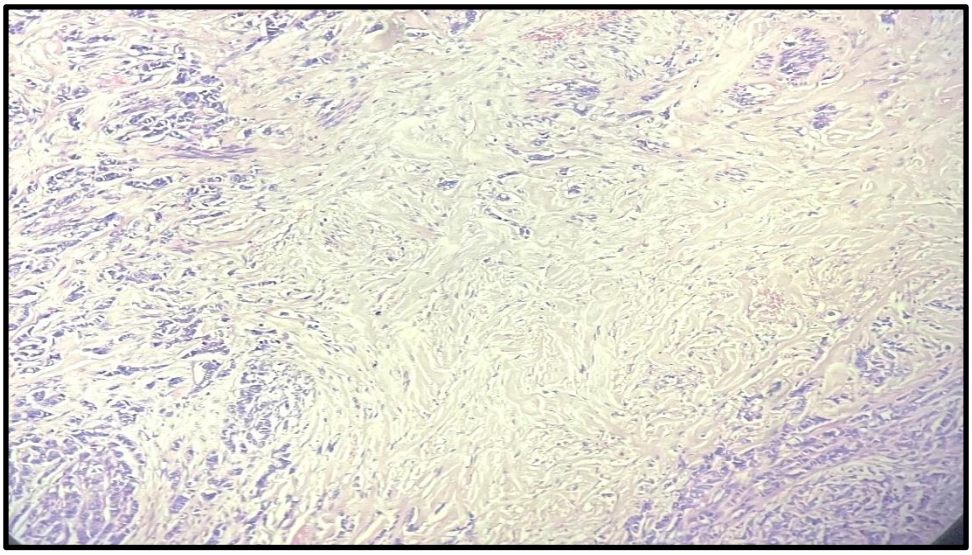

Figure 29: Microphotograph of invasive breast carcinoma showing desmoplastic stroma (H&E) (400x)

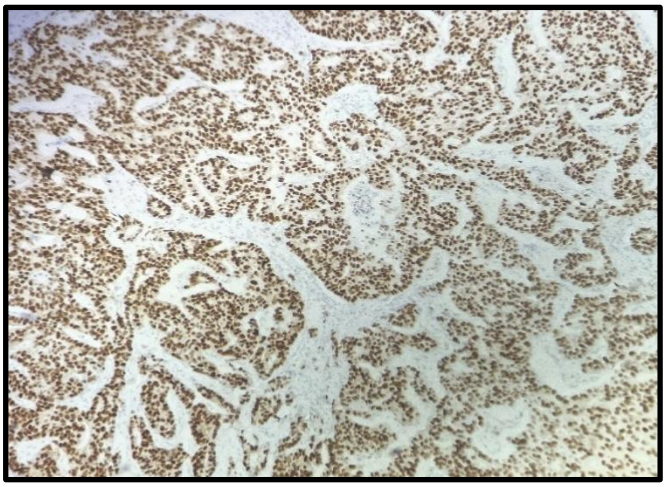

Figure 30: Microphotograph of IHC marker ER showing nuclear positivity in invasive breast carcinoma NOS (100x)

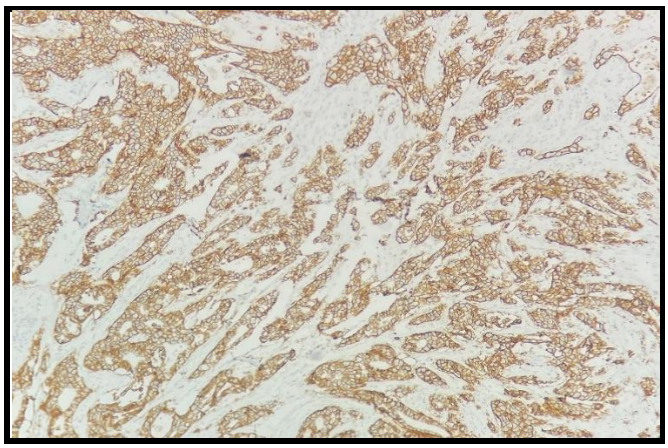

Figure 31: Microphotograph of IHC marker HER2/neu showing membranous positivity in invasive breast carcinoma NOS (100x)

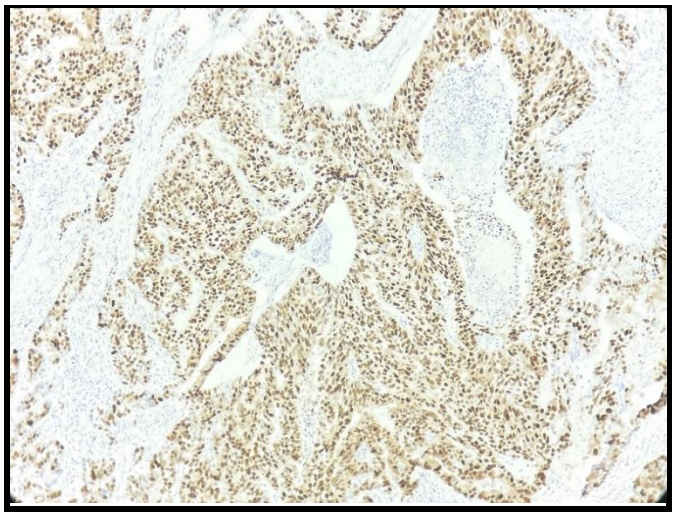

Figure 32: Microphotograph of IHC marker PR showing nuclear positivity in invasive breast carcinoma NOS (100x)

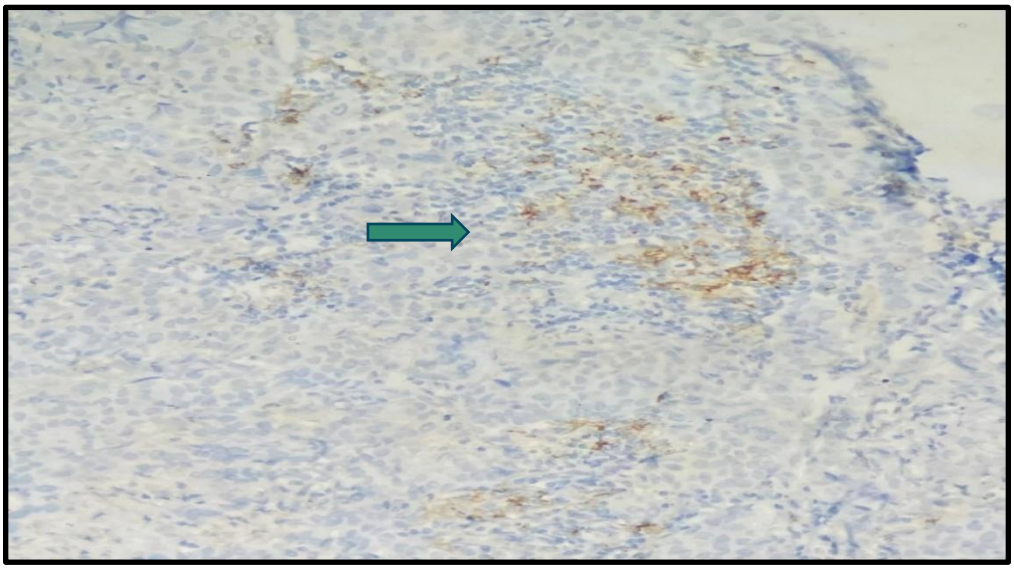

Figure 33: Microphotograph of IHC marker PD-L1 showing membranous staining in tumor cells of invasive breast carcinoma NOS (100x)

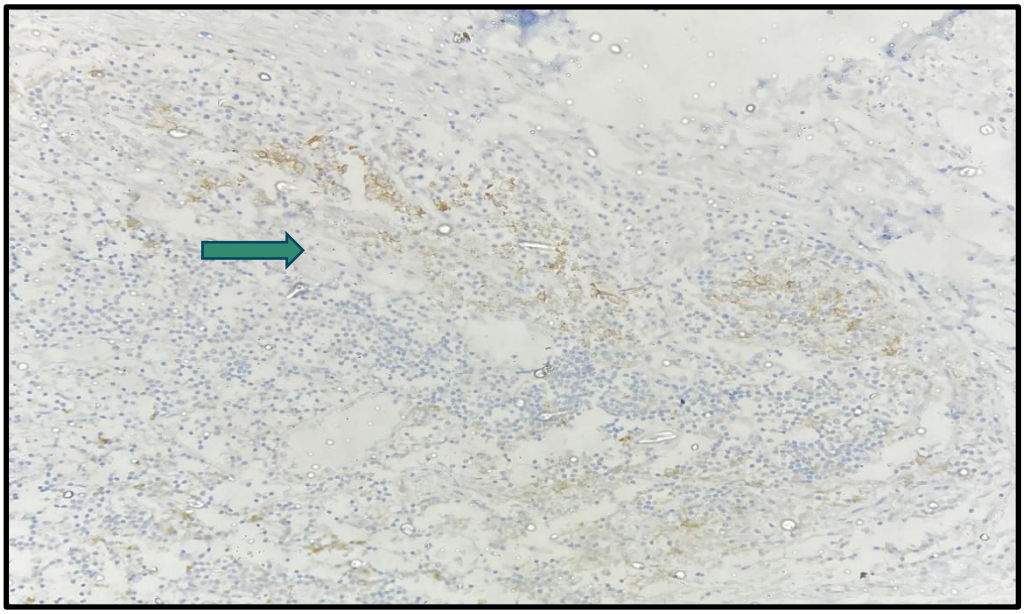

Figure 34: Microphotograph of IHC marker PD-L1 showing cytoplasmic and membranous staining in immune cells in invasive breast carcinoma NOS (100x)

DISCUSSION

The leading cause of cancer in women worldwide, both in high- and low-resource settings, is breast carcinoma, which accounts for over 1 million of the estimated 10 million cancers found each year in both sexes globally.[9,2]

Since breast cancer-related deaths are caused by metastatic disease, a deeper understanding of the molecular basis of this disease would be helpful in the diagnosis, treatment, and prognosis of breast cancer. [61] The awareness campaigns have started a drift towards a younger age at diagnosis, but what is more concerning is the prevalence of aggressive tumors, "ER -ve," "PR -ve," "HER2/neu+ve," or "triple negative tumors" in this age group. Given their rising prevalence, a focus on these aggressive tumors is urgently required. [62]

However, there has been a significant advancement in the diagnosis and treatment of carcinoma breast, including Breast conservation surgeries (BCS), Neoadjuvant chemotherapy, tumor classification based on overexpression of the HER2/neu protein and the estrogen, progesterone, and HER2 receptors, and incorporation of these into standard treatment protocols. [61]

It has recently been established that the interplay between the malignant cells and their microenvironment facilitates the growth of tumors. Several variables that affect the signaling pathways involved in tumor invasion and metastatic spread engage in this interaction. Understanding how stromal cells and cancer cells interact in the tumor microenvironment may help in the search for new treatment targets and prospective indicators. [8]

To create individualized care, a thorough search for potential disease markers is required, particularly for those having prognostic and therapeutic implications. [63]

The expression of PD-L1 in the tumor microenvironment indicates immune resistance to the body's natural antitumor response. PD-L1 shows expression in various cells, including TCs and ICs. In recent years, research on PD-L1 expression in breast cancer has become more significant. According to this research, each subgroup of breast cancer has varying levels of PD-L1 expression. As a result, different studies had different frequencies of PD-L1 expression. The prognostic and predictive significance of PD-L1 remains a topic of ongoing debate in published research. The various techniques used to measure PD-L1 expression (i.e., IHC expression, mRNA expression, paraffin tissue blocks, tissue microarray, and various IHC staining monoclonal kits) and different scoring systems result in disparate findings in publications. According to Gonzalez-Ericsson et al., the SP142, SP263, and 22C3 tests produced different results on TNBC. The SP142 assay detects lower PD-L1 expression on tumor cells (TC) and immune cells (IC) compared to other PD-L1 assays. [64]

Multiple prognostic and predictive clinical and pathological markers guide the therapeutic management of invasive breast cancer (IBC). The intrinsic subtype of invasive breast carcinoma (IBC) plays a key role in determining the use of endocrine therapy, chemotherapy, and targeted therapy (such as anti-HER2 treatment). These subtypes, which have both predictive and prognostic significance, include triple-negative breast cancer (TNBC), HER2-enriched (HER2+) IBC, and luminal tumors. Beyond conventional systemic therapies, novel agents such as CDK4/6 inhibitors, which target specific oncogenic pathways, have demonstrated the potential to improve the prognosis of breast cancer patients.

Immunotherapeutic approaches, especially immune checkpoint inhibitors (ICIs), have transformed oncology and proven effective in managing various cancers. Targeting the programmed death-1 (PD-1) protein and its ligand PD-L1 has become the standard of care for

several advanced or metastatic malignancies, including non-small cell lung cancer, urothelial carcinoma, Merkel cell carcinoma, classic Hodgkin lymphoma, head and neck squamous cell carcinoma, cutaneous squamous cell carcinoma, and renal cell carcinoma.

In metastatic triple-negative breast cancer (mTNBC) with PD-L1 expression on tumor-associated immune cells, studies have shown that combining the anti-PD-L1 drug atezolizumab with nab-paclitaxel significantly improves survival compared to nab-paclitaxel alone. [59]

In this study, we examined PD-L1 expression in Breast carcinoma cases to determine whether PD-L1 is connected to a specific clinicopathological feature and hormonal status and whether it is an independent prognostic marker.

1. Distribution of study population according to PD-L1 positivity and Comparison of expression of PD-L1 in various studies

In the study we conducted, PD-L1 positivity was found in 28% of cases of breast carcinoma. PD-L1 expression ranges from 80%, 14.6%, 35.9%, 11%, and 52.6%, respectively, in research by Gupta et al., [65] Punhani et al., [19] Gajaria et al. [11] Amin et al. [13] Dey et al. [60] and Lou J et al.[66] Forty of the 50 patients that Gupta et al. [65]examined had PD-L1 positive. According to Punhani et al. [19], PD-L1 positive was seen in 22 out of 150 patients and of the 184 cases in the study by Gajaria et al. [11], 66 (35.9%) had PD-L1 positive. PD-L1 IHC in the study population was found to be high in 81 (52.6) out of 154 cases in the study of Dey et al. [60]. In comparison, it was found in 12 out of 109 cases in the study of Amin et al. [13] and 24 out of 64 cases in the study of Lou J et al. [66] Different antibody clones and scoring systems may be the cause of the difference in PD-L1 expression observed in different studies, according to Punhani et al. [19]According to Gupta et al. [65] the variance in PD-L1 expression between studies may be caused by tissue collection. Although our study included all subtypes of breast cancer, limited research has

specifically explored the relationship between PD-L1 expression and TILs in cases of triple-negative breast carcinoma (TNBC). These variations in PD-L1 expression in different studies indicated the need for further studies with large sample sizes.

2. Distribution of the study population based on Age and its correlation with PD-L1 expression.

PD-L1 expression was higher in postmenopausal patients in this study, with a Mean age of 55.7 years and a median age of 55. PD-L1 expression and patient age did not, however, correlate statistically significantly. The research done by Punhani et al. [19], Dey et al. [60], and Lou J et al. [66] yielded similar results. The majority of cases were diagnosed in those under 50 years old (P 0.003), according to Gajaria et al. [11]. In the study done by Gupta et al. [65], they observed that PD-L1 expression showed a statistically significant association with age with a median age of 53.8. Also, Amin et al. [13], in their study, observed that PD-L1 positivity is seen in age >49 years and is statistically significant, with PD-L1 expression being almost similar in <50 and > 50 years.

3. Distribution of the study population based on tumor and its correlation with PD-L1 expression.

The 10-year survival rate for women with tumors less than 1 cm and no lymph nodes is 90%; if the cancer is more significant than 2 cm, the 10-year survival rate is 77%. [33] In this study, tumor diameters varied from 1 to 16 cm. The highest number of cases in the studies by Punhani et al. [19], Gajaria et al. [11], Amin et al. [13], and Lou J et al. [66] belonged to size 2-5(T2)cm. However, this was not statistically significant. The current research found that 07 (50%) cases with PD-L1 positive belong to the tumor size T2 category. PD-L1 and tumor size did not significantly correlate in our investigation, as indicated by the p-value of 0.32. In studies conducted by Gupta

et al. [65] and Dey et al. [60], a significant correlation between PD-L1 and tumor size(T2) was found.

4. Distribution of the study population based on histologic type and its correlation with PD-L1 expression.

According to the WHO classification, the following types were included: Infiltrating ductal carcinoma-NOS(IDC-NOS), Infiltrating Lobular Carcinoma(ILC), Encapsulated Papillary Carcinoma(EPC), Mucinous Carcinoma, and Invasive papillary Carcinoma(IPC). Most cases were Infiltrating Ductal carcinoma-NOS (46 out of 50 cases, or 92%), but none of these types were statistically significant with PD-L1 expression. Punhani et al. [19], Amin et al. [13], and Dey et al. [60] found that 94%, 84.4%, and 89.6% of cases were IDC-NOS, respectively, but that there was no statistical significance between PD-L1 and histological type. Gupta et al. [65], Gajaria et al. [11], and Lou J et al. [66] examined all cases of invasive ductal carcinoma, and no other special types were included.

5. Distribution of the study population based on Histologic grade and its correlation with PD-L1 expression.

Patients with higher grades of breast cancer have a worse chance of survival. [33] Grade 2 tumors accounted for the most significant fraction in the current study (46%). A study by Gupta et al. [65] and Dey et al. [60] found the same results, with 37 out of 50 cases (74%) and 99 out of 154 cases (64.3%) being Grade 2. In contrast to the work by these two authors, which established a statistically significant link, our investigation did not find any statistical significance between PD-L1 and histological grade (p-value = 0.53). PD-L1 expression was observed to be linked with higher tumor grade, i.e., Grade 3, with a p-value<0.05 in the Punhani et al. [19] and Amin et al. [13] study. Out of the 184 study participants, 177 cases of grade 2 were included in the Gajaria

et al. [11] study. In contrast, PD-L1 expression was linked to higher tumor grade (i.e., Grade 2-3) with a p-value of 0.03 in the Lou J et al. [66] study. They found that tumor grade predicts a poor prognosis, as evidenced by increased expression of PD-L1 in higher histologic grades.

6. Distribution of the study population based on Nodal metastasis and its correlation with PD-L1 expression.

Distant metastasis is more likely to occur when nodal metastasis is present. However, removing involved Lymph nodes doesn't lower the risk of future metastatic diseases. Approximately 10-20% of women without axillary lymph node mets experience recurrence of breast cancer with distant metastasis. [33,26] After 10-25 years of detection, recurrence is raised with elevated lymph node involvement at baseline, ranging from 12.7% to 24.6%. [67] In the current study, out of 30 lymph node metastasis cases, 08 cases (57.1%) showed PD-L1 positive expression, which meant that there is no statistically significant correlation between PD-L1 and lymph node metastasis, as the p-value is 0.30. In the study performed by Punhani et al. [19] and Dey et al. [60], similar findings were noted in the present study. In the study, Amin et al. [13] and Lou J et al. [66], 57 out of 109 and 34 out of 64 did not show nodal metastasis, respectively. Gupta et al. [65] found that 30 cases (60%) of 50 lymph node-positive groups and 21 showed PD-L1 positivity. A statistical correlation was found in this study with a p-value of 0.02.

7. Distribution of the study population based on ER, PR AND HER2 STATUS and its correlation with PD-L1 Status.

PD-L1 and HER2/Neu positive status were statistically significant in the current study, with a p-value of 0.03. However, no statistical significance existed between PD-L1 and PR/ER status. According to Amin et al.'s [13] study, no statistical correlation was seen between PD-L1 and ER expression, PR expression and HER2/neu expression in their research. In the study by Gupta

et al. [65], a statistical correlation was seen between PD-L1, ER-positive expression, and HER2/neu Negative expression. Still, there was no correlation with PR positive expression in their study. At the same time, the other studies did not assess hormonal receptors.

Table- 28 Comparison of statistical results of HER2/neu with other studies

AUTHOR	SAMPLE SIZE	p-Value for HER2/neu Positive Expression	p-Value for HER2/neu Negative Expression
Present study	50	**0.03**	--
Amin et al	109	0.80	--
Gupta et al	50	--	**0.05**
Punhani et al	150	--	>0.05
Dey et al.	154	--	>0.05

SUMMARY

- Mastectomy specimens of primary breast carcinoma obtained in the histopathology section of the Department of Pathology between May 1, 2023, and December 31, 2024, were included in this cross-sectional study.

- Histopathological diagnosis of all cases included in this study was based on routine microscopic examination of H&E stain.

- Data regarding the patient's age and tumor size was collected, whereas slides were reviewed for histological grade, type and lymph node involvement.

- The IHC staining for ER/PR/HER2/neu receptor, along with PD-L1, was studied in all the cases of invasive breast carcinoma.

- PD-L1 expression was analyzed about various prognostic markers, including patient age, size of tumor, histological type, grade, lymph node status, and the presence of ER, PR, and HER2/neu.

- The study included patients aged 30 to 80, with an average age of 55.7 years.

- PD-L1 positivity was seen in 14 (28%) cases among the 50 cases studied, and 36 (72%) cases showed PD-L1 negative immune reactivity.

- PD-L1 expression was strongly associated with HER2/neu positive expression in this study with a significant statistical correlation.

- Statistically, a Non-significant correlation was observed between PD-L1 expression and patient age, tumor size, histological type, tumor grade, lymph node status, or ER/PR status.

CONCLUSION

This study was carried out to assess PD-L1 expression in Indian patients with breast cancer. According to our research, PD-L1 expression is associated with poor prognostic variables for breast cancer, notably HER2/neu status. The study population's overall PD-L1 expression was 28%. The number of HER2/neu patients among PD-L1 positive groups was 10 (71.4%). This suggests that a poor prognosis is linked to PD-L1 expression. Nevertheless, no statistically significant association was discovered between prognostic and clinicopathological factors, such as ER/PR status, lymph node status, tumor size, histology type, histological grade, and patient age. Because of this, it cannot be employed as a prognostic marker. It can be helpful to assess the indication for the use of anti-PD-L1 inhibitors in advanced breast disease.

Existing research on PD-L1 expression has yielded contradictory results, and there is limited data on its expression in breast cancer patients, particularly in the Indian subcontinent. This study aims to enhance the understanding of PD-L1 expression in breast cancer, specifically among Indian women, while also contributing to existing knowledge on its correlation with key prognostic factors.

With the growing emphasis on personalized treatments, developing new targeted therapies has gained significant attention. Immunohistochemistry (IHC) remains a cost-effective and straightforward technique for assessing the expression of emerging biomarkers, including PD-L1.

The creation of novel targeted therapeutics is receiving increased attention in the current era of customized treatment. Immunohistochemistry is a comparatively more straightforward and

less expensive method for assessing the expression of newly developed markers, such as PDL1.

Therefore, PD-L1 expression is a new marker and standardization of immunohistochemical (IHC) reporting for PD-L1 is essential to ensure reproducibility and reliability in evaluating breast carcinoma. Consistent and accurate PD-L1 assessment could significantly impact the application of novel targeted immunotherapies in treating breast carcinoma.

Limitations of the current study:

A larger-scale investigation is necessary because there is variability in the expression of PD-L1 with other factors.

Recommendations:

It is recommended that PD-L1 immunohistochemistry reporting be standardized to make it consistent and dependable for assessing breast cancer. More extensive studies with extended follow-up periods are recommended to better define the PD-L1 role as a prognostic marker in breast cancer. The implications of programmed death-ligand 1(PD-L1) may also be significant for new targeted immunotherapies against breast carcinoma.

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ANNEXURE I

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/ 933/2023-24 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 NOVEL IMMUNOHISTOCHEMICAL MARKER PROGRAMMED DEATH LIGAND-1 IN BREAST CARCINOMA".

NAME OF THE STUDENT/PRINCIPAL INVESTIGATOR: DR. MONIKA PAWAR

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 _____ at _____ (place) and it has been explained to me in my own dialect that I am suffering from _____ disease (condition) and this disease/condition mimic following diseases. Further Doctor informed me that he/she is conducting a dissertation/research titled **ASSESSMENT OF EXPRESSION OF NOVEL IMMUNOHISTOCHEMICAL MARKER PROGRAMMED DEATH LIGAND 1(PD-L1) AND ITS CORRELATION WITH CLINICOPATHOLOGICAL PARAMETERS IN BREAST CARCINOMA** under the guidance of **Dr Satish Arakeri** requesting my participation in the study. Apart from routine treatment procedures, the pre-operative, operative, post-operative, and follow-up observations will be utilized for the study as reference data.

The doctor has informed me that my participation in this study will help in the evaluation of the results of the study, which will be a useful reference for the treatment of other similar cases shortly. Also, I may be benefited from getting relieved from suffering or 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 a person other than my legal hirer or me except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on the information provided by me, I can ask for any clarification during treatment/study related to diagnosis, the procedure of treatment, result of treatment, or the prognosis. After understanding the nature of the dissertation or research, the diagnosis made, mode of treatment, I the undersigned Shri/Smt _____, under my full conscious state of mind, agree to participate in the said research/dissertation.

Signature of the patient:

Signature of the Doctor:

Witness: 1. Witness: 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 ಪದ್ಧತಿ/ಸಂಶೋಧನೆ ನಡೆಸುತ್ತಿದ್ದಾರೆ ಶೀರ್ಷಿಕೆಯುಳ್ಳ_ **ASSESSMENT OF EXPRESSION OF NOVEL IMMUNOHISTOCHEMICAL MARKER PROGRAMMED DEATH LIGAND 1(PD-L1) AND ITS CORRELATION WITH CLINICOPATHOLOGICAL PARAMETERS IN BREAST CARCINOMA** 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: OP/IP no.:

Age:

Occupation :

Residence :

Presenting Complaints:

Past history:

Personal history:

Family history:

Treatment history:

LOCAL EXAMINATION:

Site of lesion:

Size of lesion:

Lymph node status:

CLINICAL DIAGNOSIS:

INVESTIGATIONS:

Histopathological report of tissue sections:

Status of Estrogen, Progesterone and Her2/neu receptor:

Status of PD-L1 stain:

KEY TO MASTER CHART

Sr. No. -Serial Number

HPR number –Histopathology number

ER – Estrogen receptor immunostaining

PR – Progesterone receptor immunostaining

HER2 neu – Herceptin receptor immunostaining

PD-L1 – Programmed death ligand 1

Sr. No	Hpr number	Age	Gender	Histologic Type	Histologic Grade	Tumor Size	Lymph node	ER	PR	Her-2-neu	PD-L1
1	2811/21	52	F	INFILTRATING DUCTAL CARCINOMA NOS	3	T1	Involved	Positive	Positive	Negative	Negative
2	3900/21	60	F	INVASIVE DUCTAL CARCINOMA NST	2	T2	Not involved	Negative	Positive	Positive	Negative
3	4003/21	75	F	INVASIVE DUCTAL CARCINOMA NST	2	T2	Not involved	Positive	Negative	Negative	Negative
4	4238/21	65	F	INVASIVE DUCTAL CARCINOMA NST(DCIS)	2	T2	Not involved	Positive	Positive	Positive	Negative
5	4340/21	57	F	INVASIVE DUCTAL CARCINOMA NST	1	T2	Not involved	Negative	Negative	Negative	Negative
6	4560/21	37	F	INVASIVE BREAST CARCINOMA NST MEDULLARY PATTERN	3	T1	Not involved	Positive	Positive	Positive	Positive
7	4452/21	35	F	INFILTRATING DUCTAL CARCINOMA NOS	2	T2	Not involved	Negative	Negative	Negative	Positive
8	5823/21	72	F	INFILTRATING DUCTAL CARCINOMA NOS	1	T3	Not involved	Positive	Positive	Positive	Negative
9	5907/21	50	F	INFILTRATING DUCTAL CARCINOMA NOS	1	T3	Involved	Positive	Negative	Negative	Negative
10	194/22	50	F	INVASIVE DUCTAL CARCINOMA NST(DCIS)	2	T2	Involved	Positive	Positive	Positive	Negative
11	1591/22	47	F	INVASIVE DUCTAL CARCINOMA NST(DCIS)	2	T2	Involved	Negative	Negative	Negative	Negative
12	1719/22	56	F	INFILTRATING DUCTAL CARCINOMA NOS	2	T1	Not involved	Negative	Negative	Negative	Positive
13	1874/22	54	F	INVASIVE BREAST CARCINOMA NST	1	T2	Not involved	Positive	Positive	Negative	Negative
14	1890/22	57	F	INFILTRATING DUCTAL CARCINOMA NST(DCIS)	2	T1	Involved	Positive	Positive	Positive	Negative
15	2625/22	35	F	INVASIVE LOBULAR CARCINOMA(DCIS)	1	T2	Involved	Positive	Positive	Positive	Positive
16	3191/22	37	F	INVASIVE DUCTAL CARCINOMA NST	2	T2	Involved	Negative	Negative	Positive	Negative
17	3458/22	52	F	INFILTRATING DUCTAL CARCINOMA NOS	1	T1	Not involved	Positive	Positive	Positive	Positive
18	3518/22	72	M	INFILTRATING DUCTAL CARCINOMA NST	2	T2	Involved	Positive	Positive	Positive	Positive
19	3906/22	37	F	INFILTRATING DUCTAL CARCINOMA NST	2	T2	Involved	Positive	Positive	Negative	Negative
20	4228/22	57	F	INFILTRATING DUCTAL CARCINOMA NST	1	T2	Involved	Positive	Positive	Positive	Positive
21	4848/22	47	F	INFILTRATING DUCTAL CARCINOMA NOS	1	T3	Involved	Positive	Positive	Negative	Negative
22	4918/22	68	F	BILATERAL INVASIVE DUCTAL CARCINOMA WITH MIXED DUCTAL AND LOBULAR FEATURES	3	T2	Involved	Negative	Negative	Positive	Negative
23	6024/22	56	F	INVASIVE BREAST CARCINOMA NST	1	T1	Not involved	Positive	Positive	Negative	Negative

24	7243/22	83	F	INVASIVE SOLID PAPILLARY CARCINOMA	1	T3	Involved	Positive	Positive	Negative	Negative
25	7906/22	41	F	INVASIVE DUCTAL CARCINOMA NST	1	T1	Not involved	Negative	Positive	Positive	Negative
26	836/23	55	F	INVASIVE BREAST CARCINOMA NST	1	T3	Involved	Positive	Negative	Positive	Positive
27	981/23	48	F	INVASIVE BREAST CARCINOMA NST	2	T3	Involved	Negative	Negative	Positive	Positive
28	942/23	40	F	INVASIVE BREAST CARCINOMA NST	1	T4	Involved	Negative	Negative	Positive	Negative
29	1434/23	67	F	INVASIVE DUCTAL CARCINOMA NOS	3	T4	Not involved	Negative	Negative	Positive	Negative
30	2828/23	46	F	INVASIVE DUCTAL CARCINOMA NST(DCIS)	2	T2	Involved	Positive	Positive	Negative	Negative
31	2872/23	66	F	INVASIVE BREAST CARCINOMA NST(DCIS)	2	T2	Involved	Positive	Positive	Negative	Negative
32	2582/23	80	F	INVASIVE DUCTAL CARCINOMA NST	2	T4	Involved	Positive	Positive	Negative	Negative
33	3090/23	63	F	CARCINOMA WITH APOCRINE DIFFERENTIATION(DCIS)	2	T2	Involved	Negative	Negative	Negative	Negative
34	3352/23	41	F	INVASIVE BREAST CARCINOMA NOS	2	T3	Involved	Positive	Negative	Negative	Negative
35	3933/23	77	F	INVASIVE DUCTAL CARCINOMA NOS(DCIS)	2	T2	Not involved	Positive	Positive	Negative	Negative
36	4341/23	61	F	INVASIVE BREAST CARCINOMA NOS	1	T4	Involved	Positive	Positive	Positive	Negative
37	4195/23	43	F	INFILTRATING DUCTAL CARCINOMA NOS	2	T1	Not involved	Negative	Negative	Negative	Negative
38	240/23	58	F	INFILTRATING DUCTAL CARCINOMA NOS	1	T1	Not involved	Positive	Positive	Positive	Negative
39	273/23	50	F	INVASIVE DUCTAL CARCINOMA NST	2	T1	Involved	Positive	Positive	Positive	Negative
40	5298/23	42	F	INVASIVE BREAST CARCINOMA NOS	3	T1	Involved	Positive	Positive	Positive	Positive
41	5428/23	65	F	INVASIVE DUCTAL CARCINOMA NST(DCIS)	1	T4	Involved	Negative	Negative	Negative	Negative
42	5889/23	75	F	INVASIVE BREAST CARCINOMA NOS	1	T2	Not involved	Positive	Positive	Negative	Positive
43	5904/23	40	F	INVASIVE BREAST CARCINOMA NOS(DCIS)	1	T3	Involved	Positive	Positive	Negative	Negative
44	6366/23	58	F	INVASIVE BREAST CARCINOMA NOS	3	T4	Involved	Positive	Positive	Negative	Negative
45	6534/23	55	F	INVASIVE DUCTAL CARCINOMA NOS(DCIS)	2	T2	Involved	Negative	Negative	Negative	Positive
46	466/23	58	F	INVASIVE DUCTAL CARCINOMA NOS	1	T1	Not involved	Negative	Negative	Positive	Positive
47	7359/23	62	F	ENCAPSULATED PAPILLARY CARCINOMA(DCIS)	2	T2	Not involved	Positive	Positive	Negative	Negative
48	19/24	54	F	MUCINOUS CARCINOMA TYPE B(DCIS)	1	T2	Not involved	Positive	Positive	Negative	Negative
49	178/24	60	F	INVASIVE BREAST CARCINOMA NST (DCIS)	3	T2	Involved	Negative	Negative	Positive	Positive
50	135/24	70	F	INVASIVE BREAST CARCINOMA NST(DCIS)	2	T2	Involved	Negative	Negative	Positive	Negative

Monika Pawar

“ASSESSMENT OF EXPRESSION OF NOVEL IMMUNOHISTOCHEMICAL MARKER PROGRAMMED DEATH-LI...

BLDE University

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