ASSESSMENT OF TUMOUR PROLIFERATION BY USE OF THE MITOTIC ACTIVITY INDEX, Ki67 AND PHOSPHOHISTONE H3 EXPRESSION IN INFILTRATING DUCTAL CARCINOMA OF BREAST

BY

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IN

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UNDER THE GUIDANCE OF

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PROFESSOR & HOD

DEPARTMENT OF PATHOLOGY

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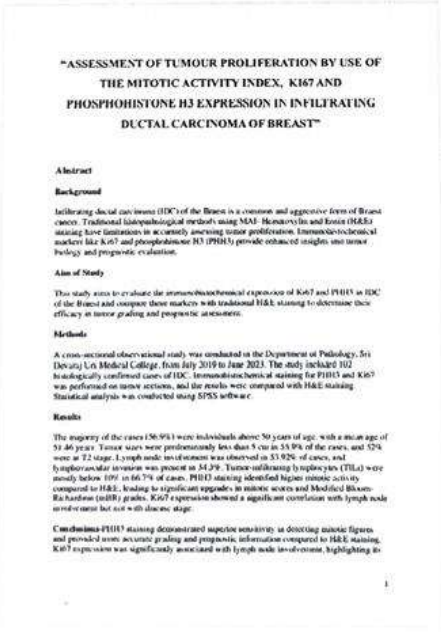

"ASSESSMENT OF TUMOUR PROLIFERATION BY USE OF THE MITOTIC ACTIVITY INDEX, K167 AND PHOSPHOHISTONE H3 EXPRESSION IN INFILTRATING DUCTAL CARCINOMA OF BREAST"

Abstract

Background

Infiltrating ductal carcinoma (IDC) of the Breast is a common and aggressive form of Breast cancer. Traditional histopathological methods using MAI- Hematoxylin and Eosin (H&E) staining have limitations in accurately assessing tumor proliferation. Immunohistochemical markers like Ki67 and phosphohistone H3 (PHH3) provide enhanced insights into tumor biology and prognostic evaluation.

Aim of Study

This study aims to evaluate the immunohistochemical expression of Ki67 and PHH3 in IDC of the Breast and compare these markers with traditional H&E staining to determine their efficacy in tumor grading and prognostic assessment.

Methods

A cross-sectional observational study was conducted in the Department of Pathology, Sri Devaraj Urs Medical College, from July 2019 to June 2021. The study included 102 histologically confirmed cases of IDC. Immunohistochemical staining for PHH3 and Ki67 was performed on tumor sections, and the results were compared with H&E staining. Statistical analysis was conducted using SPSS software.

Results

The majority of the cases (56.9%) were individuals above 50 years of age, with a mean age of 51.86 years. Tumor sizes were predominantly less than 5 cm in 55.9% of the cases, and 52% were at T2 stage. Lymph node involvement was observed in 53.92% of cases, and lymphovascular invasion was present in 34.3%. Tumor-infiltrating lymphocytes (TILs) were mostly below 10% in 66.7% of cases. PHH3 staining identified higher mitotic activity compared to H&E, leading to significant upgrades in mitotic scores and Modified Bloom-Richardson (mBR) grades. Ki67 expression showed a significant correlation with lymph node involvement but not with disease stage.

Conclusion: PHH3 staining demonstrated superior sensitivity in detecting mitotic figures and provided more accurate grading and prognostic information compared to H&E staining. Ki67 expression was significantly associated with lymph node involvement, highlighting its

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Date: Signature of the Candidate

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LIST OF ABBREVIATIONS

BC – Breast cancer

IHC – Immunohistochemistry

ER – Estrogen Receptor

PR – Progesterone Receptor

Her 2 – Human epidermal growth factor receptor 2

IDC – Infiltrating Ductal Carcinoma

ELD – Extralobular ducts

TD – Terminal ducts

L – Lobules

WHO – World Health Organisation

AJCC – American Joint Committee on Cancer

H&E – Haematoxylin and Eosin

MAI-Mitotic Activity Index

mBR- modified Bloom Richardson grade

PHH3-Phospho Histo H3

NPI – Nottingham Prognostic index

TBS – Tris buffer Solution

HR – Hormone Receptors

DPX - Dibutylpthalate Polystyrene Xylene

TILS- Tumor Infiltrating lymphocytes

T:S Ratio- Tumor stromal ratio

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ABSTRACT

Background

Infiltrating ductal carcinoma (IDC) of the breast is a common and aggressive form of breast cancer. Traditional histopathological methods using MAI- Hematoxylin and Eosin (H&E) staining have limitations in accurately assessing tumor proliferation. Immunohistochemical markers like Ki67 and phosphohistone H3 (PHH3) provide enhanced insights into tumor biology and prognostic evaluation.

AIMS

To assess PHH3 and Ki67 expression in infiltrating ductal carcinoma of the breast and correlate it with mitotic activity index. Additionally, to investigate their association with histological grade and staging of the carcinoma.

OBJECTIVES

1. To determine the expression of PHH3 and Ki67 in infiltrating ductal carcinoma breast
2. To correlate mitotic activity index with the expression of PHH3 and Ki67 in infiltrating ductal carcinoma breast
3. To study the association between mitotic activity index, Ki67 and phosphohistone H3 expression with Histological grade and staging of ductal carcinoma breast.

Methods

A cross-sectional observational study was conducted in the Department of Pathology, Sri Devaraj Urs Medical College, from July 2019 to June 2023. The study included 102 histologically confirmed cases of IDC. Immunohistochemical staining for PHH3 and Ki67 was performed on tumor sections, and the results were compared with H&E staining. Statistical analysis was conducted using SPSS software.

Results

The majority of the cases (56.9%) were individuals above 50 years of age, with a mean age of 51.46 years. Tumor sizes were predominantly less than 5 cm in 55.9% of the cases, and 52% were at T2 stage. Lymph node involvement was observed in 53.92% of cases, and lymphovascular invasion was present in 34.3%. Tumor-infiltrating lymphocytes (TILs) were mostly below 10% in 66.7% of cases. PHH3 staining identified higher mitotic activity compared to H&E, leading to significant upgrades in mitotic scores and Modified Bloom-Richardson (mBR) grades. Ki67 expression showed a significant correlation with lymph node involvement but not with disease stage.

Conclusions

PHH3 staining demonstrated superior sensitivity in detecting mitotic figures and provided more accurate grading and prognostic information compared to H&E staining. Ki67 expression was significantly associated with lymph node involvement, highlighting its prognostic value. The findings support the integration of PHH3 staining in routine diagnostic practices to improve the accuracy of breast cancer grading and prognosis.

Keywords

Infiltrating ductal carcinoma, breast cancer, immunohistochemistry, Ki67, PHH3, H&E staining, mitotic figures, prognostic markers.

INTRODUCTION

INTRODUCTION

Breast cancer (BC) is the most common cancer in women worldwide. The predicted 2.3 million new cases in 2020 represent 11.7% of all cancer cases, surpassing lung cancer as the leading cause.[1] The epidemiological literature predicts about 2 million BC cases worldwide by 2030.[2] In India, incidence rose about 50% between 1965 and 1985.[3] In 2016, 118,000 incident cases and 526,000 prevalent cases were estimated in India, with 98.1% affecting women. From 1990 to 2016, the age-standardized incidence rate of BC in women increased 39.1% across all states.[4] As per 2020 Globocan data, BC in India contributes to 13.5% (178,361) of all cancer cases and 10.6% (90,408) of related deaths, with a lifetime risk of 2.81.[1]

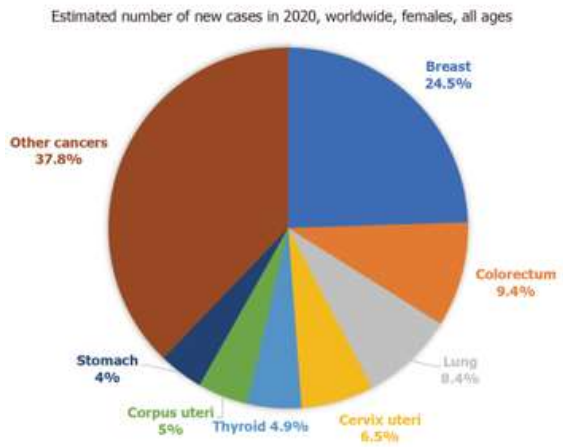

Figure 1: WHO- Estimated number of new cancer cases in 2020, worldwide

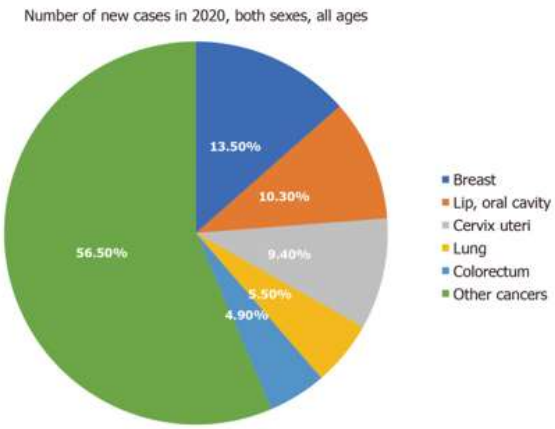

Figure 2: WHO- Globocon 2020 India

Recent statistics imply Indian women get the disease earlier than Western women. The National Cancer Registry Program examined 1988–2013 cancer registry data to track cancer incidence. BC incidence increased significantly in all population-based cancer registries.[5] Bangalore, Bhopal, Chennai, and Delhi had cervical cancer as the leading cancer location in 1990, while Mumbai had BC. By 2000–2003, all registries except rural Barshi had breast cancer as their top priority. The Bhopal, Chennai, and Delhi registries showed significant BC increases.[6] A study reported 5-year overall survival rates of 95% for stage I, 92% for stage II, 70% for stage III, and 21% for stage IV.[7] The survival rates for breast cancer patients in India are somewhat lower than those in Western countries due to parameters such as an earlier age of onset, a more advanced stage of illnesses at diagnosis, a delay in obtaining definitive therapy, and inadequate or fragmented care.[8] The World Cancer Report 2020 states that the most successful therapies for controlling breast cancer are early identification and fast treatment.[9] A comprehensive analysis conducted in 2018, which examined 20 studies, emphasized that the expenses associated with treating breast cancer increase as the disease progresses to more advanced stages upon diagnosis. This underscores the potential economic advantages of detecting the cancer at an early stage, which can lead to cost savings.[10]

Breast cancer's origin is linked to a multifaceted interplay of diverse factors, both modifiable and non-modifiable. Genetics, environment, nutrition, hormones, and hereditary components collectively shape its development. Risk factors encompass a prior breast cancer diagnosis, familial predisposition, obesity, tall stature, smoking, alcohol use, early onset of menstruation, delayed menopause, lack of physical activity, never having given birth, and hormone replacement therapy. Conversely, factors linked to reduced breast cancer risk involve having multiple children, breastfeeding history, engaging in physical activity, weight management, and preventive surgical or medical measures.[11]

Female individuals are primarily affected by breast cancer, with a considerably higher occurrence compared to males. The incidence rates of this phenomenon are highly connected with age and race. White women had the greatest overall incidence rates, followed by Black, Asian, and Hispanic women in that order. Moreover, women who have a close family member (such as a parent or sibling) who has had breast cancer are two to three times more likely to develop the disease themselves throughout the course of their life.[6]

Symptoms of breast cancer-[12]

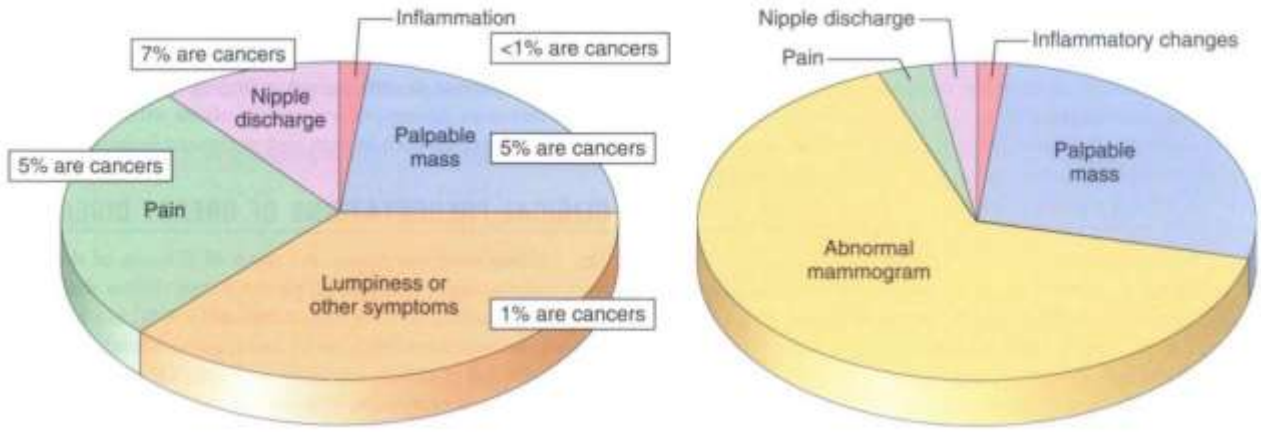

Figure 3- The presenting symptoms of breast disease can be categorized into two groups: common breast symptoms and presentations of breast cancer.

Pathogenesis of Breast cancer [13,14]

There is a rising prevalence of breast cancer instances globally. It primarily affects women who have gone through menopause. Breast carcinoma can arise in women with genetic abnormalities or can manifest spontaneously. Environmental factors are observed to influence the occurrence of hereditary forms of breast cancer, while both environmental and genetic factors contribute to the development of carcinoma in sporadic cases. Developed countries exhibit a significantly higher occurrence (six times more) of breast cancer in comparison to poor countries.

Genetic mutations in genes such as PTEN, P53, BRCA1, and BRCA2 are associated with an increased susceptibility to and likelihood of developing breast cancer. Understanding the etiopathogenesis, early identification, treatment decision-making, and outcome assessment are significant challenges in cases of breast cancer. The identification of the most vulnerable genes that contribute to the development of breast cancer is crucial for understanding the causes and mechanisms of both sporadic and familial types of the disease. Several factors contribute to an elevated risk and likelihood of developing breast cancer, such as environmental variables, lifestyle differences, hormone changes, and hereditary factors.

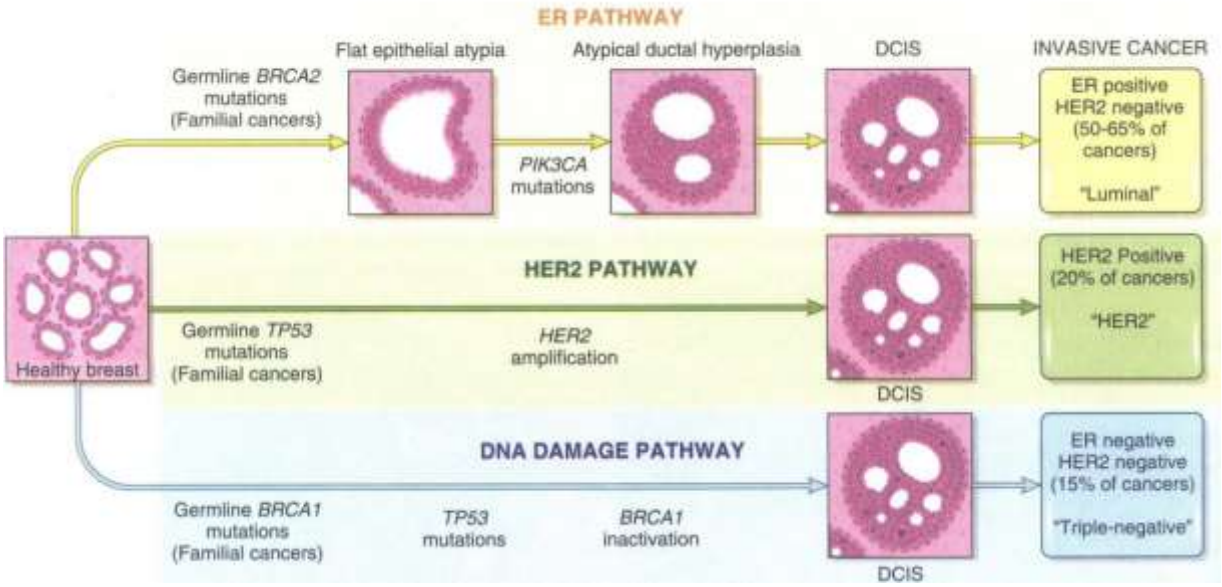

Figure 4- The primary route of progression for ductal breast cancer[12]

The predominant pathway (shown by the yellow arrow) is associated with ER-positive malignancies. The morphologically identifiable precursor lesions include atypical ductal hyperplasia (ADH) and ductal carcinoma in situ (DCIS). These lesions have some genomic events in common with invasive ER-positive malignancies, such as mutations in PIK3CA. Through the analysis of gene expression patterns, certain types of tumors are categorized as "luminal". This is the most prevalent type of cancer that occurs in persons with inherited BRCA2 gene mutations. Less frequent are tumors that have Her2 overexpression due to gene amplification (shown by the green arrow). These malignancies can exhibit either positive or negative expression of the estrogen receptor (ER). This is the predominant form of cancer that develops in individuals with inherited TP53 gene mutations. The rarest but most unusual kind of breast cancer is negative for estrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2), commonly referred to as "triple negative" breast cancer (shown by the blue arrow). These tumors exhibit a deficiency in BRCA1 and p53 function and display genomic instability. They are linked to inherited BRCA1 mutations. [12]

Carcinoma breast shows diversity in its molecular mechanisms which have multiple processes that ultimately will result in the initiation, and progression of the disease and its metastatic nature. There are three major groups into which carcinoma of the breast can be divided into which are the luminal subtypes along with positivity in hormone receptors (HR+), oncogene HER2 (HER2+), and the triple negative variant. New subtypes have been added recently. With the help of this additional genes and the mutations, they give the molecular mechanisms and the pathway leading to tumorigenesis.[15]

Several oncogenes implicated in carcinogenesis have been found to significantly contribute to the development of breast cancer and its ability to metastasize. Resistant phenotypes are seen emerging due to mutations and dysregulation of apoptotic pathway which are seen in driver oncogenes, which will ultimately affect the survival and therapy. Hence, targeting of the drivers and downregulating them is pursued in various cancers, including carcinoma of the breast. In patients with HER2-positive subtypes, targeted endocrine therapies are given, which are showing good outcome.[15]

Recently, there have been advancements in the development of targeted therapies that focus on specific molecular mechanisms. These therapies involve the use of inhibitors that target DNA repair, particularly in breast carcinomas with BRCA mutation. Additionally, there is a CDK4/6 inhibitor that is effective for both hormone receptor-positive and HER2 negative types of breast cancer. [15]

Uncontrolled proliferation of cells is a key feature of the development and progression of cancer, which affects the effectiveness of cytotoxic chemotherapy. Tumor proliferation, a complex process, is tightly controlled by components including growth factors, hormones, genetic factors, epigenetic alterations, and the tumor microenvironment. These factors jointly coordinate cell division throughout the many phases of the cell cycle (see Fig.). Gaining insight into the fundamental mechanisms that regulate cell growth has the potential to discover specific targets for therapy that can effectively limit the growth of tumors. The assessment of a tumor's proliferative activity usually involves estimating the fraction of tumor cells in different phases of the cell cycle, including both interphase and mitotic phases.[16]

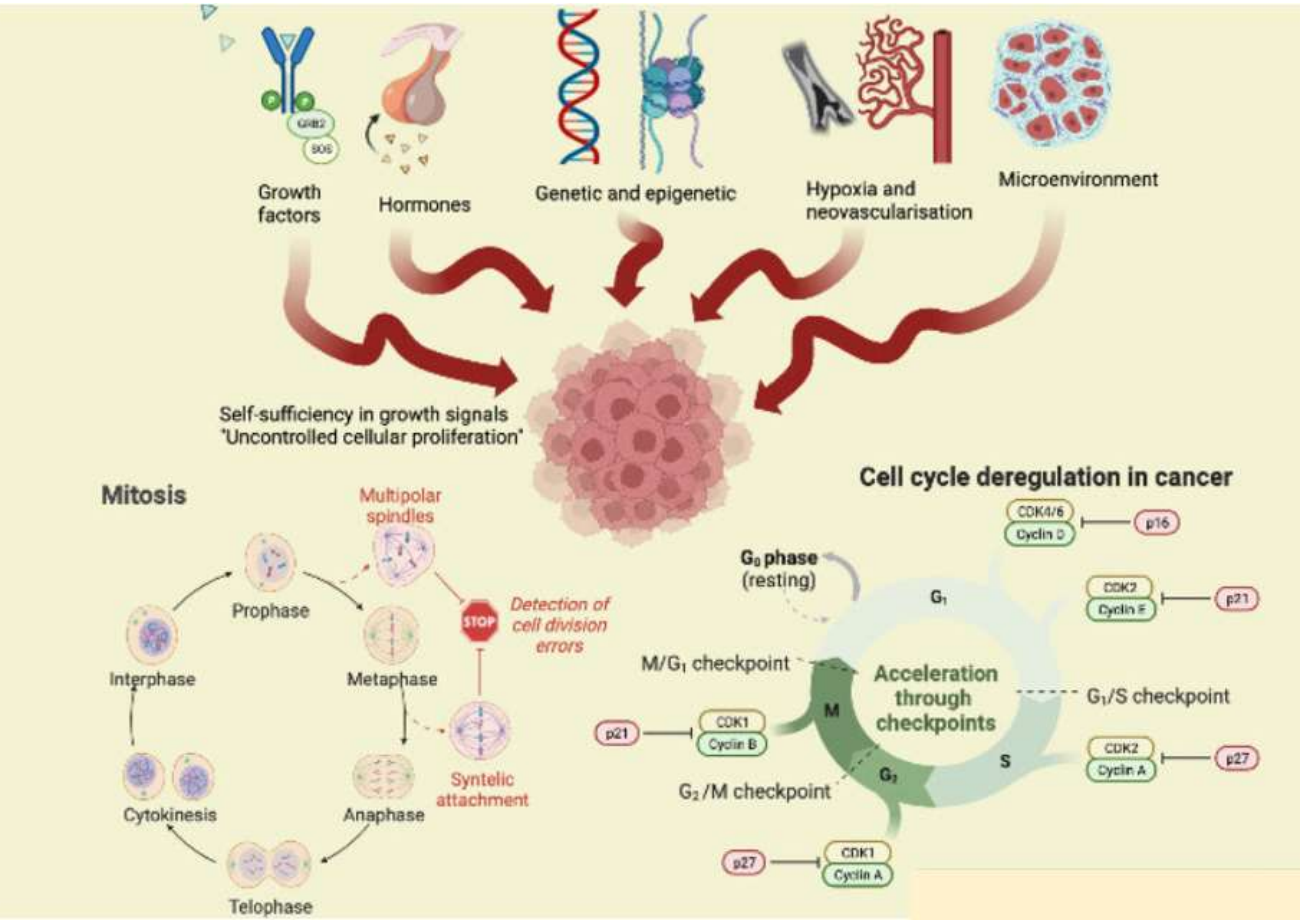

Figure 5- The rate of cell division in breast cancer (BC) tumors[16]

The rate of cell division in breast cancer tumors is of great significance, as it not only aids in the diagnosis but also plays a crucial role in determining the prognosis and therapy options.[17]The Nottingham grading system (NGS) for breast cancer assessment is based on the evaluation of three components: (i) mitotic scores, which indicate the proliferative activity of the cancer cells; (ii) the degree of tubule formation; and (iii) pleomorphism.[18] The use of NGS plays a vital role in several prognostic tools used to estimate the risk of breast cancer patients. These tools include the Nottingham prognostic index, Nottingham Px[19], and the tumor, node, metastasis (TNM) prognostic staging system, which has recently integrated NGS.[20]Ki67, an immunohistochemistry (IHC) marker that shows cell proliferation, has received much focus as a prognostic predictor in breast cancer (BC). Moreover, genes linked to cell growth play a significant role in prognostic multigene signatures.

Methods for assessing proliferation in breast cancer involve determining the percentage of cells in the cell cycle using molecular tests, examining dividing cells in the mitotic phase through either morphological analysis (counting mitotic figures) or molecular tests such as immunohistochemistry (IHC) with mitotic-specific markers (e.g., PHH3). Although complicated multigene tests can measure groups of genes related with proliferation, the most straightforward and easily available way for assessing breast cancer proliferation is undoubtedly by counting mitoses in tissue slices stained with hematoxylin and eosin (H&E).[16]

Unrestrained cellular growth is a defining characteristic of cancer and plays a vital role in predicting tumor activity[21]. Cellular proliferation not only affects the rate at which a tumor grows and indicates how aggressive it is, but it also plays an increasingly important role in predicting the effectiveness of treatment.[22] Several techniques have been developed to assess the rate at which tumors grow, with the most notable ones being mitotic count (MC), Ki67 labeling index, proliferating cell nuclear antigen (PCNA), and phosphohistone H3 (PHH3).[23,24] Nevertheless, these methods have their constraints (see to online supplementary table S1). For example, MIB1, which specifically targets the Ki-67 antigen, covers the full process of cell division, providing information not only on cells that are actively dividing but also on those in the resting phase of the cell cycle.[23,24] However, there are still difficulties because there is no agreement on the methodologies used to score, the values used as cutoffs, and the trustworthiness of antibodies, especially for Ki67.[25–27] PCNA only targets the S phase of the cell cycle, providing relatively limited insights into tumor progression [3, 4]. PHH3 is a marker that specifically identifies mitotic cells. However, its usefulness in breast cancer (BC) is restricted due to the need for further immunohistochemistry (IHC) procedures and the uncertain reliability of its sensitivity. [28,29]

Although there have been improvements, the classic approach of visually identifying mitotic figures is still the most commonly used in everyday practice. This is because it is simple and quick, allowing for the assessment of growth pace and tumor behavior.
[30]. Mitotic figures remain a crucial element in the diagnosis and prognosis of many cancers, such as BC. They are included in the histological grading system, along with tubule development and nuclear pleomorphism.[18,31–33]. Mitotic count (MC) is a strong histomorphological predictor of results in this grading system. It is sometimes given as either Mitotic Index (MI) or Mitotic Activity Index (MAI).[34–36] Within the context of breast cancer (BC), the mitotic count (MC) is commonly assessed in 10 high-power fields (HPF) and converted into scores as a component of the Nottingham grading system. These scores are then adjusted according to the diameter and area size of the microscope field. [18,31,37]

Nevertheless, even with the use of a uniform counting procedure, there continues to be a prevalence of low concordance rates. Interpretative schemes show that there are low rates of agreement for MC, especially in tumors with high scores, highlighting the difficulties in accurately assessing them.[38] In ordinary practice, there are often substantial discordance rates when it comes to characterizing mitotic forms on hematoxylin and eosin (H&E)-stained slides, as this process is subjective. [35,38–41]Standardization efforts have been made, but challenges persist, including defining the optimal area size for MC assessment and adapting criteria for digitized whole slide images (WSI). [42–45]Modifications in criteria and incorporation of defined mitotic figure counts into histological grading are needed to address these challenges effectively.

Traditionally, MC can be expressed as either Mitotic Index (MI) or Mitotic Activity Index (MAI). MI is commonly described as the proportion of cells undergoing mitosis compared to cells that are not undergoing mitosis, regardless of the phase of the cell cycle, in a specific location of a tumor. It is typically expressed as a percentage or as the number of mitotic events per 1000 neoplastic cells. [34] MAI, or Mitotic Activity Index, is a measurement of the number of mitotic figures inside a specific region of a tumor. It is expressed as an index, calculated by dividing the number of mitotic figures by the area that was enumerated.[34–36]

Antimitotic protein monoclonal-2 (MPM-2) and antiphosphohistone-H3 (PHH3) are the most commonly used markers for identifying mitotic figures. MPM-2 detects phosphoprotein epitopes on specific molecules involved in mitosis, such as topoisomerase IIa, microtubule-associated proteins, and Cdc2-inhibitory kinases. Research has demonstrated that the use of phosphohistone H3 (PHH3) labeling is a more powerful predictor of prognosis when compared to conventional measures such as axillary lymph node status, tumor size, nuclear grade, and histological grade.[46–52] Hence, there is significant interest in comparing the predictive significance of traditional pathological prognosticators with proliferation markers, such as PHH3 has been identified as a potential biomarker for breast cancer, as indicated by studies.[53,54]

PHH3 is a fundamental protein found at the later stages of the G2 and M phases, providing a more precise evaluation of mitotic activity in theory.[53] A recent study has proposed it as a potential surrogate marker for mitotic count. PHH3.[54] PHH3 is particularly valuable for pinpointing the specific area of intense cell division, making it a suggested method for confirming the presence of cell division in the

diagnosis of thin melanoma.[55]In well-differentiated neuroendocrine tumors of the pancreas, PHH3 IHC was shown to enhance agreement between different observers when it comes to assessing mitotic count and final grade, as compared to Hematoxylin and Eosin (H&E) staining.[56] Recent studies have emphasized that the level of nuclear PHH3 expression is a powerful prognostic indicator for lymph node-negative breast cancer patients who are under 55 years old and receiving systemic adjuvant chemotherapy. This marker is found to be more significant than other indicators such as ER/PR status, Oncotype Dx, and Mammaprint.[57]

The utilization of PHH3 IHC stain analysis greatly improves consensus among observers when calculating the mitotic rate and enables a quick and unbiased determination of the number of mitotic events.[58] It helps differentiate between apoptotic cell debris and mitoses and improves the chances of identifying mitotic cells with abnormal morphologies, such as metaphase or anaphase, which may not be detected by H&E staining alone. Nevertheless, studies have demonstrated that PHH3 analysis can elevate the mean mitotic rate by 86-200%.[59–63] The immunohistochemical (IHC) staining technique for PHH3 has been suggested as a possible substitute indicator for mitotic count. It has been examined in several types of tumors such as melanoma, meningioma, pulmonary carcinoid, and well-differentiated neuroendocrine tumors of the pancreas.[55,56,64–66] Furthermore, the examination of PHH3 requires less time and is more straightforward to interpret compared to the standard method of mitotic counting using H&E stain.

NEED FOR STUDY

The heterogeneity of breast cancer poses a challenge in predicting its malignant behavior, distinguishing it from other types of human malignancies.[67] Therefore, the evaluation of proliferation is essential for categorizing and forecasting the basic characteristics of breast tumors. The two most commonly accepted techniques for quantifying cell proliferation are Ki67 and the mitotic activity index (MAI). MAI, which is included in standard breast cancer pathology reports as part of histologic grading, is regarded as the most critical factor for predicting prognosis. [68]Ki67 is frequently used in laboratories to distinguish tumors that have a high likelihood of recurrence, necessitating further chemotherapy, and is a factor in the classification of breast cancer subtypes.[69,70] Nevertheless, despite their crucial functions, both Ki67 and MAI demonstrate significant limitations in terms of reproducibility, which present diagnostic difficulties.

Ki67, which is especially challenging in diverse tumors such as breast cancer, causes discrepancies in the choice of field for evaluation. In addition, the interpretation of Ki67 as positive can vary widely based on the subjective criteria of individual raters, leading to a lack of consistency in the results.[27]Another significant drawback of Ki67 is its limited ability to accurately depict proliferation, as it is present in all active stages of the cell cycle (G1, S, and G2 phases). Although widely acknowledged as a marker for cell proliferation, its reliability has been called into question by various studies, mostly due to the unknown fate of cells in the G1 phase.[21,25]

Although MAI is considered the most reliable marker for measuring proliferative potential, its repeatability has constantly been described as low. Low reproducibility in this context is caused by challenges in accurately identifying areas of cell division in Hematoxylin and Eosin (H&E)-stained slides. This is further complicated by the presence of cells that resemble dividing cells, such as hyperchromatic, karyorrhectic, or apoptotic cells. These factors result in inconsistent interpretations, even among pathologists who have received specialized training.[29]

The difficulties related to traditional proliferative markers can be overcome by employing phosphohistone H3 (PHH3). PHH3, a nuclear core histone protein found in DNA chromatin, has a significant impact on the condensation of chromosomes and the advancement of the cell cycle during mitosis and meiosis. This occurs when serine-10 and serine-28 residues are phosphorylated. Phosphorylation takes place during the late G2 phase to early prophase, while dephosphorylation happens progressively from late anaphase to early telophase. PHH3 staining is unique to cells that are actively undergoing mitosis, providing specificity to cell proliferation. [29]

PHH3 has been extensively verified in multiple studies including different types of malignancies, demonstrating its high sensitivity and specificity as a marker for mitotic figures (MFs). Additionally, it has shown a substantial connection with outcomes. There is a significant association between MAI and PHH3 in breast cancer.[71] These data suggest that PHH3 may be more reliable and provide a more accurate measure of proliferation compared to the current marker Ki67.
According to a study conducted by Gerring et al.[72] PHH3 proved to be a more powerful indicator of survival at 5 years after diagnosis, surpassing Ki67 (with a hazard ratio of 4.35 compared to 2.44). Additionally, it effectively distinguished the likelihood of mortality in patients over the age of 45. Nevertheless, the study employed Tissue Microarrays (TMAs), which may not comprehensively depict the diversity of tumors.

Among all types of cancer in humans, breast cancer is particularly known for its heterogeneity, making it challenging to accurately forecast its behavior. Evaluating the rate of cell division is crucial for categorizing and forecasting the biological characteristics of breast tumors.[28]

Unrestrained cellular growth is a defining characteristic of cancer and serves as a significant indicator of tumor behavior.[21] Cellular proliferation not only impacts the rate of tumor growth and reflects the aggressiveness of the tumor, but it also plays an increasingly significant role in its ability to forecast and guide treatment.[22] Various techniques have been outlined for evaluating the rate of cell division in tumors, with the most significant ones being mitotic count (MC), Ki67 labelling index, proliferating cell nuclear antigen (PCNA), and phosphohistone H3 (PHH3). The techniques used to quantify cell proliferation include the mitotic activity index (MAI), Ki67 staining, and phosphohistone H3 (PHH3) staining.

Even among expert pathologists, it is challenging to distinguish between mitotic figures and hyperchromatic, karyorrhectic, or apoptotic cells. The use of IHC proliferation markers can resolve issues with conventional proliferative indicators.

•Histone H3 is a crucial protein found in the nucleus that forms the core of DNA chromatin. It plays a significant role in the condensation of chromosomes and the advancement of the cell cycle during mitosis and meiosis. This occurs after the phosphorylation of specific serine residues, namely serine-10 and serine-28.[28]Phosphorylation takes place from the late G2 phase to the early prophase, whereas

dephosphorylation proceeds gradually from late anaphase to early telophase. FourDuring metaphase, histone H3 is consistently phosphorylated and shows a positive result for PHH3. In contrast, interphase cells either do not produce PHH3 or express it to a lesser extent. This feature enables PHH3 to specifically stain cells that are actively undergoing mitosis, making it a marker for cell proliferation.[28]

PHH3 has been confirmed to be sensitive and specifically involved in marking mitotic figures and overall survival rate in cancers such as colorectal adenocarcinoma, lung neuroendocrine carcinoma, astrocytoma, meningioma, and uterine smooth muscle tumors. The number 90 is enclosed in square brackets.

Several studies have shown the predictive significance of Ki67. Nevertheless, the practical usefulness of this marker has been questioned due to its limited ability to be replicated accurately. The flaws in Ki67 assessment can be related to the absence of a consensus among specialists regarding the score and the lack of a clearly defined cut-off point for making clinical decisions.[73]

However, the connection between PHH3 and KI67 in infiltrating ductal carcinoma of the breast is restricted. Hence, this study aims to investigate the relationship between the Mitotic activity index, PHH3, and Ki67 in infiltrating ductal carcinoma of the breast.

AIMS & OBJECTIVES

AIMS AND OBJECTIVES

AIMS

To assess PHH3 and Ki67 expression in infiltrating ductal carcinoma of the breast and correlate it with mitotic activity index. Additionally, to investigate their association with histological grade and staging of the carcinoma.

OBJECTIVES

4. To determine the expression of PHH3 and Ki67 in infiltrating ductal carcinoma breast
5. To correlate mitotic activity index with the expression of PHH3 and Ki67 in infiltrating ductal carcinoma breast
6. To study the association between mitotic activity index, Ki67 and phosphohistone H3 expression with Histological grade and staging of ductal carcinoma breast.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

ANATOMY[74,75]:

In order to understand the diseases that affect the breast and develop the necessary surgical plan, it is essential to have a comprehensive grasp of the anatomical structure of the breast. When inspected, the majority of breasts display varying degrees of asymmetry. Other deformities include kyphosis, scoliosis, and other pectus deformities.

The majority of the breast tissue consists of glandular and adipose (fatty) components. Nevertheless, the proportion of fatty tissue to glandular tissue varies among individuals. Estrogen, a sex hormone, exerts a substantial influence on the development of breasts. As menopause approaches, estrogen levels decrease, leading to a reduction in the size of glandular tissues.

During the early stages of life, the breast tissue is located between the second and sixth ribs. However, as the breasts age and lose firmness, they may extend below the sixth rib. The pectoralis major muscle forms the base of the breast or the posterior wall. The Cooper ligaments are responsible for attaching the breast to the pectoralis major fascia. Nevertheless, due to their pliability, these ligaments allow for breast mobility. Over time and as women age, the Cooper ligaments tend to elongate, resulting in a sagging breast. Gravity causes the lower part of the breast to appear more voluminous than the upper part. The Spence tail extends along the lateral borders of the breast and axilla.

The nipple is often positioned slightly above the inframammary crease and is observed at the level of the 4th rib along the midclavicular line.

GLANDS:

The breast's underlying tissue consists of glandular and adipose components. The ratio between fat and glandular components undergoes continuous changes influenced by factors such as age, menopausal status, and parity. As menopause approaches, a decrease in estrogen levels leads to the shrinking of glandular tissue and the expansion of fatty tissue.

STRUCTURE OF NIPPLE:

The nipple plays a vital role in the process of nursing. In order to ensure efficient nursing, it is necessary for a nipple to have a minimum length of seven millimeters. Nevertheless, the topography of the nipple exhibits significant variation, ranging from flat to short, and even inverted, hence posing challenges for some women when it comes to breastfeeding.

NERVES:

The branches of the intercostal nerves T3-T5 innervate the breast with sensory input. The cervical lower plexus is one of several nerves that provide sensory innervation. The sensation of the nipple originates from the lateral cutaneous branch of the T4 nerve.

BLOOD SUPPLY:

The profound underlying arterioles that provide blood to the breast tissue interact with the subdermal plexus, which is responsible for giving blood to the surface of the breast.
The breast is supplied with blood from:
The thoracoacromial artery is a blood vessel.
The internal mammary perforators are located between the second and fifth ribs.
The lateral thoracic artery.
The thoracodorsal artery is the fourth artery.
The terminal branches of the internal perforators range from the 3rd to the 8th.
The internal mammary artery's superomedial perforators contribute to at least 60% of the overall blood flow.

THE LYMPHATIC SYSTEM:

The breast has extensive lymphatic drainage that spreads across its superficial and deep regions. The superficial lymphatics consist of the areolar and subareolar plexus. The axillary lymph nodes are ultimately accessed by the superficial lymphatics as they move towards the back and middle.

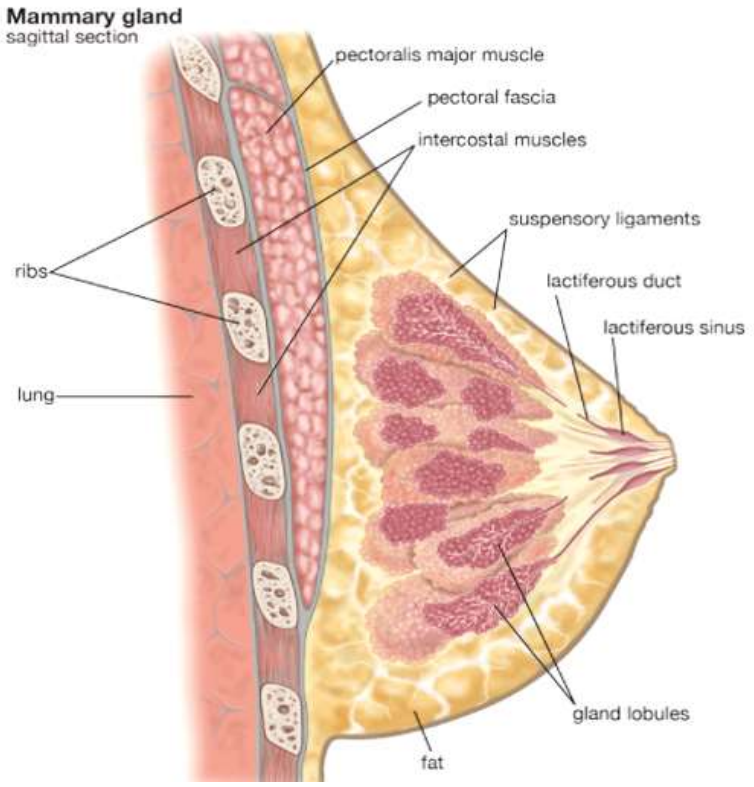

Figure 6: Normal breast anatomy[76]

NORMAL HISTOLOGY OF BREAST:

The breast's normal histology is made of acini and ducts which are arranged in the form of lobules and the stromal component comprising of predominantly adipose along with fibrous components. The two major constituents are stromal and epithelial elements. The dual layered epithelial lining by lobular systems and the ducts, which is rested on basement membrane is surrounded by stromal tissue. The inner layer of the ducts consists of columnar to cuboidal cells, whereas the outer layer is composed of myoepithelial cells. The basement membrane surrounds the ductules, ducts, and acini.[77]

The lobular units of terminal ducts consist of:

1. Terminal ductules, epithelium of which is differentiated into secretory acini which is seen in lactation and pregnancy.
2. Collecting ducts (Intralobular)
3. Intralobular stroma (specialized).

All the lobes drain into their own lactiferous ducts which finally opens into nipple.[78]

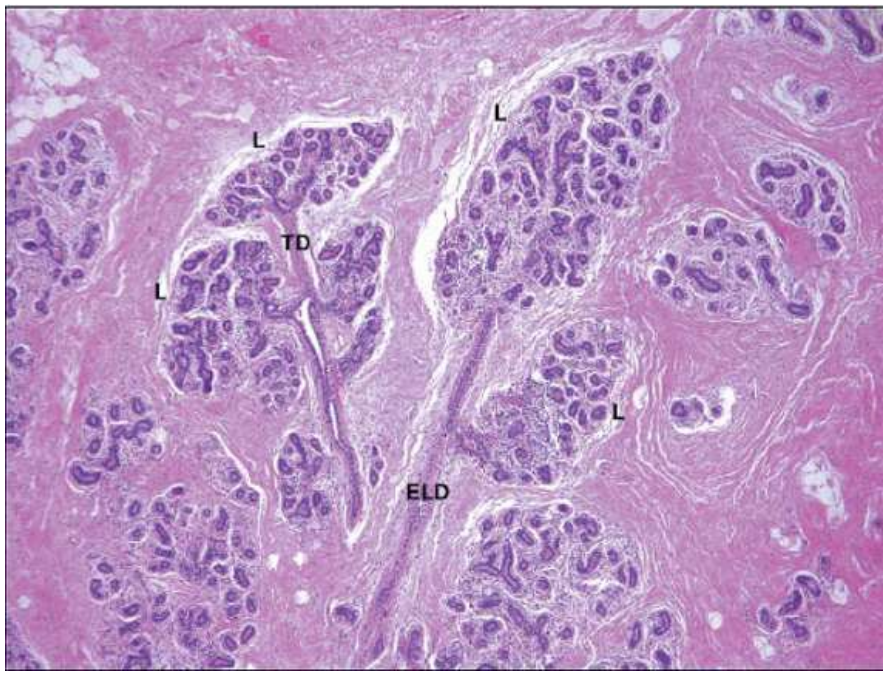

Figure 7: Normal histology of breast. (ELD – Extralobular ducts, TD – Terminal ducts, L – Lobules)

ETIOLOGY AND RISKFACTORS:[13,79]

Many factors are there which play role in development of carcinoma breast. Some of the important factors are:

1. Geographical place: Western population is seen to be more affected than in Indian population.
2. Familial history- 5-10% of carcinoma breast cases are seen to show autosomal dominant pattern of inheritance.
3. Endogenous hormones: Late first pregnancy (>35 years), early menarche, delayed menopause, nulliparous women, non-lactational women show increased risk of breast cancer.
4. Molecular genetics: Mutations in genes such as PTEN, P53, BRCA1 & BRCA2 shows increased risk for breast cancer.
5. Lifestyle patterns: Obesity, lack of physical exercise, smoking & alcohol intake show increased incidence for breast cancer development.
6. Benign lesions: Patients who are previously diagnosed with any benign breast lesion are at increased risk of developing malignancy.
7. Environmental risk factors: Prolonged exposure to harmful ionizing radiation.
8. Hormone therapy: Women who are on medical contraceptive pills, who are put on hormone replacement therapy also show increased risk.

ETIOPATHOGENESIS:[13,14]

There is a rising prevalence of breast cancer instances globally. It primarily affects women who have gone through menopause. Breast carcinoma may arise in women with genetic abnormalities or can manifest sporadically. Environmental factors are observed to influence the occurrence of hereditary forms of breast cancer, while both environmental and genetic factors contribute to the development of carcinoma in sporadic cases. Developed countries exhibit a significantly greater prevalence (six times higher) of breast cancer compared to poor countries.

Genetic mutations in genes such as PTEN, P53, BRCA1, and BRCA2 are associated with an increased susceptibility to and likelihood of developing breast cancer. Understanding the etiopathogenesis, early identification, treatment decision-making, and outcome assessment are significant challenges in cases of breast cancer. The identification of the most vulnerable genes that contribute to the development of breast cancer is crucial for understanding the causes and mechanisms of both sporadic and familial types of the disease. Several factors contribute to an elevated risk and likelihood of developing breast cancer, including environmental influences, lifestyle variations, hormone fluctuations, and genetic predisposition.

MOLECULAR MECHANISM OF CARCINOGENESIS:[15]

Carcinoma breast shows diversity in its molecular mechanisms which has multiple processes that ultimately will result in the initiation, progression of the disease and the metastatic nature. There are three major groups into which carcinoma of the breast can be divided into which are the luminal subtypes along with positivity in hormone receptors (HR+), oncogene HER2 (HER2+), and the triple negative variant. New subtypes are added recently. With the help of this additional genes and the mutations, they give the molecular mechanisms and the pathway leading to tumerogenesis.

Many oncogenes which are responsible for carcinogenesis are seen to play major role in carcinogenesis and metastatic ability in breast cancer. Resistant phenotypes are seen emerging due to mutations and dysregulation of apoptotic pathway which are seen in driver oncogenes, which will ultimately affect the survival and therapy. Hence, targeting of the drivers and downregulating them is pursues in various cancers, including carcinoma of the breast. In patients with HER2 positive subtypes, targeted endocrine therapies are given, which are showing good outcome.

Recently, novel targeted medicines have been developed that act as inhibitors in the DNA repair process. These therapies specifically target breast carcinomas with BRCA mutation, as well as CDK4/6 inhibitors that are effective for both hormone receptor positive and HER2 negative breast cancer cases.

Table 1- WHO 2019 classification of breast[80]

Category	**Subtypes**
Invasive Breast Carcinoma	Infiltrating duct carcinoma, NOS; Oncocytic; Lipid rich; Glycogen rich; Sebaceous; Lobular, NOS; Tubular; Cribriform, NOS; Mucinous adenocarcinoma; Mucinous cystadenocarcinoma, NOS; Invasive micropapillary; Metaplastic, NOS
Rare & Salivary Gland Type Tumors	Secretory; Acinar cell; Mucoepidermoid; Polymorphous adenocarcinoma; Adenoid cystic (Classic, Solid basaloid, High grade); Tall cell with reversed polarity
Neuroendocrine Neoplasms	Neuroendocrine tumor (NOS, Grade 1, Grade 2); Neuroendocrine carcinoma (NOS, Small cell, Large cell)
Epithelial Myoepithelial Tumors	Pleomorphic adenoma; Adenomyoepithelioma (NOS, with carcinoma); Epithelial myoepithelial carcinoma
Noninvasive Lobular Neoplasia	Atypical lobular hyperplasia; Lobular carcinoma in situ (Classic, Florid, Pleomorphic)
Ductal Carcinoma in Situ (DCIS)	DCIS (NOS, Low grade, Intermediate grade, High grade)
Benign Epithelial Proliferations	Usual ductal hyperplasia; Columnar cell lesions; Atypical ductal hyperplasia
Adenosis & Benign Sclerosing Lesions	Sclerosing adenosis; Apocrine adenoma; Microglandular adenosis; Radial scar/complex sclerosing lesion

Papillary Neoplasms	Intraductal papilloma; DCIS, papillary; Encapsulated papillary carcinoma (in situ, with invasion); Solid papillary carcinoma (in situ, with invasion); Intraductal papillary adenocarcinoma with invasion
Adenomas	Tubular, NOS; Lactating; Duct, NOS
Mesenchymal Tumors	
Vascular Tumors	Hemangioma (Perilobular, Venous, Cavernous, Capillary); Angiomatosis; Atypical vascular lesion; Postradiation angiosarcoma; Angiosarcoma
Fibroblastic/Myofibroblastic Tumors	Nodular fasciitis; Myofibroblastoma; Desmoid type fibromatosis; Inflammatory myofibroblastic tumor
Peripheral Nerve Sheath Tumors	Schwannoma, NOS; Neurofibroma, NOS; Granular cell tumor (NOS, malignant)
Smooth Muscle Tumors	Leiomyoma (NOS, Cutaneous, Nipple, and areola); Leiomyosarcoma, NOS
Adipocytic Tumors	Lipoma, NOS; Angiolipoma, NOS; Liposarcoma
Other	Pseudoangiomatous stromal hyperplasia
Fibroepithelial Tumors	Fibroadenoma, NOS; Phyllodes tumor (NOS, Periductal stromal tumor, Benign, Borderline, Malignant); Hamartoma
Tumors of the Nipple	Nipple adenoma; Syringoma, NOS; Paget disease
Malignant Lymphoma	Diffuse large B cell, NOS; Burkitt (NOS, Endemic, Sporadic, Immunodeficiency associated); Breast implant-associated anaplastic large cell lymphoma; Mucosa-associated lymphoid tissue lymphoma; Follicular, NOS
Metastatic Tumors	
Tumors of the Male Breast	Gynecomastia; Carcinoma (Invasive, In situ)

HISTOLOGICAL SUBTYPES:

INVASIVE/INFILTRATING DUCTAL CARCINOMA OF BREAST:

Invasive/Infiltrating ductal carcinoma is the most prevalent category of breast cancers. This category comprises a collection of tumors that do not exhibit any distinct histological types, such as lobular variation or tubular variant. Other synonyms for this condition include invasive ductal carcinoma, invasive ductal carcinoma not otherwise described, and infiltrating ductal carcinoma. These tumors exhibit infiltration into the adjacent stroma and tissues and have the potential to spread to other parts of the body. The user's text is incomplete and does not provide any information.[12,80]

GROSS FEATURES:

Different examples exhibit variations in macroscopic aspects. The tumor's dimensions can vary significantly, ranging from 1 cm to 10 cm. The contours may exhibit regular, irregular, nodular, or stellate configuration. The clear distinction between the boundaries of the tumor and the surrounding stroma is typically not observed. These tumors will have a solid to rigid texture when touched. Occasionally, when using a knife, one may experience a rough or grainy sensation. The cut surface exhibits a grey-white hue.

MICROSCOPY:[81]

The cells of the tumor are seen typically in trabecular pattern, cords & in clusters. These will show predominantly solid and sometimes syncytial pattern of infiltration into adjacent stroma. Individual tumor cells show abundant amount of eosinophilic cytoplasm, nucleus is regular, uniform, pleomorphic & showing prominent nucleoli. Mitotic figures can be seen at places. Many times an associated ductal carcinoma insitu (DCIS) component can also be seen. Stroma shows proliferation of fibroblastic tissue, also noted are areas of connective tissue and hyalinization. Necrosis is also noted at places.

This image shows a moderately magnified picture of a section of breast cancer discovered in a middle-aged lady. The cancer corresponds to the most prevalent kind, known as ductal carcinoma, NOS (not else specified). Significantly, groups of cancerous epithelial cells infiltrate the healthy breast stroma, resulting in its deterioration. The image shows a solitary dilated duct that has been filled and increased by identical cancerous cells, indicating the presence of ductal carcinoma in situ (CIS). The presence of calcifications in breast cancer in situ aids in its identification during mammography. Early detection of cancer greatly enhances the likelihood of achieving a total remission. The standard approach to

treating breast cancer usually includes a multifaceted approach that combines surgery, hormone therapy, chemotherapy, and radiotherapy. The specific treatment plan is customized based on the kind and size of the tumor.

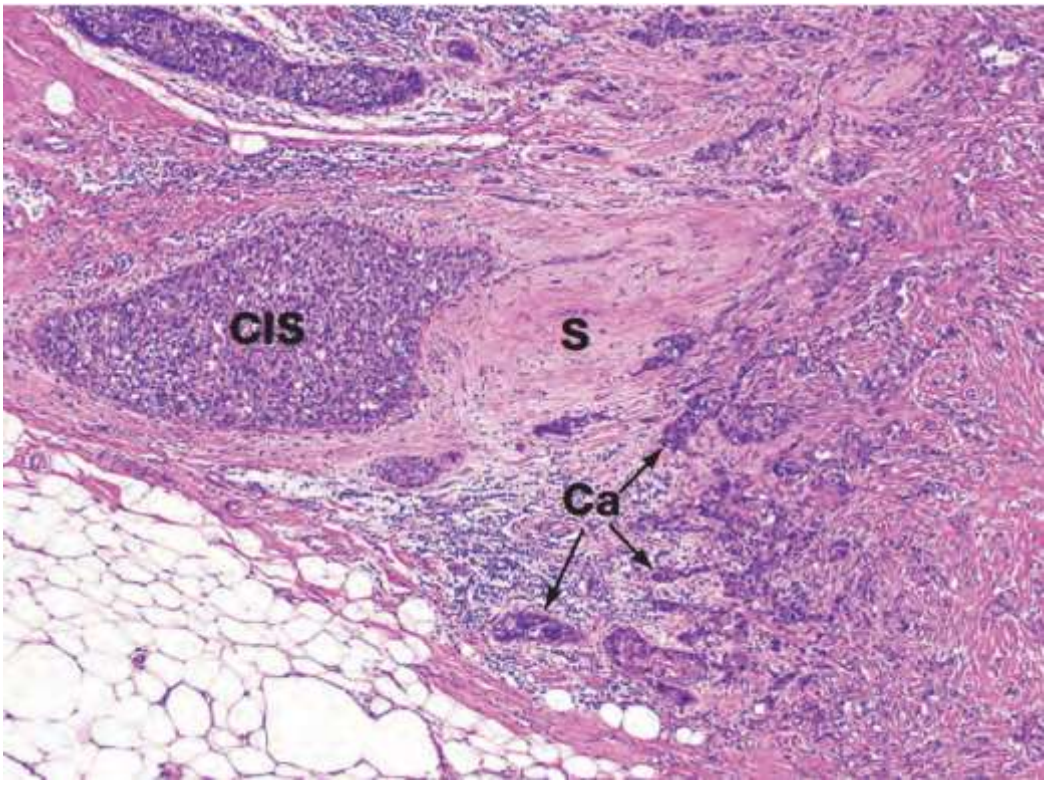

Figure 8: Note the infiltration of clusters of cancerous epithelial cells into the healthy breast stroma, resulting in its destruction.[81]

LOBULAR CARCINOMA:

This entity accounts for around 5-15% of breast cancers. Typically observed as a central tumor with an in situ lobular component. They have a highly uneven appearance with indistinct boundaries. Tumor cells are characterized by their tiny size and are organized in a linear pattern known as an Indian file arrangement. [82,83]

TUBULAR CARCINOMA:

This entity comprises 2% of breast cancers and they are small in size of <2cms. These tumors show better prognosis and are less aggressive. Majority of the tumors show ER positivity. Characteristic microscopic feature is the lumina are lined by epithelial cells arranged in one single layer. [82,83]

CRIBRIFORM TYPE OF CARCINOMA:

One of the types of invasive malignancy with an intraductal cribriform pattern is called invasive cribriform carcinoma (ICC). 50% of the tumor may show a tubular pattern. It constitutes about 0.3%–0.8% of breast cancers and consists of a cribriform pattern in >90% of the lesion. The tumor has angulated islands, in which bridges of cells form a well-defined sieve-like pattern. The tumor, which has a majority of cribriform patterns and few tubular patterns, is also an invasive cribriform carcinoma. A mixed variant of invasion type of cribriform carcinoma is a tumor composing of <50% of other types of patterns other than tubular carcinoma. It metastasizes very rarely to the axillary lymph nodes and carries a good prognosis.[74]

CARCINOMA WITH MEDULLARY FEATURES:

It is a broad category that has medullary type of cancers (MC), atypical type of medullary cancers, and no special type subset of invasive carcinomas. Common features are pushing type of borders, growth pattern like a syncytium, cells, nuclei showing high grade & a dense infiltration by lymphocytes. They represent about <1% of all breast carcinomas.[76]

METAPLASTIC CARCINOMA:

The incidence of metaplastic carcinomas is just 0.3% of all of the invasive carcinoma. They are composed of other cellular components apart from the glandular component. The sarcomatous components vary from spindle cell component, myxoid, bone, and cartilage. Gross features vary from well-defined lesions to irregular masses with speculated margins. Microscopically there are two main subtypes: monophasic "sarcomatoid," also known as spindle cell carcinoma with squamous component or without squamous components, and the other one is biphasic "sarcomatoid" carcinoma. The tumor probably is derived from myoepithelial cells. Based on the myoepithelial cell's presence or absence, metaplastic carcinoma differentiates into epithelial and mesenchymal elements.[76,77]

THE AMERICAN JOINT COMMITTEE ON CANCER (AJCC) STAGES FOR BREAST CANCER: [84]

Table 2-Definition of Primary T Categories

Category	Description
pT (Tumor)	**Primary Tumor**
pTX	Primary tumor cannot be assessed
pT0	No evidence of primary tumor
pTis	Carcinoma in situ (DCIS, LCIS, or Paget's disease)
pT1	Tumor ≤ 2 cm in greatest dimension
pT1mi	Tumor ≤ 1 mm
pT1a	Tumor > 1 mm but ≤ 5 mm
pT1b	Tumor > 5 mm but ≤ 10 mm
pT1c	Tumor > 10 mm but ≤ 20 mm
pT2	Tumor > 2 cm but ≤ 5 cm
pT3	Tumor > 5 cm
pT4	Tumor of any size with direct extension to chest wall and/or to the skin (ulceration or skin nodules)
pT4a	Extension to chest wall
pT4b	Ulceration and/or ipsilateral satellite nodules and/or edema of the skin (excluding Paget's disease of the nipple)
pT4c	Both T4a and T4b
pT4d	Inflammatory carcinoma

Table 3: N – Regional lymph nodes (pN):

pN (Nodes)	Regional Lymph Nodes
pNX	Regional lymph nodes cannot be assessed
pN0	No regional lymph node metastasis
pN0(i-)	No regional lymph node metastasis histologically, negative IHC
pN0(i+)	Isolated tumor cells in regional lymph node(s) no greater than 0.2 mm
pN1mi	Micrometastasis (greater than 0.2 mm, none greater than 2.0 mm)
pN1	Metastasis in 1-3 axillary lymph nodes and/or in internal mammary nodes with microscopic disease detected by sentinel lymph node biopsy but not clinically apparent
pN2	Metastasis in 4-9 axillary lymph nodes, or in clinically apparent internal mammary nodes in the absence of axillary lymph node metastasis
pN2a	Metastasis in 4-9 axillary lymph nodes (at least one tumor deposit >2.0 mm)
pN2b	Metastasis in clinically apparent internal mammary nodes in the absence of axillary lymph node metastasis
pN3	Metastasis in 10 or more axillary lymph nodes, or in infraclavicular (level III axillary) lymph nodes, or in clinically apparent ipsilateral internal mammary lymph nodes in the presence of one or more positive axillary lymph nodes, or in more than 3 axillary lymph nodes and in internal mammary nodes with microscopic disease detected by sentinel lymph node biopsy but not clinically apparent, or in ipsilateral supraclavicular lymph nodes
pN3a	Metastasis in 10 or more axillary lymph nodes (at least one tumor deposit >2.0 mm), or metastasis to the infraclavicular (level III axillary) lymph nodes
pN3b	Metastasis in clinically apparent ipsilateral internal mammary lymph nodes in the presence of one or more positive axillary lymph nodes; or metastasis in more than 3 axillary lymph nodes and in internal mammary nodes with microscopic disease detected by sentinel lymph node biopsy but not clinically apparent
pN3c	Metastasis in ipsilateral supraclavicular lymph nodes

Table 4: Distant metastasis (M):

pM (Metastasis)	Distant Metastasis
pM0	No distant metastasis
pM1	Distant metastasis

Table 5: TNM Stage grouping:

Pathologic Stage	T stage score	N stage score	M stage score
Stage 0	Tis	N0	M0
Stage I	IA	T1	N0
	IB	T0	N1mi
		T1	N1mi
Stage II	IIA	T0	N1
		T1	N1
		T2	N0
	IIB	T2	N1
		T3	N0
Stage III	IIIA	T0	N2
		T1	N2
		T2	N2
		T3	N1 or N2
	IIIB	T4	N0, N1 or N2
	IIIC	any T score	N3
Stage IV	IV	any T score	any N score

MICROSCOPIC GRADE:

Considering both architecture and cytology have been found to correlate with prognosis, Elston and Ellis modified the original Bloom and Richardson and Bansal et al.[85] grading schemes based on tubule formation and nuclear degree atypia. This is the Modified Bloom-Richardson grading system (MBR). It also incorporates the mitotic activity to the previous classification. The grade is calculated by summing the numbers obtained for formation of tubules, nuclear pleomorphic features and count of the mitotic activity.[12]

Grading is advocated for all, regardless of morphological type, as it serves to prognosticate the metastasis and survival, independent of the lymph node's status, and predicts chemotherapy response

Table 6: Modified Bloom Richardson grading

Component	Description	Scoring
Tubule/Gland Formation	Percentage of tubular or glandular acinar spaces. Structures with clear central lumina enclosed by polarized cells are counted.	>75%: Score 1 10–75%: Score 2 <10%: Score 3
Nuclear Pleomorphism	Size and variation in tumor cell nuclear size and shape. Scored in the least differentiated area of the tumor. Regularity of nuclear size compared to normal epithelial cells.	Similar in size to normal epithelial cells, minimal pleomorphism: Score 1 5–2x larger than epithelial cells, moderate pleomorphism: Score 2 >2x larger, considerable variation, prominent nucleoli: Score 3
Mitotic Counting	Number of defined mitotic figures in the most proliferative area of the tumor. Scored based on the number of mitotic figures in a given area.	Score 1–3 based on number of mitotic figures (cut-off points dependent on field area size)

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

Table 7: Score threshold for mitotic counts according to WHO breast[80]

Field	Field	Mitotic count (score)		
Diameter (mm)	Area (mm^2)	1	2	3
0.40	0.126	≤4	5–9	≥10
0.41	0.123	≤4	5–9	≥10
0.42	0.138	≤5	6–10	≥11
0.43	0.145	≤5	6–10	≥11
0.44	0.152	≤5	6–11	≥12
0.45	0.159	≤5	6–11	≥12
0.46	0.166	≤6	7–12	≥13
0.47	0.173	≤6	7–12	≥13
0.48	0.181	≤6	7–13	≥14
0.49	0.188	≤6	7–13	≥14
0.50	0.196	≤7	8–14	≥15
0.51	0.204	≤7	8–14	≥15
0.52	0.212	≤7	8–15	≥16
0.53	0.221	≤8	9–16	≥17
0.54	0.229	≤8	9–16	≥17
0.55	0.237	≤8	9–17	≥18
0.56	0.246	≤8	9–17	≥18
0.57	0.255	≤9	10–18	≥19
0.58	0.264	≤9	10–19	≥20
0.59	0.273	≤9	10–19	≥20
0.60	0.283	≤10	11–20	≥21
0.61	0.292	≤10	11–21	≥22
0.62	0.302	≤11	12–22	≥23
0.63	0.312	≤11	12–22	≥23
0.64	0.322	≤11	12–23	≥24
0.65	0.332	≤12	13–24	≥25
0.66	0.342	≤12	13–24	≥25
0.67	0.352	≤12	13–25	≥26
0.68	0.363	≤13	14–26	≥27
0.69	0.374	≤13	14–27	≥28

NOTTINGHAM PROGNOSTIC INDEX[86,87]

Table 8: Nottingham prognostic index in breast cancer

NPI	Score	5 year survival	Prognosis
I	≤2.4	96%	Excellent
II	>2.4 - ≤3.4	93%	Good
III	>3.4-5.4	78%	Moderate
IV	>5.4	44%	Poor

NPI = (0.2 X S) + N +G

Lymph nodes = number of lymph nodes, 0=1, 1-3 = 2, >3 = 3

PROGNOSTIC & PREDICTIVE FACTORS:[77,88]

1. Tumor size refers to the maximum measured diameter of the tumor. A larger tumor size is correlated with a higher likelihood of distant metastasis and lower survival rates..
2. Histological type - Infiltrating ductal carcinoma is the commonest breast carcinoma constituting 22%. Inflammatory carcinoma has lower survival rates among different histological types, but with systemic chemotherapy, the prognosis is better, with 25 to 50% survival rates.
3. The presence of necrosis is a separate prognostic factor. Central necrosis and fibrosis were observed in large tumors with higher T stage and negligible in early breast cancers. They significantly lack hormone receptors and are associated with a higher grade.
4. The existence of mononuclear inflammatory cell infiltrates within and around the tumor indicates the host's immune response to the tumor cells and is linked to a more favorable prognosis, regardless of hormone receptor status, grade, and other clinic-pathological characteristics. Macrophages have demonstrated their efficacy in combating cancer cells.
5. Lymphatic invasion is linked to an increased likelihood of lymph node metastases and a higher stage of the tumor. It helps the clinician determine whether adjuvant treatment should be considered for individuals who cannot undergo chemotherapy.
6. Vascular invasion refers to the infiltration of tumor cells into the inner space of an artery or vein. It is linked to the spread of cancer to distant parts of the body, larger size of the tumor, higher level of severity, and fewer chances of survival. Vascular invasion is observed in patients with systemic or metastatic illness.
7. Perineural invasion is linked to lymphovascular invasion and a more advanced tumor grade

8. Stromal features - Tumors with less stromal reactivity typically display a greater histology grade and higher nuclear grade. On the other hand, tumors that display a strong stromal response, such as fibrosis and desmoplasia, have a star-shaped appearance, well-defined boundaries, are of low severity, and are more likely to have hormone receptors
9. Axillary node status is frequently linked to both disease-free and overall survival rates. Tumors exhibiting higher grade, histological type, stage, and lymphovascular invasion are associated with an elevated risk of axillary lymph node metastasis.

Table 9: Molecular subtypes [89–91]

Category	Subcategory/Details	Data
Lifetime Risk	**Luminal A and B**	6.79%
	HER2 Positive	1.78%
	Triple Negative	1.2%
Overall Frequency	**Luminal A**	30 - 40%
	Luminal B	20 - 30%
	HER2 Positive	12 - 20%
	Triple Negative	15 - 20%
Molecular Subtype by Age	**< 40**	Triple Negative > Luminal B > HER2 > Luminal A
	40 - 59	Triple Negative > Luminal B > HER2 > Luminal A
	60 - 69	Luminal A > HER2 > Luminal B > Triple Negative
	> 70	Luminal A > Luminal B and HER2 > Triple Negative
Triple Negative	**Demographics**	Younger age, African ancestry, BRCA1 mutations
	Mutation Carriers	BRCA1, BRCA2, TP53 mutation carriers
	Mutation Prevalence in Triple Negative	20 - 30% of triple negative patients have BRCA1 or BRCA2 mutations

This table summarizes the key data points regarding the frequency, lifetime risk, and molecular subtypes of breast cancer, as well as specific characteristics of triple-negative breast cancer.

PHH3, KI67 and MAI in BREAST CANCER

In a study conducted by **Elham Mirzaiian et al** examines the efficacy of Phosphohistone H3 using immunohistochemistry (IHC) in various stages of breast cancer. The study compares this method with the traditional mitotic count obtained using hematoxylin and eosin (H&E) staining, and also explores any potential changes in tumor grading.

There were a total of 90 instances of invasive breast cancer that were examined. The average mitotic counts were 8.6 and 6.4 per 10 high-power fields (HPF) in the IHC and H&E groups, respectively. Although the IHC approach had a slightly higher average count, a significant correlation of R=0.914

was detected. Using PHH3 immunohistochemistry (IHC), two out of 33 grade I cases were elevated to grade II, and three grade II cases were elevated to grade III, with no instances of lowering the grade.[92]

Even though phospho-histone-H3 (PHH3) expression in cancer patients has been studied a lot, there is still disagreement about how reliable it is. So, **Qian Hao et al**. did a meta-analysis to bring together all the results and figure out how important PHH3 expression is for predicting how well a cancer patient will do in the future. This study looked at the link between PHH3 expression levels and overall survival (OS), disease-free survival, and recurrence-free survival. It did this by searching PubMed, Web of Science, Embase, and the Cochrane Library for all 19 papers that included a total of 4803 patients. The results show that having a lot of PHH3 can tell you if a cancer patient will have a short overall survival rate (HR=2.66, 95% CI=1.74–4.08, P<0.001), a short disease-free survival rate (HR=3.40, 95% CI=1.47–7.87, P=0.004), or a short recurrence-free survival rate (HR=2.80, 95% CI=1.61–4.85, P<0.001). A subgroup study revealed that high levels of PHH3 were strongly linked to both breast cancer (HR=5.66, 95% CI=2.72–11.78, P<0.001) and urogenital tumors (HR=3.01, 95% CI=1.78–5.09, P<0.001). Also, there was no important difference between Asian (HR=1.98, 95% CI=1.083.63,P=0.026) and Caucasian (HR=3.01, 95% CI=1.87–4.85, P<0.001) groups when it came to OS and PHH3 expression.[93]

A study conducted by **Monica et al** compared mitotic counts obtained from conventional hematoxylin-eosin-saffron (HES) stained sections with counts obtained from sections stained using immunohistochemistry for Anti-Phospho-Histone H3 (Ser10) (PHH3), a marker that indicates cells in the late G2 to M phase. The study utilized a sample of 250 individuals with symptomatic breast cancer that were collected and organized on tissue microarrays (TMA). Every case was subjected to staining using both Hematoxylin and Eosin (HES) and Phospho-Histone H3 (PHH3), after which the number of mitotic counts was evaluated. In addition, mitotic counts obtained from HES-stained entire sections of the same cases were accessible for comparison.
Among the 250 cases examined, 18 (7.2%) were classified as histopathological grade 1, 141 (56.4%) as grade 2, and 91 (36.4%) as grade 3. The majority, comprising 178 cases (72.2%), showed the ductal histological type, whereas 30 cases (12.0%) were lobular carcinomas. These demographic characteristics closely resemble those of the original group described by Engstrøm et al. The histological examination showed that the number of mitotic counts on tissue microarray (TMA) sections stained with PHH3 consistently exceeded those stained with HES. However, there was a good correlation (0.72) between the two approaches. The average number of mitotic cells labeled

with PHH3 was 14.4, which was significantly greater than the average of 4.2 for mitotic cells stained with HES. However, when comparing TMA and entire sections, the mitotic counts obtained from both staining procedures exhibited a somewhat comparable pattern, with Pearson correlations of 0.61 and 0.63, respectively. Significantly, the molecular subtyping analysis showed clear variations in mitotic count patterns, with the Luminal A subtype consistently displaying low numbers, which suggests a positive prognosis. Additional research indicated that PHH3 mitotic counts vary among histopathological grades, with grades 1 and 3 showing more specificity compared to grade 2. Furthermore, the analysis consistently shown higher mitotic counts in PHH3-stained slides compared to HES-stained slides. However, a significant link between the two approaches was revealed, with a Pearson correlation coefficient of 0.72. The PHH3 numbers showed strong correlations with histopathological grade and molecular subtypes. Nevertheless, there was no clear association observed with breast cancer-specific survival. The study's sample size was relatively small, and the use of TMA posed an additional limitation. However, these findings indicate the necessity for additional investigation of PHH3 as a potential substitute for observing mitotic activity. .[94]

The usefulness of phosphohistone H3 as a proliferation marker for evaluating invasive breast cancers: A comparative study with Ki67 was looked into by **Ye Kim et al.** The study looked at Ki67 and PHH3 staining in 218 surgery cases of breast cancer found at Severance Hospital between 2012 and 2013. The expression of PHH3 successfully found mitotic activity that MAI had missed, which led to an increase in the M grade at diagnosis in 29 of the 218 cases that were studied. It was clear that PHH3 could consistently find areas where cells were multiplying because the numbers of mitoses were very similar between 10 high-power fields and 10 low-power fields ($R2 = 0.999$; $P = 0.001$). Additionally, PHH3 was more consistent than Ki67, as shown by a higher inter-class correlation coefficient among five raters ($0.904 > 0.712$; $P = 0.008$). Notably, even though the follow-up time was pretty short (median 46 months; 7 recurrences), PHH3 was the only variable that was significantly linked to disease-free survival ($P = 0.043$). Other common clinicopathologic factors, such as Ki67 ($P = 0.356$), were not significantly linked.[28]

The study by **Julia et al**. looked at how well different people agreed on how to rate the mitotic activity index (MAI), Ki67 expression, and PhH3 in a group of ER-positive breast cancer patients.The study looked at tumor samples from 159 women with luminal breast cancer. Three breast cancer pathologists looked at the MAI and PhH3 scores. Two of the pathologists looked at the Ki67 scores on their own. We counted the number of PhH3-positive cells in a 2 mm^2 area, using a cutoff of 13 positive cells or

more to tell the difference between low and high proliferative tumors. The global scoring method was used to measure Ki67 expression. Ki67 rates below 20% were considered low. The intraclass correlation coefficient (ICC) and Cohen's j statistics were used to measure agreement between observers. It was looked at what would happen to histological scoring if MAI was replaced with PhH3. All three viewers were able to count PhH3-positive cells very accurately (ICC of 0.86). It was pretty clear that all viewers agreed on the categorical PhH3 counts (j = 0.78, j = 0.68, and j = 0.80), but not so much on the MAI (j = 0.38, j = 0.52, and j = 0.26) and Ki67 (j = 0.55) scores. When PhH3 was used for histological scoring, there was more agreement in the grades (PhH3, j = 0.52, j = 0.48, and j = 0.52; MAI, j = 0.43, j = 0.35, and j = 0.32) and more grade III tumors (14%, 18%, and 27%).[95]

Gerring et al. did a study to directly compare Ki67 and phosphohistone H3 and find the marker that was most useful for predicting the future. Tissue microarrays from 108 breast cancer patients were stained with antibodies for Ki67 and phosphohistone H3. The results showed that phosphohistone H3 was a better predictor of outcome than Ki67 in a multivariable model that took into account common breast cancer prognostic factors. Phosphohistone H3 staining was a strong indicator of 5-year survival after diagnosis (HR=4.35, P<10^-5), better than Ki67 (HR=2.44, P=0.004) and a key way to group patients over 45 years old by their risk of death. It's important to note that phosphohistone H3 always showed clear and strong staining, while Ki67 staining levels changed over time.[72]

Jennifer S. et al. look into the differences between the H&E mitotic count, the PHH3 mitotic count, and the Ki-67 index in invasive breast cancer. It was decided to look back at cases of aggressive breast cancer from 2013 to 2014. The mBR standards were used to give both the H&E and PHH3 mitotic counts scores between 1 and 3. The Ki-67 index was put into three groups: low (<10%), middle (10–20%), and high (>20%). There were 451 cases that were looked at. When PHH3 and H&E mitotic count were compared, the mBR score changed in 24% of cases. In those cases, 23% of the scores went up and 1% went down. There were 431 cases with both Ki-67 and PHH3 available. In 51% of those cases, both the H&E and PHH3 mitotic scores matched Ki-67, with PHH3 having a stronger link. Researchers came to the conclusion that PHH3 makes it easier to find mitotic figures in breast cancer. As a useful check, PHH3 immunohistochemistry helps figure out the final mitotic score, which leads to more accurate mBR scoring and grades. Notably, 48 of the 451 patients (10.6%) in this study had a major improvement, which could have changed their treatment plans and made chemotherapy a possibility.[96]

In this research, **Cui X et al**. looked at how well Phosphohistone H3 (PHH3) could grade 97 breast biopsy samples in a row. Even at low magnification, PHH3 antibodies showed clear, strong nuclear staining in cells that were dividing. There was a strong link between the PHH3 mitotic index and both the hematoxylin and eosin (H&E) mitotic index and the Ki-67 proliferation index. Also, PHH3 immunostaining made the paired κ-value and agreement of the MI a lot better, even though the PHH3 MI was used to improve a lot of breast cancer cases. The results of this study show that PHH3 is a more sensitive and accurate mitotic measure than regular H&E staining, with less variation between observers. This shows that it could be useful in clinical practice. Adding PHH3 to breast cancer grading should be looked at again if it regularly works better than traditional H&E staining.[71]

Ibrahim et al. did a study in which 97 cases of aggressive breast cancer were looked at. This showed that pathologists found significantly more mitotic figures using the PHH3 method (median SD, 20–33) compared to the H&E method (median SD, 16–25), $P < 0.001$.When ICC = 0.820 was used instead of ICC = 0.514 for normal H&E, there was a lot of agreement between pathologists. It took a lot longer to score mitotic figures stained with H&E alone than it did to score mitotic figures stained with PHH3–H&E. The study's results showed that PHH3-labeled mitotic figures were easy to see and made it quick and easy to find hotspots. They also showed up mitotic figures at low power without any trouble. When doctors used PHH3–H&E ($k = 0.842$), they agreed more than when they used H&E ($k = 0.605$). PHH3 was a reliable indicator of life on its own. Breast cancer specific survival (BCSS) was shorter and distant metastasis free survival (DMFS) was worse when PHH3 was present.[16]

MATERIALS AND METHODS

MATERIAL AND METHODS

STUDY DESIGN: Cross-Sectional observation study.

SOURCE OF DATA: Cases of invasive ductal breast carcinoma received in the Department of Pathology, Sri Devaraj Urs Medical College, Tamaka, Kolar from July 2019 to June 2023 have been included in this study

STUDY TOOLS: Immunohistochemical staining for PHH3 and KI67 in histologically diagnosed cases of invasive ductal carcinoma of breast

STUDY SETTING: This study has been conducted in Department of Pathology, Sri Devaraj Urs Medical College and have diagnosed patients with invasive ductal carcinoma pf breast who have undergone surgical excision in attached R.L.J Hospital

STUDY POPULATION: All histologically proven cases of invasive ductal breast carcinoma in Department of Pathology, Sri Devraj Urs Medical College, Tamaka, Kolar

INCLUSION CRITERIA:

Patients diagnosed with infiltrating ductal carcinoma of breast

EXCLUSION CRITERIA:

Patients subjected to neoadjuvant radiotherapy / chemotherapy before excision of invasive breast carcinoma.

Patients who underwent chemotherapy for other cancer over the past 5 years.

STUDY DURATION: July 2019 to June 2023

METHOD OF COLLECTION OF DATA INCLUDING SAMPLE PROCEDURE:

SAMPLE SIZE

- Considering p as 40.30% of cases for PHH3 positive as reported in study by Kim et al.[28]
- Equation sample size is(n) = $Z_{1-\alpha}^2 p(q)/d^2$
- Here $Z_{1-\alpha}$ = Standard normal variant
- P = Expected prevalence in population based on previous studies
- d = Absolute error of 10%
- q = 100-p
- Sample size required for cross-sectional study will be **102** for invasive breast carcinoma with 95% confidence

METHOD OF COLLECTION: All histopathologically confirmed cases of Invasive ductal carcinoma breast on surgically excised specimens from July 2019 to June 2023 have been included in the study. The H&E slides from tumor proper have been screened for histopathological parameters like grading using the Modified Bloom Richardson score and TNM staging was done according to the new AJCC staging criteria. Immunohistochemical staining for PHH3 and Ki67 was done on sections from the tumor proper for all the cases of Invasive Ductal carcinoma breast using appropriate positive and negative controls.

METHODOLOGY: Specimens fixed in 10% neutral buffered formalin have been taken. Grossing and sampling have been done according to standard protocols. All tissue blocks showing IDC breast on standard Haematoxylin & Eosin (H&E) histology were selected for immunohistochemistry(IHC).

IMMUNOHISTOCHEMICAL STAINING:

IHC staining was done on paraffin-embedded 4-micrometer tissue slices that had been fixed in 10% formalin for 48 hours at 25 degrees Celsius. After deparaffinizing in Xylene and rehydrating with a number of ethanols (100, 95, 90, 80, and 70%) at room temperature for 5 minutes, tissue sections were used to get antigens out under high steam pressure. The sections were then washed in distilled water for 1-2 minutes after being left to cool for 10 minutes, do not let the sections dry out

After which endogenous Per-oxidase the sections in 3% H_2O_2 for 10 minutes. Wash in tris buffered solution(TBS) at a pH of 7.4 for 2 minutes. The sections are then covered with individual primary antibodies for 45 minutes to 1 hour based on room temperature. Then again wash slides for 2 minutes in TBS. The sections are then covered with secondary antibody for 30 minutes and then wash the slides 2 times in TBS for 2 minutes. The sections are then covered with Diaminobenzidine tetrahydrochloride (DAB) chromogen for 5 minutes. Wash with distilled water. The slides were then washed with deionized water, stained with hematoxylin for two to five minutes, and washed again for one minute with TBS buffer. A DPX was used to mount it.

GRADING OF IHC:[97]

Methodology involved in evaluation of slides was done by two pathologist. This dual review process was implemented to ensure reliable assessment of the histopathological features and findings.

Grading of IHC was done following a study done by calculating the **filed diameter as per the WHO criteria to calculate mitotic figures-**

Microscope used Olympus CX 23

$$\text{Diameter of one field} = \frac{\text{Field number}}{\text{Objective magnification}}$$

Filed number (FN) of eye piece + 20

Objective magnification=40

Field area= 0.5mm = 0.196 mm^2

Field area of 10 HPF = 1.96 mm^2

FOR MAI: MAI was categorized on the basis of the total number of mitotic figures in an area of 0.196 mm^2, as follows:

0– 7 mitotic count = Score 1

8–14 mitotic count = Score 2

≥15 mitotic count = Score 3.

FOR PHH3: Similar to MAI

FOR KI67:

1- A cut-off value of 14% of nuclei positively stained for Ki67 was used for Molecular classification
2- According to the International Ki67 Working Group (IKWG), a Ki67 score of less than 5% or greater than 30% is more robust for estimating prognosis in patients with stage I or II breast cancer that is ER+HER2−. Patients are categorized into low (≤ 5%), intermediate (6-29%), and high (≥30%) based on IKWG recommendations[98–100]

TILS[101,102]

TILS scoring was done according to International TILS Working Group as <10%=Low TILS, 10-40%=Intermediate TILS and >40% High TILS

T:S RATIO[103]

Tumor stromal ratio was divided into in two categories as stroma-low tumor having ≤50% stroma and >50%stroma is considered as stroma-high

The method that was folllowed for T:S Ratio was that initially using a 4x objective the most invasive tumor area of the whole slide was selected, subsequently, using 10x objective, only fields were scored where both stroma and tumor were present and, most importantly tumor cells were seen on all sides of the microscopic filed

Statistical analysis:

Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. **Chi-square test or Fischer's exact test** (for 2x2 tables only) was used as test of significance for qualitative data. Correlations were performed with **Pearson Correlation coefficient**

Graphical representation of data: MS Excel and MS word was used to obtain various types of graphs

***p* value** (Probability that the result is true) of <0.05 was considered as statistically significant after assuming all the rules of statistical tests.

Statistical software: MS Excel**,** SPSS version 22 **(**IBM SPSS Statistics, Somers NY, USA) was used to analyse data

RESULTS

RESULTS: 102 cases of IDC breast were studied

DEMOGRAPHIC DATA:

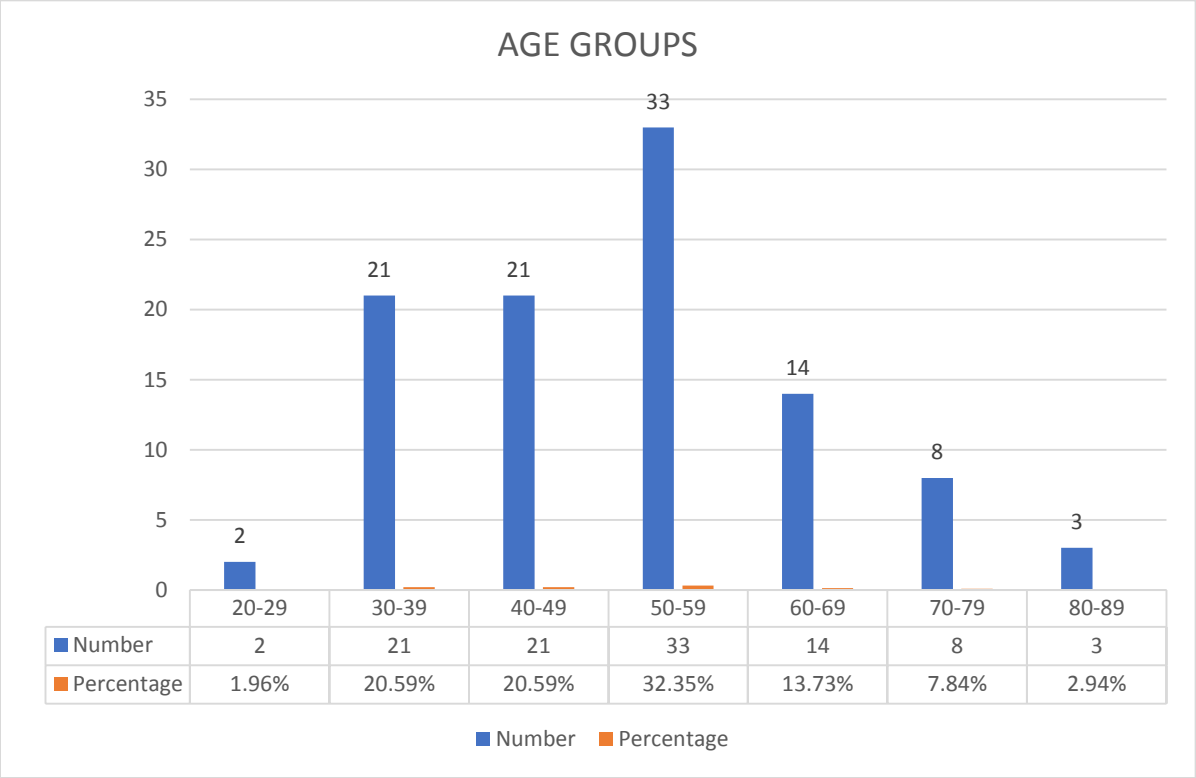

Chart 1: Distribution of cases based on age group

Chart 1 show the distribution of cases based on age group. In this study amongst 102 cases majority of cases that is 32.35%, was observed in the 50-59 age group. Both the 30-39 and 40-49 age groups each accounted for 20.59% of the cases. The 60-69 age group comprised 13.73% of the cases, while the 70-79 age group accounted for 7.84%. The 80-89 age group had 2.94% of the cases, and the lowest number of cases, 1.96%, was in the 20-29 age group.

Table 10- Distribution of cases based on age group

		N	%
Age group	<50yrs	44	43.1%
	≥50yrs	58	56.9%

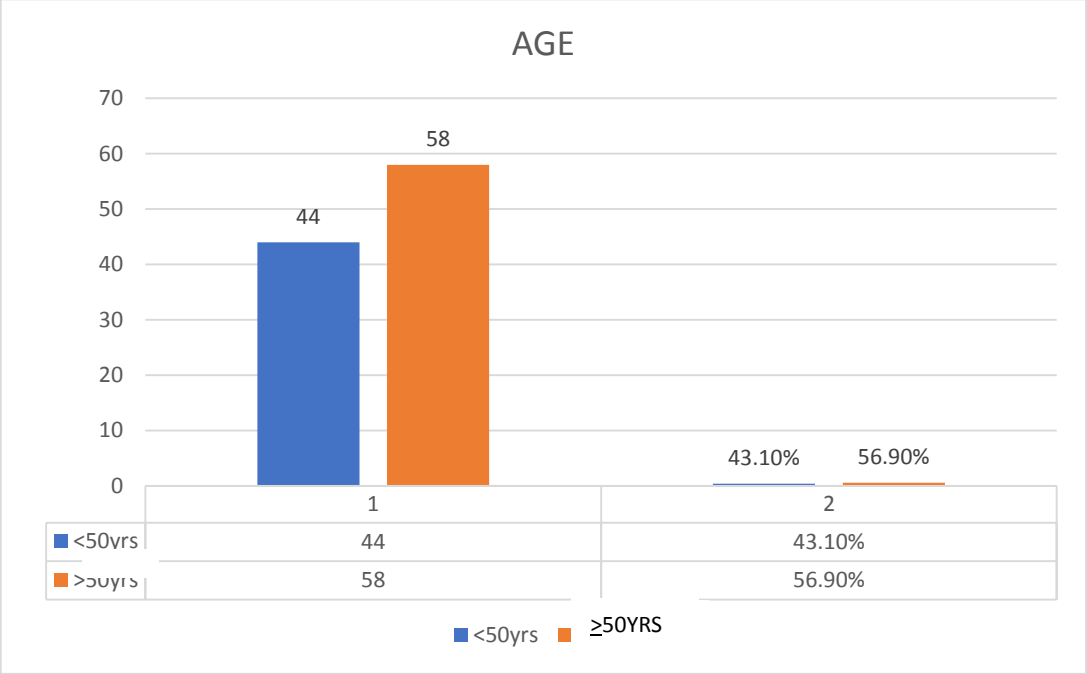

Chart 2 : Distribution of cases based on age group

Table 10 and chart 2 show the distribution of cases based on age group. In this study amongst 102 cases majority cases comprising of 56.90% were above 50 years and the mean age observed in this study was 51.46 years with a median age of 52 years. The higher incidence of breast cancer in younger women observed in this study might be due to a combination of genetic, hormonal, lifestyle, and environmental factors. These elements collectively contribute to the elevated risk of developing breast cancer at a younger age.

Table 11: Distribution of cases based on laterality

Laterality	Number of Cases	Percentage
Left	55	53.92%
Right	47	46.08%
Total	102	100%

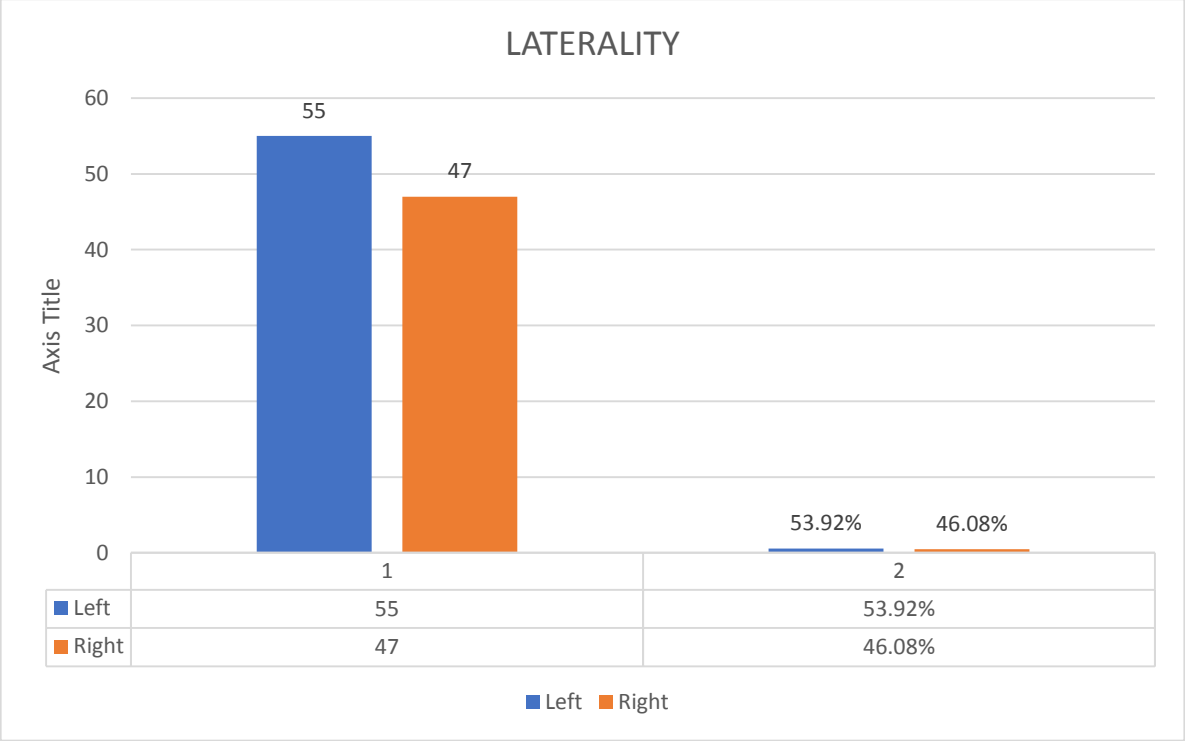

Chart 3: Distribution of cases based Laterality

Table 11 and Figure 3 show the distribution of cases based on laterality. In this study amongst 102 cases majority, 53.92% cases had breast lump on the left side and 46% cases had lump on the right side

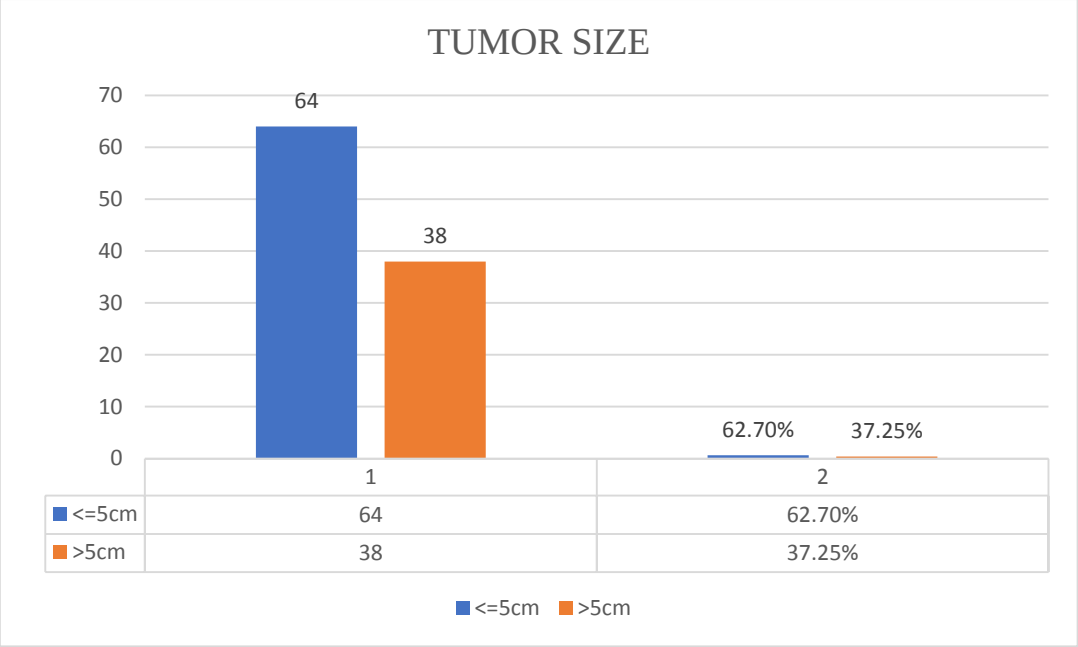

Chart 4: Distribution of cases based on Tumor size

Chart 4 show the distribution of cases based on tumor size. In this study amongst 102 cases majority of the cases were ≤5cm comprising 62.70% and only 37.25% cases had >5cm tumor size

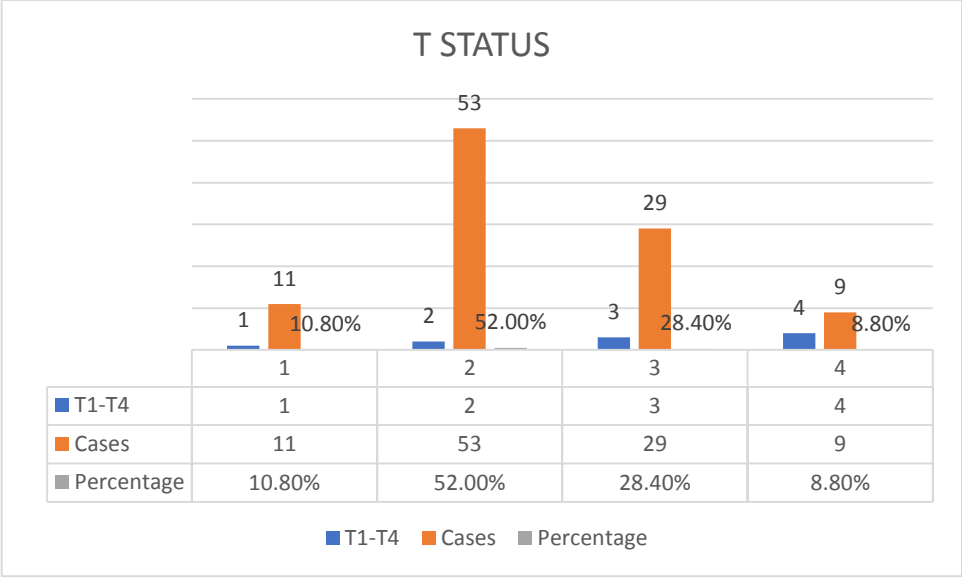

Chart 5: Distribution of cases based on T stage

Chart 5 show the distribution of cases based on T stage. In this study amongst 102 cases majority of the cases showed T2 that's is 52% of theses cases, followed by 29.4% cases in T3, 10.8% cases in T1 and least cases that is 8.8% cases were in T4

Table 12: Distribution of cases based on N stage

	N0-N3	N	%
N	0	47	46.1%
	1	24	23.5%
	2	16	15.7%
	3	15	14.7%

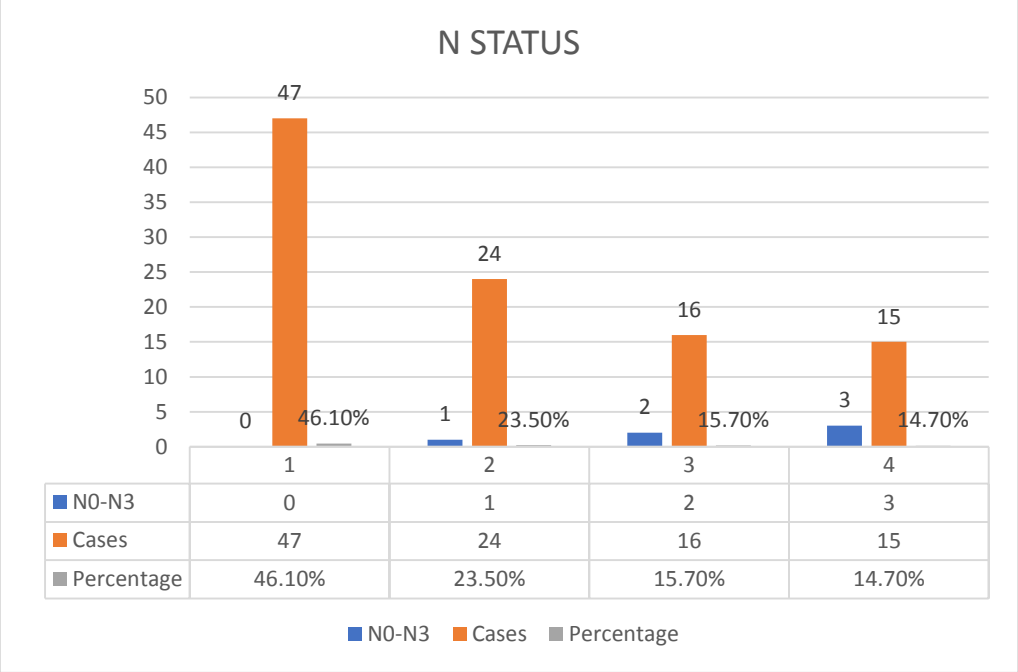

Chart 6 : Distribution of cases based on N stage

The table 12 and Chart 6 show the distribution of cases based on N stage. In this study amongst 102 cases the majority of the cases showed majority of cases in N1 that's is 46.1% of these cases, followed by 23.5% cases in N2, 15.70% cases in N2 and least number of cases that is 14.70% cases in N1

Table 13: Distribution of cases based on Lymph node status

		N	%
Lymph node status	Negative	47	46.07%
	Positive	55	53.92%

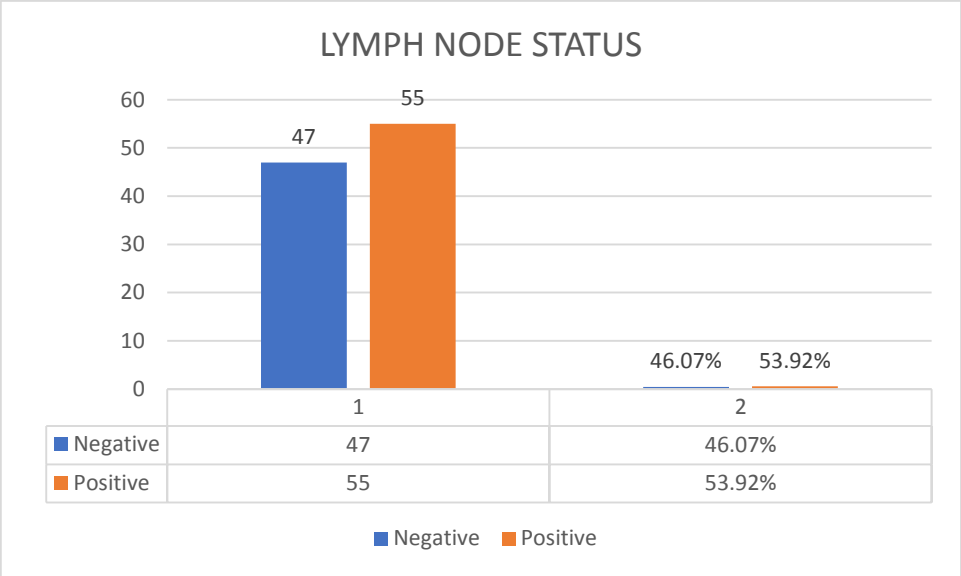

Chart 7: Distribution of cases based on Lymph node status

Table 13 and Chart 7 show the distribution of cases based on lymph node status. In this study amongst 102 cases, 55 cases showed metastatic deposits and the remaining 47 cases are free from tumor deposits

Table 14: Distribution of cases based on TILS

TILS		N	%
	<10	68	66.7%
	10-40	26	25.5%
	>40	8	7.8%

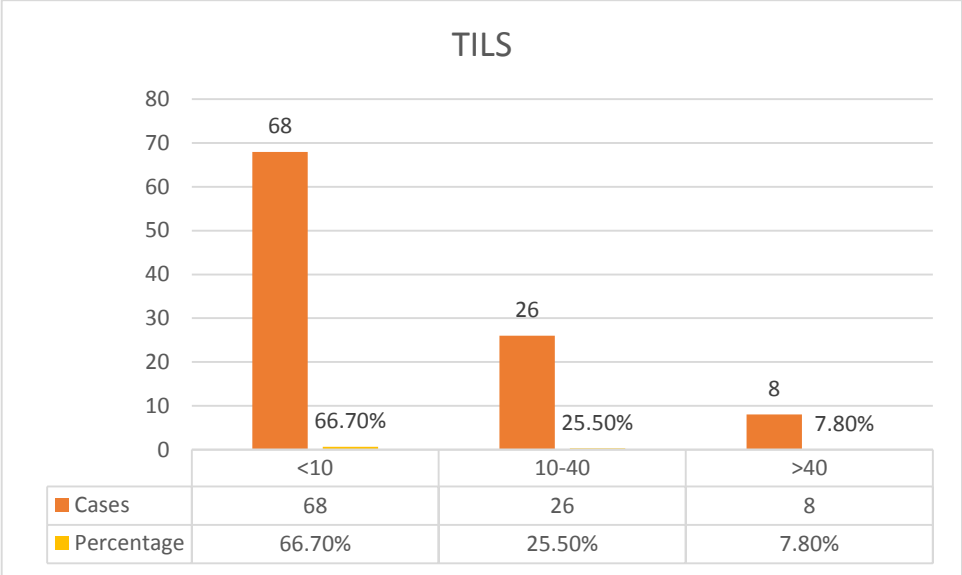

Chart 8: Distribution of cases based on TILS

The table 14 and Chart 8 shows the distribution of cases based on TILS groups in a study of 102 cases. The majority of cases, 66.7%, fall into the group with TILS less than 10. The group with TILS between 10 and 40 accounts for 25.5% of the cases. The smallest group, with TILS greater than 40, comprises 7.8% of the cases.

Table 15: Distribution of cases based on T:S RATIO

T:S RATIO		N	%
	<=50	28	27.5%
	>50	74	72.5%

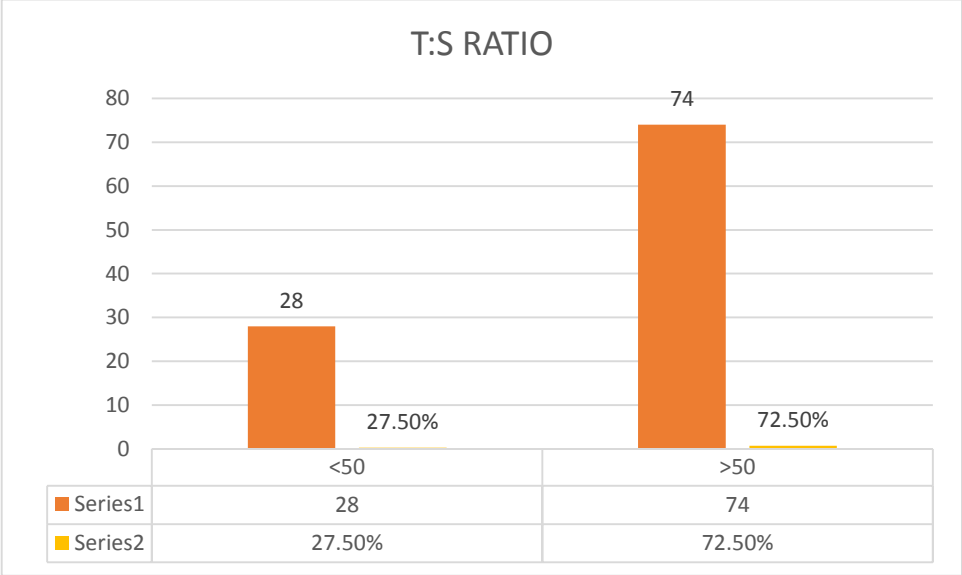

Chart 9: Distribution of cases based on T:S RATIO

Table 15 and Chart 9 show that in the study of 102 cases, the distribution based on T:S ratio groups shows that 72.5% of cases have a T:S ratio greater than 50, while 27.5% have a T:S ratio of 50 or less.

Table 16: Distribution of cases based on MAI(mitotic count – H&E)

MITOTIC COUNT score - H&E		N	%
	1	25	24.5%
	2	51	50.0%
	3	26	25.5%

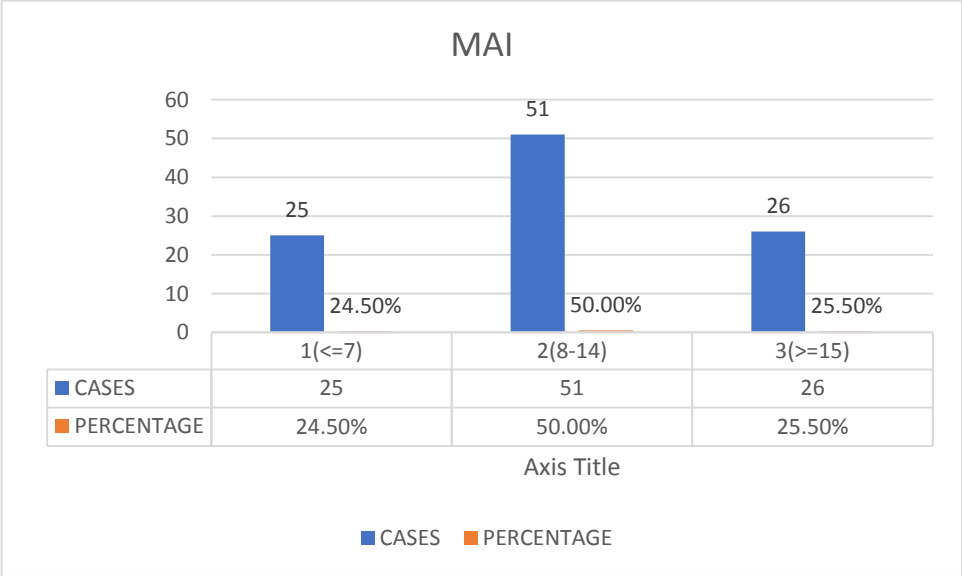

Figure 10: Distribution of cases based on MAI

The table 16 and Chart 10 shows the distribution of cases based on MAI groups in a study of 102 cases. Most cases, 50.00%, fall into MAI score 2 (8-14) followed by MAI score 3(>=15) showing 25.50% cases and only 24.50% cases were in MAI score 1(<=7)

Table 17: Distribution of cases based on Mitotic count-PHH3

MITOTIC COUNT score – PHH3		N	%
	1	7	6.9%
	2	11	10.8%
	3	84	82.4%

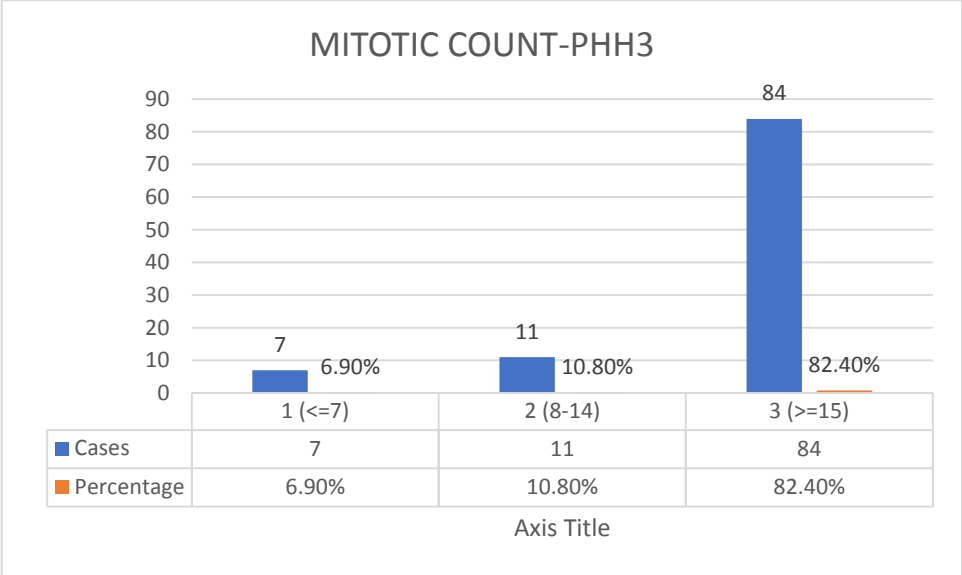

Chart 11: Distribution of cases based on Mitotic count-PHH3

The table 17 and Chart 11 shows the distribution of cases based on PHH3 groups in a study of 102 cases. Most cases, 82.40%, fall into PHH3 Group 3 (≥15). PHH3 Group 2 (8-14) accounts for 10.80% of the cases. The smallest group, PHH3 Group 1 (≤7), comprises 6.90% of the cases.

Table 18 : Summarizing the analysis of mitotic count scores using H&E staining in relation to lymph node status, stage, and mBR-H&E grade, including the *p*-values**:**

CATEGORY	SCORE 1	SCORE 2	SCORE 3	*p* value
Lymph Node Status				0.383
Node negative	27.5%	52.9%	19.6%	
Node positive	21.6%	47.1%	31.4%	
Ptnm Stage				0.526
Stage I	0.0%	57.1%	42.9%	
Stage II	30.2%	49.1%	20.8%	
Stage III	22.0%	48.8%	29.3%	
Stage IV	0.0%	100.0%	0.0%	
mBR Grade -H&E				<0.01
Grade 1	53.1%	43.8%	3.1%	
Grade 2	12.9%	53.2%	33.9%	
Grade 3	0.0%	50.0%	50.0%	

In the analysis of mitotic count scores using H&E staining in relation to lymph node status, stage, and mBR-H&E grade, several significant observations were made. For lymph node score, the distribution of mitotic count scores (1, 2, and 3) showed that 27.5% of node-negative cases had a score of 1, 52.9% had a score of 2, and 19.6% had a score of 3. Conversely, among node-positive cases, 21.6% had a score of 1, 47.1% had a score of 2, and 31.4% had a score of 3. However, the p-value of 0.383 indicates no statistically significant difference between these groups.

When examining mitotic scores across different stages, stage II cases had the highest proportion of score 1 (30.2%), score 2 (49.1%), and score 3 (20.8%). Stage III cases showed 22.0% with score 1, 48.8% with score 2, and 29.3% with score 3. The p-value for stage comparisons is 0.526, also indicating no statistically significant difference.

In contrast, a statistically significant difference was observed when comparing mitotic count scores with mBR-H&E grades. For mBR grade 1, 53.1% of cases had a mitotic score of 1, 43.8% had a score of 2, and only 3.1% had a score of 3. For mBR grade 2, 12.9% of cases had a mitotic score of 1, 53.2% had a score of 2, and 33.9% had a score of 3. Notably, for mBR grade 3, 50.0% of cases had a mitotic score of 2, and 50.0% had a score of 3, with no cases having a score of 1. The p-value of <0.01 signifies a highly significant difference in mitotic scores across different mBR-H&E grades. **These findings highlight the strong correlation between mitotic count scores and MBR grades, while the associations with lymph node status and stage are less pronounced.**

Comparison of Mitotic Count Scores (H&E) with Mitotic Count Scores (PHH3)

Table 19: Distribution of Mitotic Count Scores (H&E) by Mitotic Count Scores (PHH3):

MITOTIC COUNT score- H&E	MITOTIC COUNT score- PHH3					
	1		2		3	
	N	%	N	%	N	%
1	7	28.0%	10	40.0%	8	32.0%
2	0	.0%	1	2.0%	50	98.0%
3	0	.0%	0	.0%	26	100.0%

Table 20 : Upgrade and Downgrade Rates Between mitotic count Scores**:**

Upgrade	**N**	**%**
mitotic count **score 1 to score 2**	10	40
mitotic count **score 2 to score 3**	50	98
mitotic count **score 1 to score 3**	8	32
Downgrade		
mitotic count **score 2 to score 1**	0	0
mitotic count **score 3 to score 2**	0	0
mitotic count **score 3 to score 1**	0	0

The provided data compares Mitotic Count (MC) scores assessed using Hematoxylin and Eosin (H&E) staining with those assessed using PHH3 staining across three score categories (1, 2, and 3). For cases scored as 1 by H&E, 28.0% remained score 1, 40.0% were upgraded to score 2, and 32.0% were upgraded to score 3 with PHH3. For cases scored as 2 by H&E, 0.0% remained score 2, 2.0% were upgraded to score 2, and 98.0% were upgraded to score 3 with PHH3. For cases scored as 3 by H&E, 0.0% remained score 3, 0.0% were downgraded to score 2, and 100.0% remained score 3 with PHH3. The upgrade rates were 40.0% from score 1 to score 2, 98.0% from score 2 to score 3, and 32.0% from score 1 to score 3. No downgrades occurred. The data suggests a strong tendency for higher mitotic scores with PHH3 compared to H&E, evidenced by substantial percentages of upgrades and the absence of downgrades. The highest upgrade rate from score 2 to score 3 (98%) suggests that PHH3 may be particularly effective in identifying higher mitotic activity underreported by H&E. **These results highlight the importance of PHH3 staining for potentially more accurate assessment of mitotic activity in breast cancer studies**.

Table 21: Distribution of cases based on mBR - H and E

mBR Grade-H&E		N	%
	1	32	31.4%
	2	62	60.8%
	3	8	7.8%

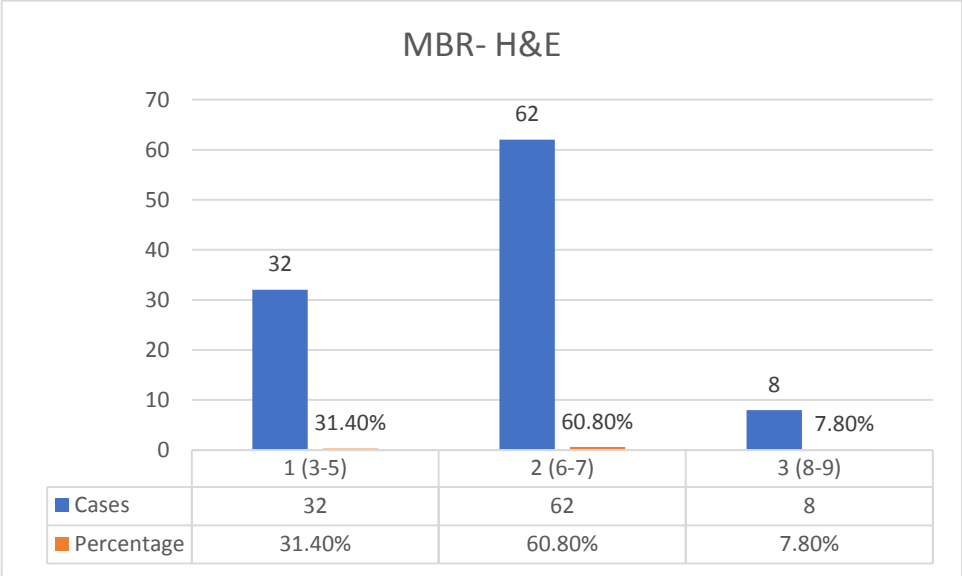

Chart 12: Distribution of cases based on mBR - H and E

Table 21 cand Chart 12 show the distribution of cases based on the mBR - H and E. For mBR grading the latest AJCC 8th edition was followed to count the mitotic counts. Out of 102 cases, 60.80% cases showed grade II followed by 31.40% showed mBR Grade 2 and only 7.80% cases showed grade 3

Table 22: Distribution of cases based on mBR – PHH3

mBR Grade-PHH3		N	%
	1	15	14.7%
	2	69	67.6%
	3	18	17.6%

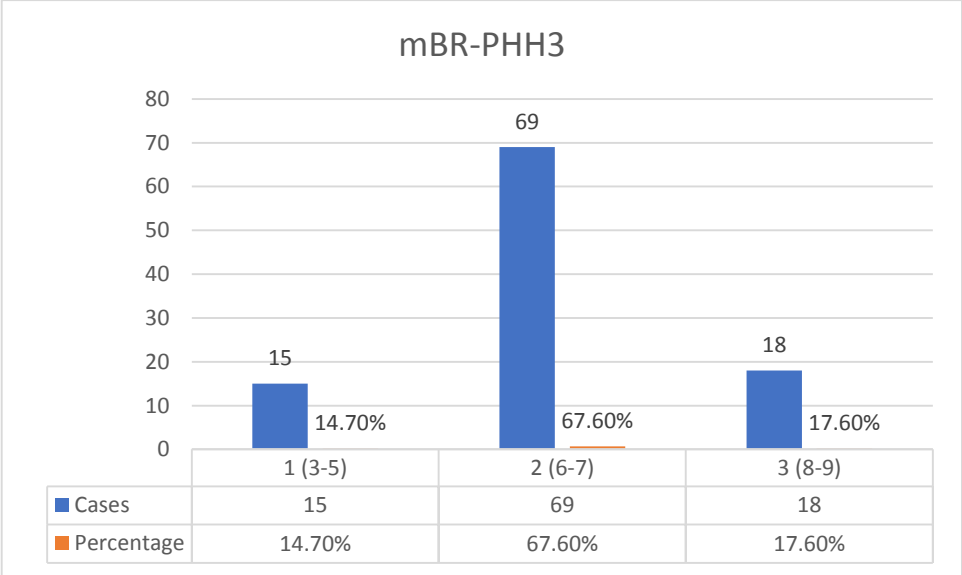

Chart 13: Distribution of cases based on mBR - H and E

Table 22 and Chart 13 show the distribution of cases based on the mBR - PHH3. For mBR grading the latest AJCC 8th edition was followed to count the mitotic counts. Out of 102 cases, 14.7% are Grade I, 67.6% are Grade II, and only 17.6% are Grade III

Comparison of mBR GRADE (PHH3) with mBR GRADE (MAI)

Table 23: Comparison of mBR GRADE (PHH3) with mBR GRADE (MAI)

mBR Grade H&E/ MAI	mBR Grade- PHH3					
	1		2		3	
	N	%	N	%	N	%
1	13	40.6%	18	56.3%	1	3.1%
2	2	3.2%	51	82.3%	9	14.5%
3	0	.0%	0	.0%	8	100.0%

Table 24-Upgrading and downgrading

Upgrade	**N**	**%**
Grade 1 to Grade 2	18	56.3
Grade 2 to Grade 3	9	14.5
Grade 1 to Grade 3	1	3.1
Downgrade		
Grade 2 to Grade 1	2	3.2

Comparing the mBR (Modified Bloom-Richardson) grade assessments between H&E and PHH3 staining methods in our study of 102 breast cancer cases reveals notable distinctions in grade distribution and transitions. H&E staining identified 13 cases (40.6%) as Grade 1, 2 cases (3.2%) as Grade 2, and none as Grade 3. In contrast, PHH3 staining showed higher proportions: 18 cases (56.3%) as Grade 1, 51 cases (82.3%) as Grade 2, and all 8 cases (100%) as Grade 3.

Out of 1-2 cases, the upgradation was seen in 28 cases and zero cases showed downgrading from PHH3 to MAI and so, PHH3 staining demonstrated more frequent upgrades compared to H&E staining. Specifically, Grade 1 to Grade 2 upgrades were observed in 56.3% of cases with PHH3, whereas H&E showed only 3.1% upgrading to Grade 3 from Grade 1. Grade 2 to Grade 3 transitions were also more pronounced with PHH3 (14.5%) compared to H&E. Downgrades were minimal in both methods, with PHH3 showing a small percentage (3.2%) from Grade 2 to Grade, this might be due to technical issues were 2 blocks/slides did not have adequate tumor tissue.

Table 25: Distribution of cases based on Lymph vascular invasion

LV invasion		N	%
	Absent	67	65.7%
	Present	35	34.3%

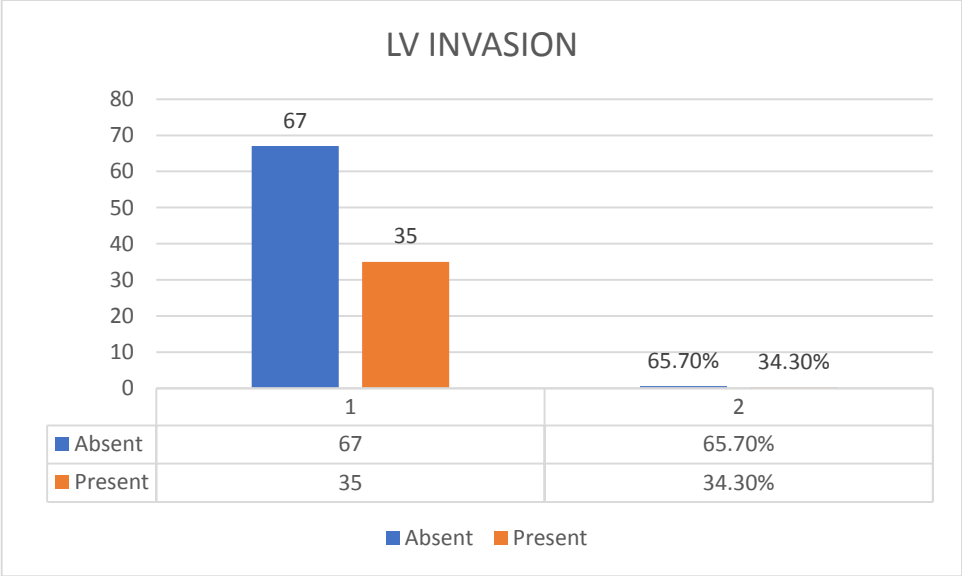

Chart 14: Distribution of cases based on Lymph vascular invasion

Table 25 and Chart 14 show the distribution of cases based on Lymph vascular invasion. In this study amongst 102 cases majority that is 65.70% of cases there was no lymph vascular invasion and in only 34.39% of cases there was lymph vascular invasion

Table 26: Distribution of cases based on NPI score

NPI		N	%
	Excellent	13	12.7%
	Good	26	25.5%
	Moderate	43	42.2%
	Poor	20	19.6%

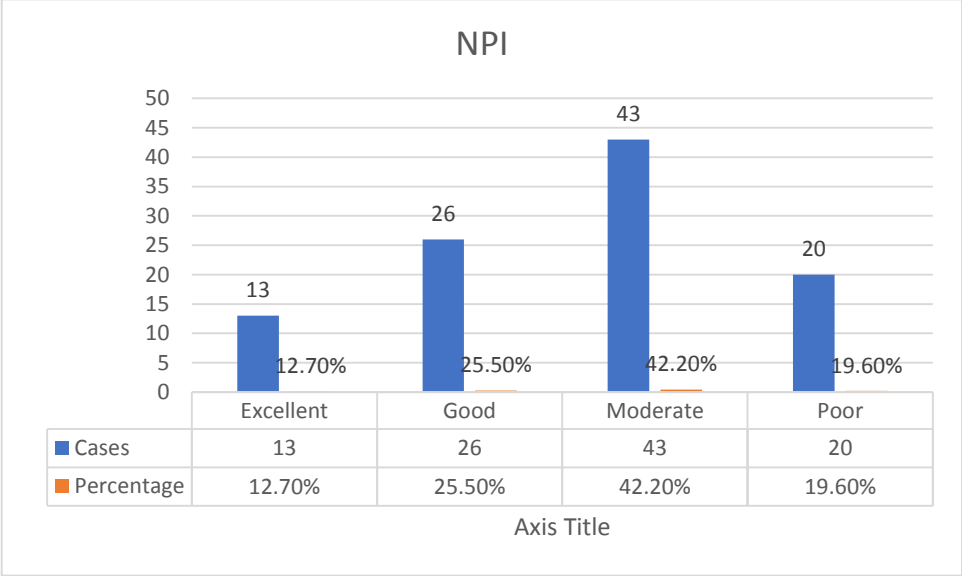

Chart 15: Distribution of cases based on NPI score

Table 26 and Chart 15 show the distribution of cases based on the NPI score . Majority of cases (42.2%)had moderate prognosis based on the NPI criteria followed by 25.5% cases showing good prognosis. 12.7% and 19.6% cases showed excellent and poor prognosis

Table 27: Distribution of cases based on MAI

		N	%
Stage	I	7	6.9%
	II	53	52.0%
	III	41	40.2%
	IV	1	1.0%

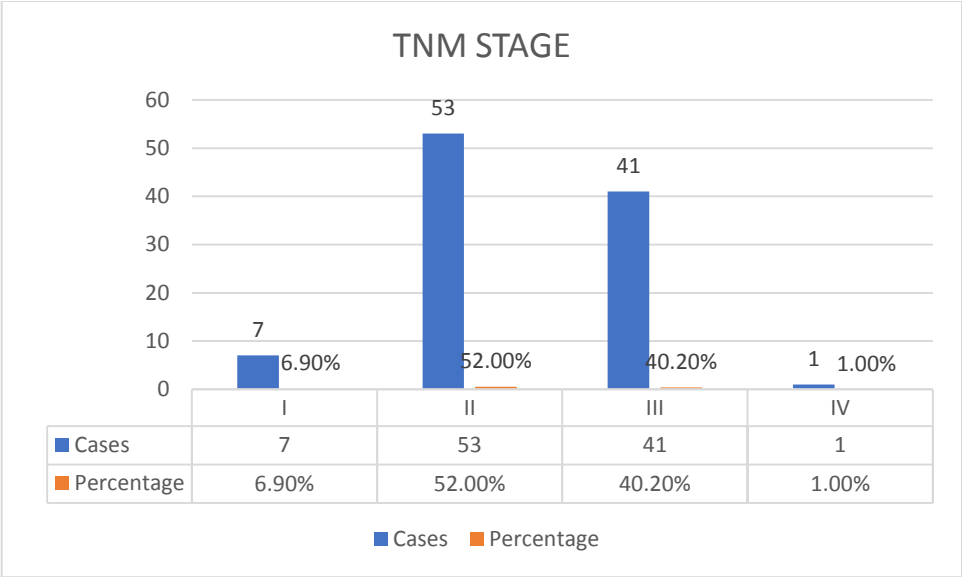

Chart 16: Distribution of cases based on TNM Stage

Table 27 and Chart 16 show the distribution of cases based on the TNM Stage. The TNM Staging was done according to the 8th AJCC criteria. Majority of the cases (52%) were in Stage 11 closely followed by Stage III. Stage I and Stage IV had 6.90% and 1% cases each.

Table 28: Distribution of cases based on Ki67

Ki67		N	%
	Low (<5)	15	14.7%
	Intermediate (6-29)	73	71.6%
	High (30)	14	13.7%

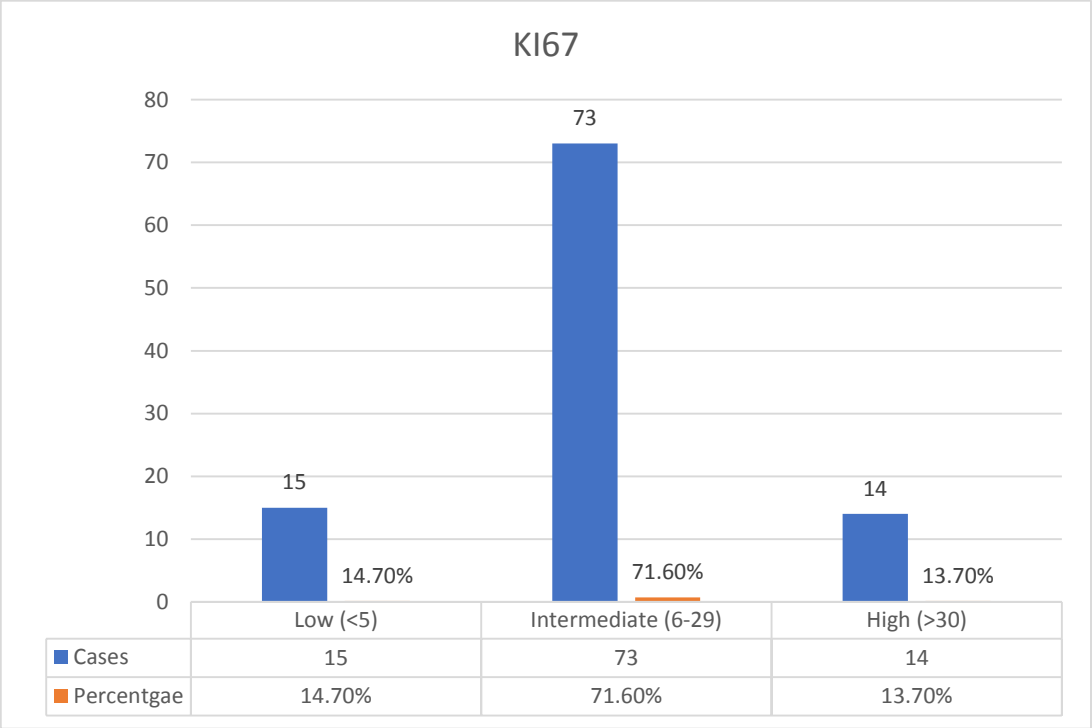

Chart 17: Distribution of cases based on Ki67 (IKWG)

Table 27 and Chart17 show the distribution of cases based on the Ki67. The classification of Ki67 as low(<5%), intermediate(6-29) and high(>30%) was done based on IKWG. In this study, the majority of cases that is 71.60% cases showed intermediate staining, followed by 14.70% showed low staining and 13.70% showed high staining

Table 29: Distribution of cases based on Ki67 (<14% and ≥14%)

Ki67		N	%
	<14%	36	35.3%
	≥14%	66	64.7%

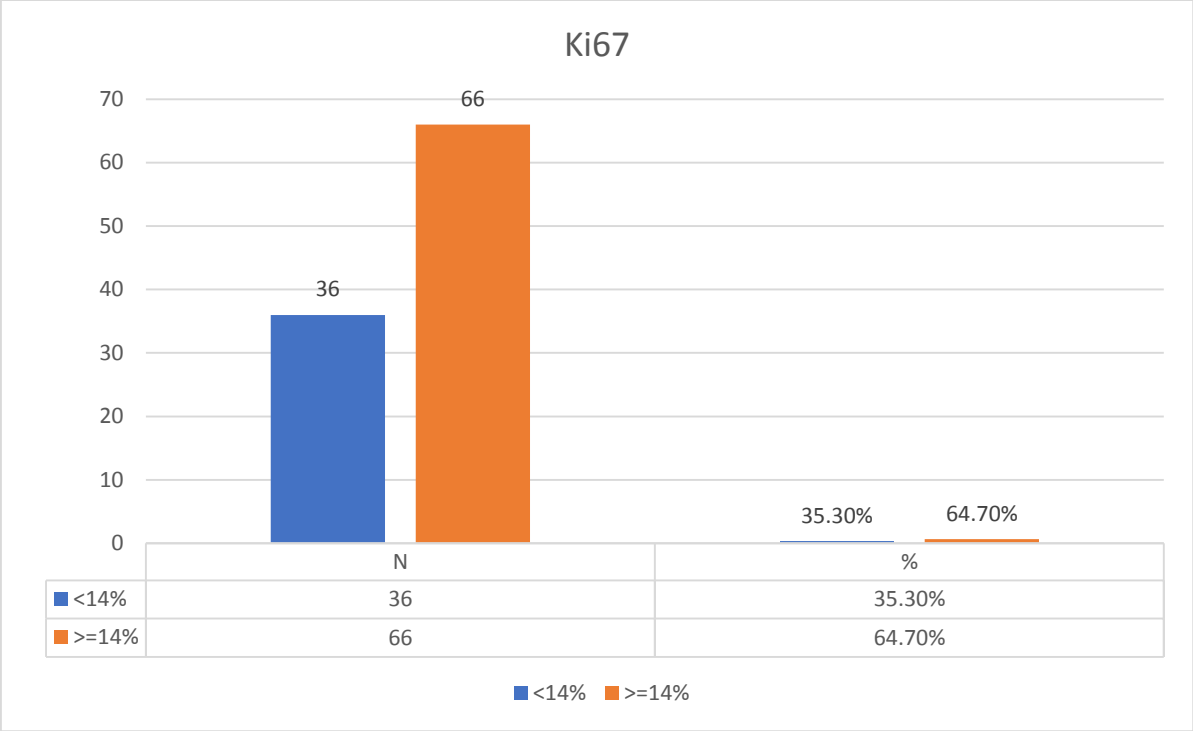

Chart 18: Distribution of cases based on Ki67 (<14% and ≥14%)

Table 29 and Chart 18 show the distribution of cases based on the Ki67. The classification of Ki67 was based on molecular subtyping as <14% and ≥14% and in this study, the majority of cases that is 64.70% showed Ki67 ≥14% and only 35.3% cases showed Ki67 <14%

TABLE 30 -Comparing Ki67 with lymph node score, stage, mBR-PHH3, and MBR-H&E, along with the corresponding *p*-values:

CATEGORY	Ki67 < 14%	Ki67 ≥ 14%	*p* value
Lymph Node Score			0.039
Node Negative	42.6%	57.4%	
Node-Positive	27.5%	72.5%	
Stage			0.162
Stage I	28.6%	71.4%	
Stage II	45.3%	54.7%	
Stage III	24.4%	75.6%	
Stage IV	0.0%	100.0%	

Table 30 shows that The analysis of Ki67 expression levels in relation to lymph node status and pTNM stage-

For lymph node status, Ki67 levels showed a statistically significant difference (p=0.039). Among node-negative cases, 42.6% had Ki67 levels <14%, while 57.4% had levels ≥14%. For node-positive cases, 27.5% had Ki67 levels <14%, and 72.5% had levels ≥14%, indicating higher proliferation rates in node-positive tumors.

Examining Ki67 levels across stages, no statistically significant differences were observed (p=0.162). Stage II had the highest percentage of cases with Ki67 <14% (45.3%) and ≥14% (54.7%). In Stage III, 24.4% had Ki67 <14%, and 75.6% had levels ≥14%, showing a trend towards higher proliferation in advanced stages.

Overall, the analysis highlights the significant association between Ki67 levels and lymph node status, reflecting higher proliferative activity in node-positive. However, no significant differences were found across different pTNM stages

Comparison of MAI with Ki67 Scores

Table 31: Comparison of MAI with Ki67 score

MAI	Ki67 SCORE					
	1		**2**		**3**	
	N	%	N	%	N	%
1	5	20.0%	7	28.0%	13	52.0%
2	8	15.7%	11	21.6%	32	62.7%
3	2	7.7%	3	11.5%	21	80.8%

Upgrade	**N**	**%**
score 1 to score 2	7	28
score 2 to score 3	32	62.7
score 1 to score 3	13	52
Downgrade		
score 2 to score 1	8	15.7
score 3 to score 2	2	7.7
score 3 to score 1	3	11.5

In comparing the Mitotic Activity Index (MAI) with Ki67, in our study it is evident that Ki67 provides superior sensitivity and accuracy in detecting proliferation rates across tumor grades. For MAI Score 1, Ki67 identifies 28.0% of cases as Ki67 Score 2 and 52.0% as Ki67 Score 3, indicating a substantial upgrade in proliferative activity. Similarly, for MAI Score 2, 62.7% of cases are identified as Ki67 Score 3. Furthermore, Ki67 demonstrates stability in its classifications, with minimal downgrades: 15.7% from MAI Score 2 to Ki67 Score 1 and 11.5% from MAI Score 3 to Ki67 Score 1. The higher detection sensitivity and clearer differentiation of Ki67, compared to MAI, underscore its superior capability in accurately and reliably assessing cellular proliferation, as evidenced by the data from 102 cases. This makes Ki67 a more precise biomarker for evaluating proliferative activity in breast cancer histopathology.

Comparison of Mitotic Count Scores (PHH3) with Ki67 Scores

Table 32: Mitotic Count Scores (PHH3) with Ki67 Scores**:**

Mitotic count score-PHH3	Ki67 SCORE					
	1		**2**		**3**	
	N	%	N	%	N	%
1	2	28.6%	1	14.3%	4	57.1%
2	1	9.1%	7	63.6%	3	27.3%
3	12	14.3%	13	15.5%	59	70.2%

Table 33 : Upgrade and Downgrade Rates Between Ki67 Scores and PHH3

Upgrade	**N**	**%**
Score 1 to Score 2	1	14.3
Score 2 to Score e 3	3	27.3
Score 1 to Score 3	4	57.1
Downgrade		
Score 2 to Score 1	1	9.1
Score 3 to Score 2	13	15.5
Score 3 to Score 1	12	14.3

The provided data compares Mitotic Count (MC) scores assessed using PHH3 staining with Ki67 scores across three Scores (1, 2, and 3), demonstrating that PHH3 is a more reliable marker for assessing mitotic activity. For cases scored as 1 by PHH3, 28.6% corresponded to Ki67 score 1, 14.3% to score 2, and 57.1% to score 3. For cases scored as 2 by PHH3, 9.1% corresponded to Ki67 score 1, 63.6% to score 2, and 27.3% to score 3. For cases scored as 3 by PHH3, 14.3% corresponded to Ki67 score 1, 15.5% to score 2, and 70.2% to score 3. The upgrade rates from Ki67 scores to PHH3 scores were 14.3% from score 1 to score 2, 27.3% from score 2 to score 3, and 57.1% from score 1 to score

3. Downgrade rates were 9.1% from score 2 to score 1, 15.5% from score 3 to score 2, and 14.3% from score 3 to score 1. These results suggest that PHH3 is more sensitive and specific in detecting higher mitotic activity, with fewer discrepancies compared to Ki67. While Ki67 scores sometimes show high proliferative activity in cases with low PHH3 mitotic counts, PHH3 consistently identifies higher mitotic activity, highlighting its superiority as a primary marker in assessing tumor biology.

When comparing Ki67 with PHH3, PHH3 proves to be a more accurate and reliable marker for assessing proliferation rates. PHH3 scores provide a clearer distinction in proliferation levels, with higher PHH3 scores aligning consistently with higher Ki67 scores, indicating a stronger relationship between mitotic activity and proliferation rates. The upgrade and downgrade patterns observed with PHH3 are more distinct, with upgrades being more pronounced and downgrades less frequent, suggesting that PHH3 is better at tracking the progression and regression of proliferation rates. Additionally, PHH3 scores provide a clearer differentiation at intermediate levels, showing a more accurate depiction of moderate proliferation compared to Ki67 alone. In conclusion, while both Ki67 and PHH3 are valuable markers for assessing proliferation rates, PHH3 offers a more precise and reliable measurement, making it a superior marker compared to Ki67

TABLE 34 -Comparison of mitotic count score PHH3 with lymph node score and pTNM

		Mitotic count score PHH3			*p* VALUE
		1	**2**	**3**	
Lymph node status	**Node negative**	7.8%	15.7%	76.5%	0.241
	Node positive	5.9%	5.9%	88.2%	
Stage	**I**	.0%	.0%	100%	0.180
	II	11.3%	17.0%	71.7%	
	III	2.4%	4.9%	92.7%	
	IV	.0%	.0%	100%	

Table 34 shows the distribution of mitotic count scores using PHH3 staining in relation to lymph node status and cancer stage, along with their associated p-values. For lymph node status, node-negative cases show a distribution of 7.8% with a mitotic count score of 1, 15.7% with a score of 2, and 76.5% with a score of 3, while node-positive cases have 5.9% with a score of 1, 5.9% with a score of 2, and 88.2% with a score of 3, with a *p*-value of 0.241 indicating no significant difference. Regarding pTNM stage, stage I and IV cases have 100% of cases with a mitotic count score of 3, stage II cases show 11.3% with a score of 1, 17.0% with a score of 2, and 71.7% with a score of 3, while stage III cases have 2.4% with a score of 1, 4.9% with a score of 2, and 92.7% with a score of 3. The *p*-value of 0.180 suggests no statistically significant difference in mitotic count scores across different stages.

Table 35: Distribution of cases based on ER Status

ER		N	%
	Negative	52	51.0%
	Positive	50	49.0%

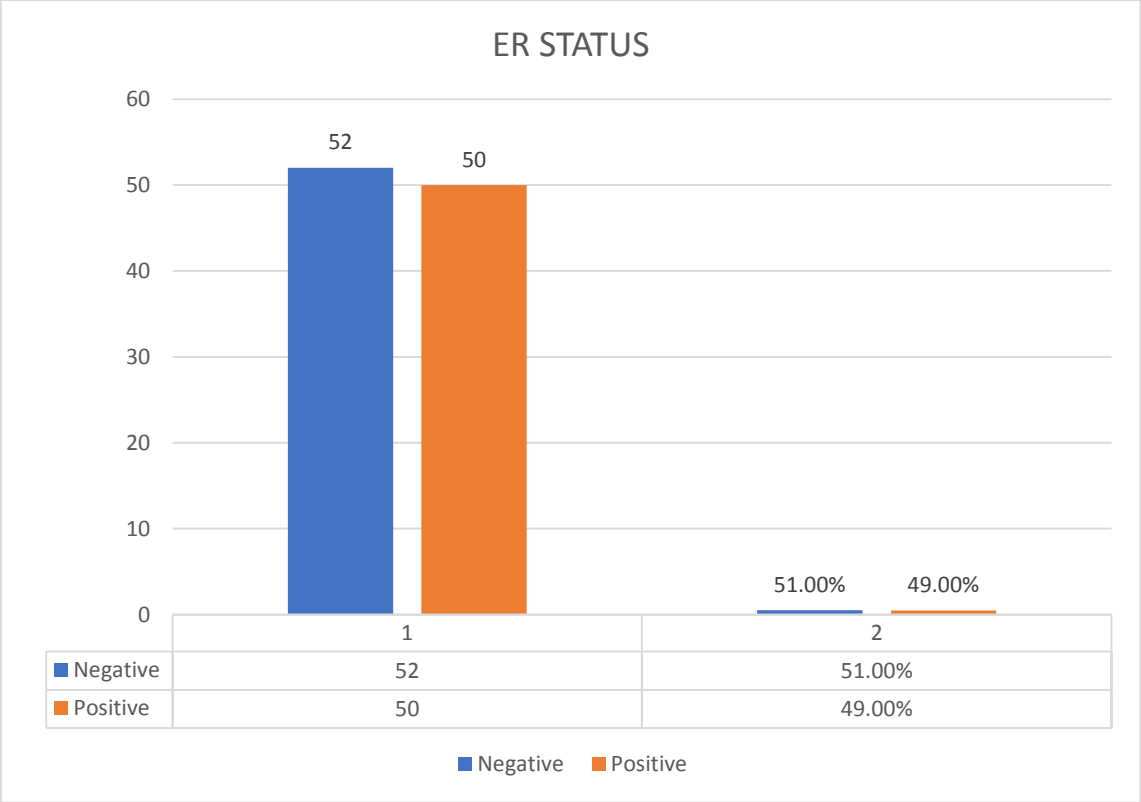

Chart 19 : Distribution of cases based on ER Status

Table 35 and Chart 19 show the distribution of cases based on the ER Status. Out of 102 cases, 51% cases were ER-negative and 49% cases were ER-positive

Table 36: Distribution of cases based on PR Status

PR		N	%
	Negative	63	61.8%
	Positive	39	38.2%

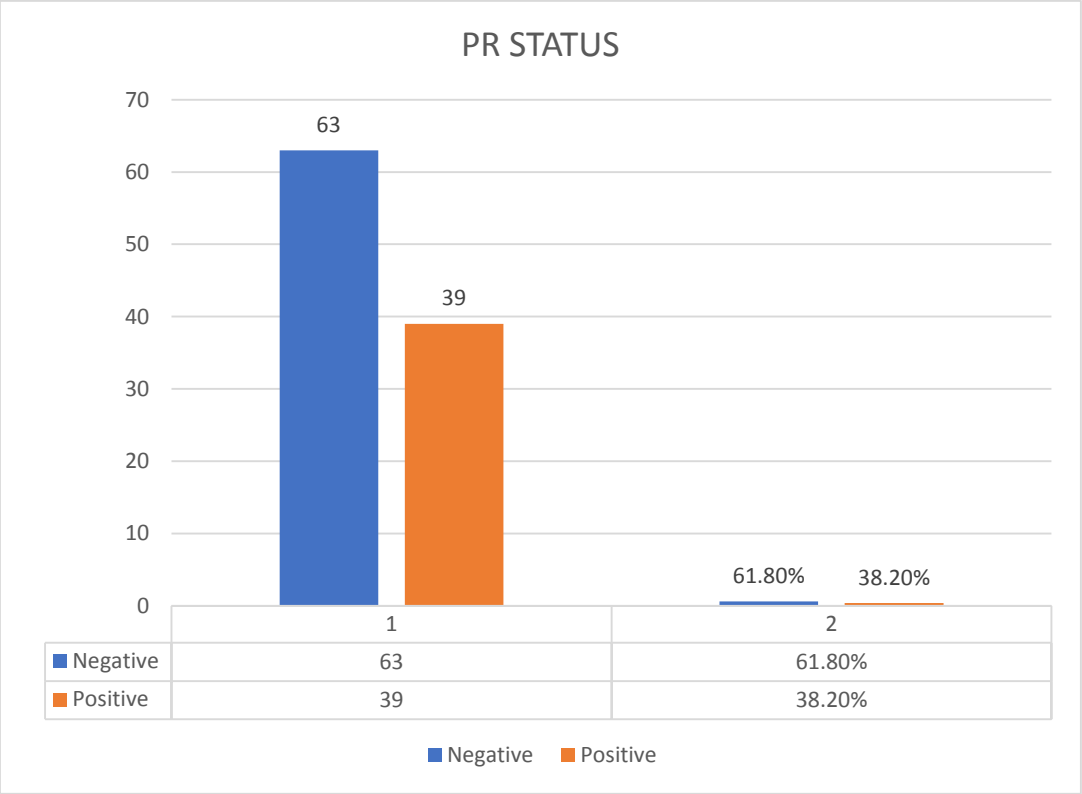

Chart 20: Distribution of cases based on PR Status

Table 36 and Chart 20 show the distribution of cases based on the PR Status. Out of 102 cases, 61.80% cases were PR-negative and 38.20% cases were PR-positive

Table 37: Distribution of cases based on Her2Neu Status

Her2Neu		N	%
	Negative	57	55.9%
	Positive	45	44.1%

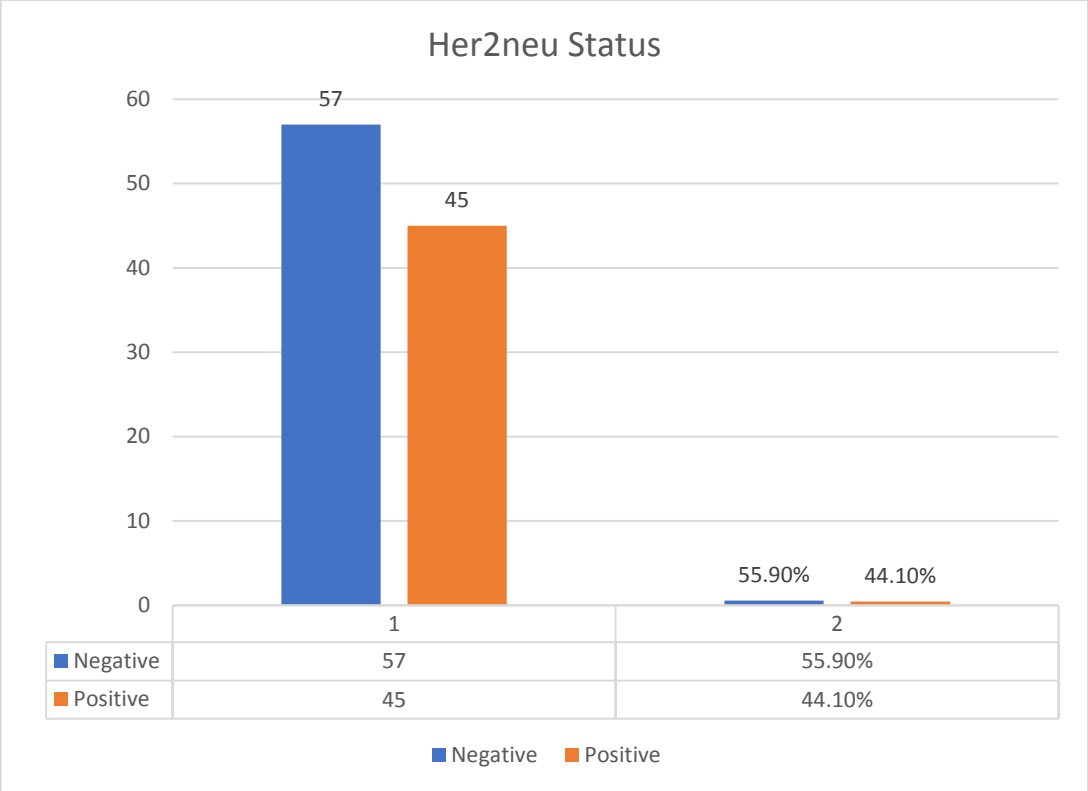

Chart 21: Distribution of cases based on Her2Neu Status

Table 37 and Chart 21 show the distribution of cases based on the Her2Neu Status. Out of 102 cases, 55.90% cases were Her2Neu Status negative and 44.10% cases were Her2Neu Status positive

GROSS FEATURES:

Macroscopic features vary among different cases. The size of the tumor may range widely from 1 cm to 10 cms. The contours may be regular/irregular/nodular/showing stellate configuration. Sharp demarcation between tumor borders and surrounding stroma may not usually be seen. These tumors will be firm to hard in consistency on palpation. Sometimes there can be gritty feel while cutting with a knife. Cut surface is grey white in color.

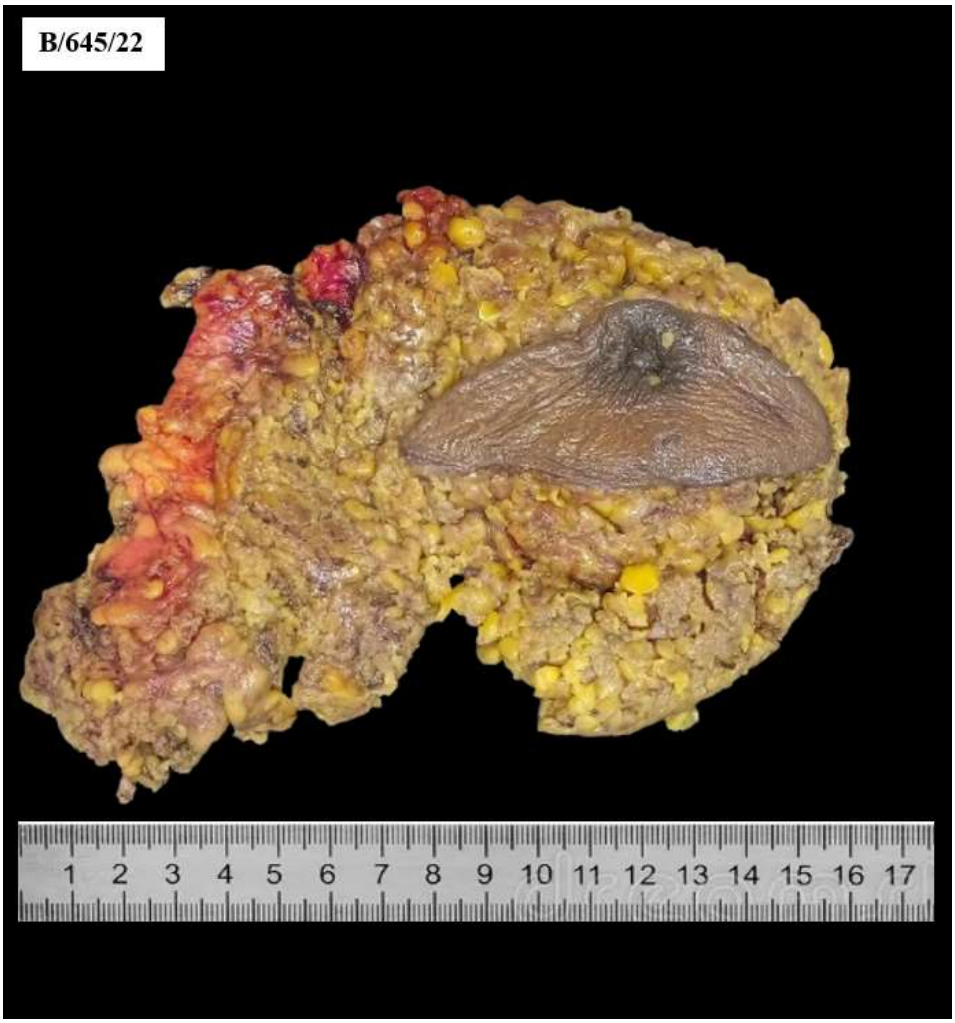

Figure 9: Photograph showing gross image of Modified radical mastectomy specimen with overlying ellipse of skin

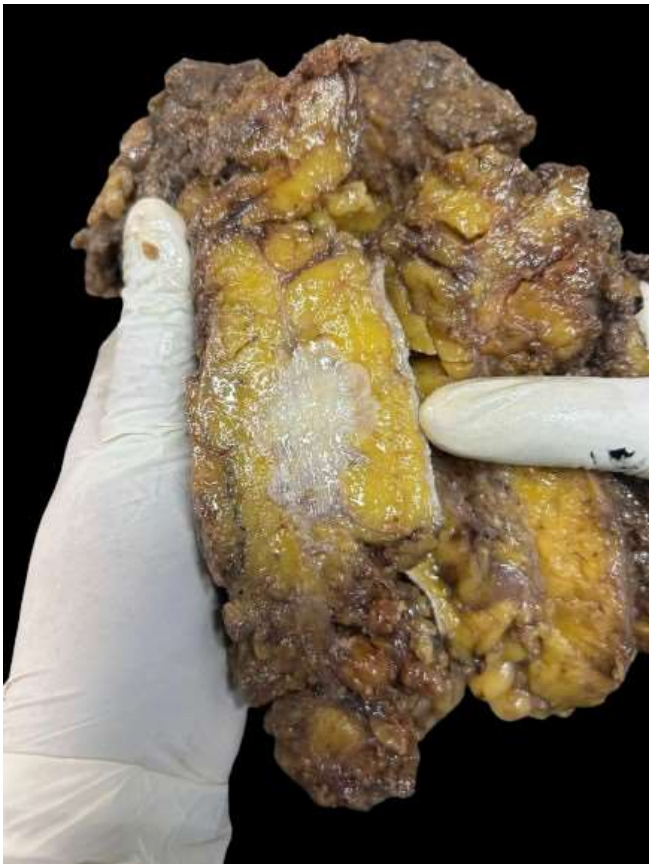

Figure 10: Photograph showing cut surface of gross specimen showing grey white tumor

MICROSCOPIC

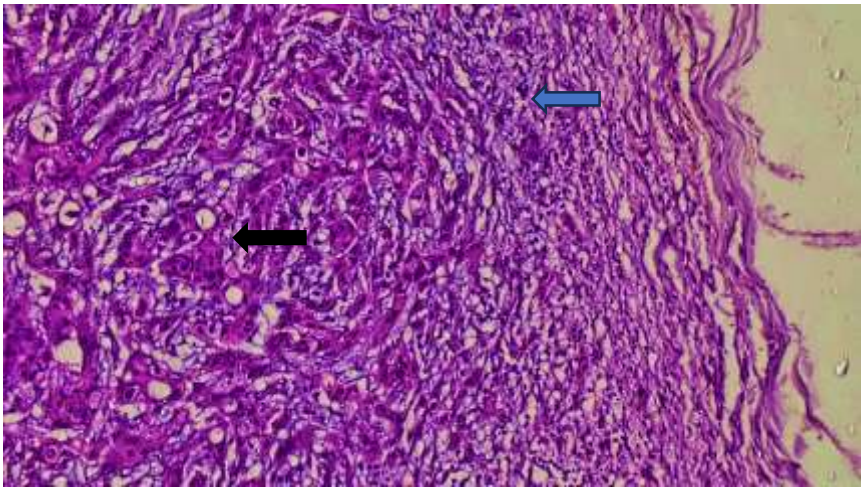

Figure 11: Microphotograph showing lymph node metastasis in IDC breast

Blue arrow- Showing the lymph node capsule and black arrow showing the tumor metastsis into the lymph node

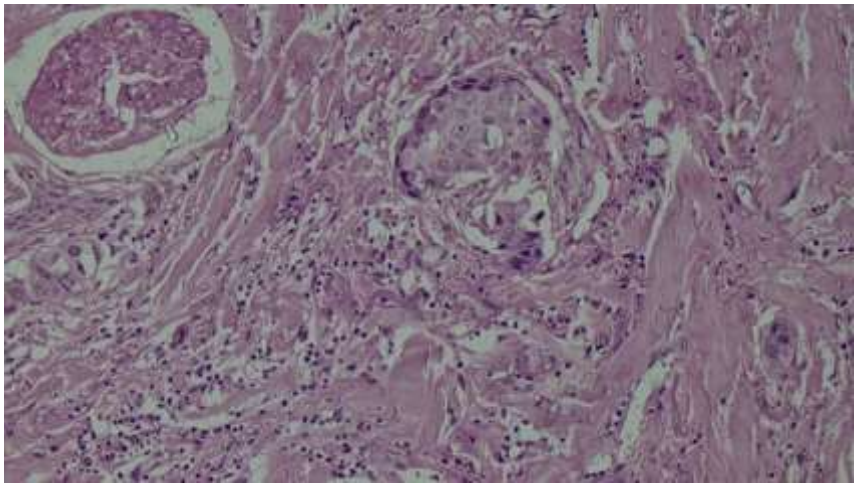

Figure 12: Microphotograph showing IDC breast with low TIILs (<10%), stain used is H&E (20x magnification)

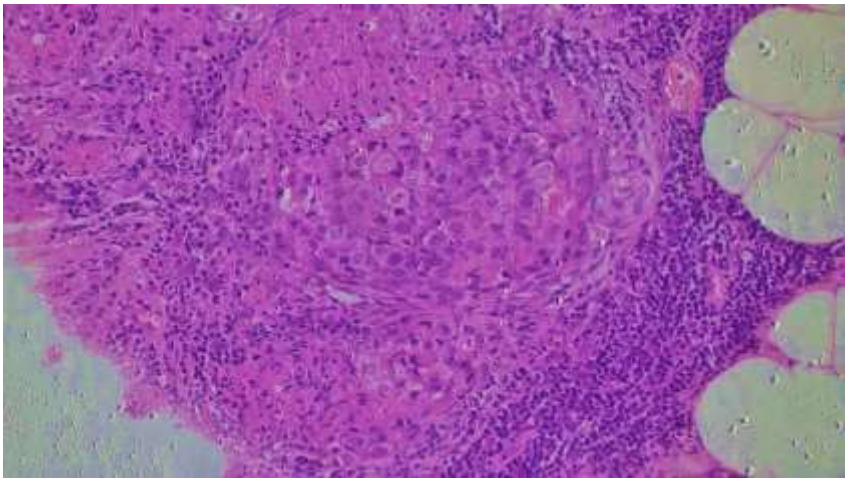

Figure 13: Microphotograph showing IDC breast with intermediate TIILs (10%-40%) (20x magnification)

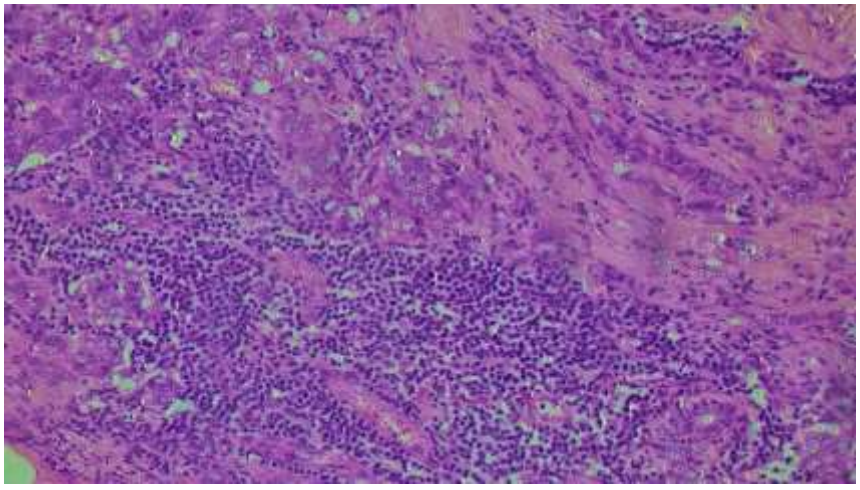

Figure 14: Microphotograph showing IDC breast with high TIILs (>40%) (20x magnification)

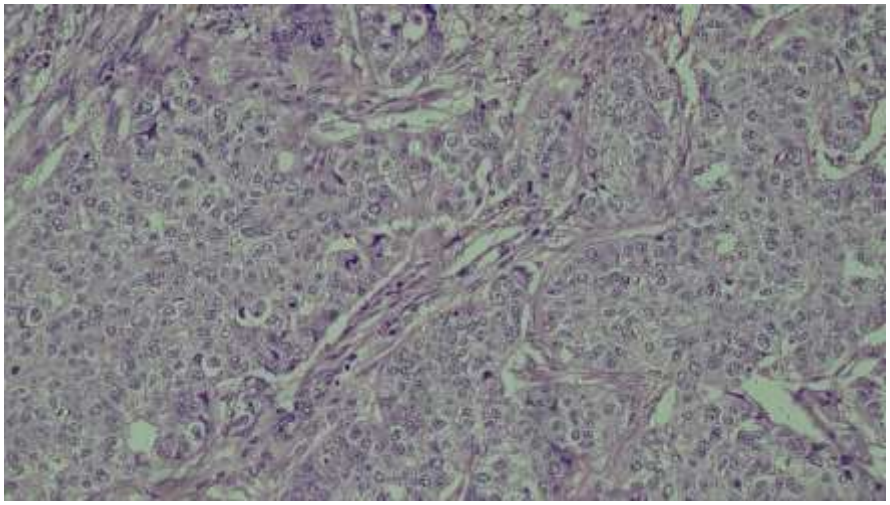

Figure 15: Microphotograph Showing IDC breast with Low T:S Ratio (≤50%) under 20x magnification

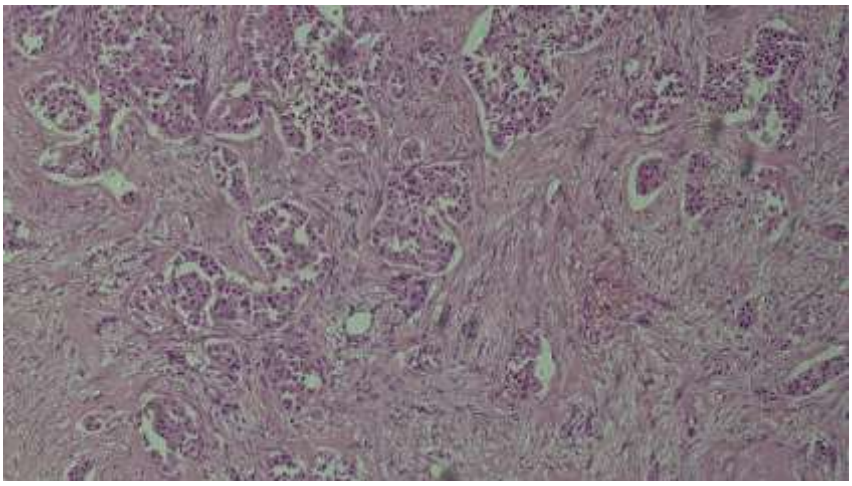

Figure 16: Microphotograph Showing IDC breast with Low T:S Ratio (>50%) under 10x magnification

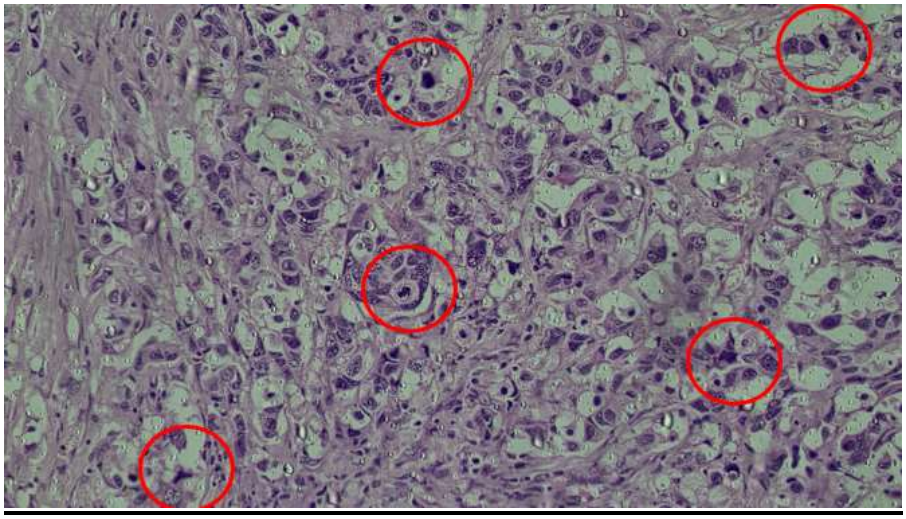

Figure 17: Microphotograph showing Mitotic figures stained with H and E stain (40x magnifiction)

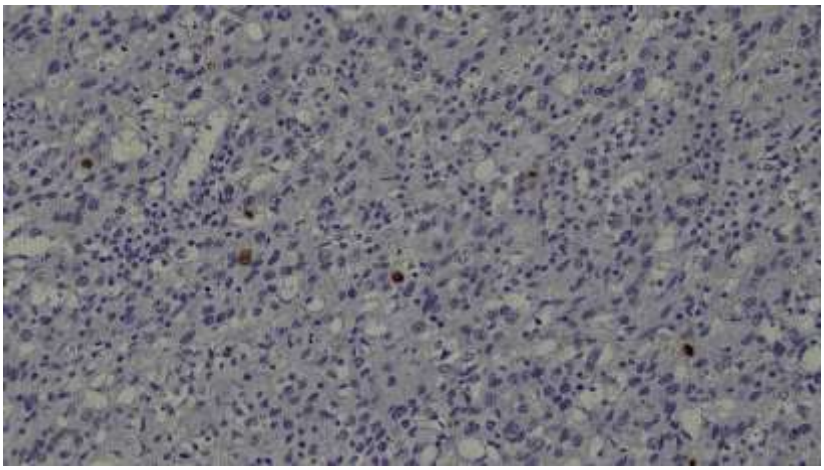

Figure 18: Microphotograph of IDC breast showingmitotic figures stained by PHH3 - Score 1 (≤7 mitotic figures) under 40x magnification

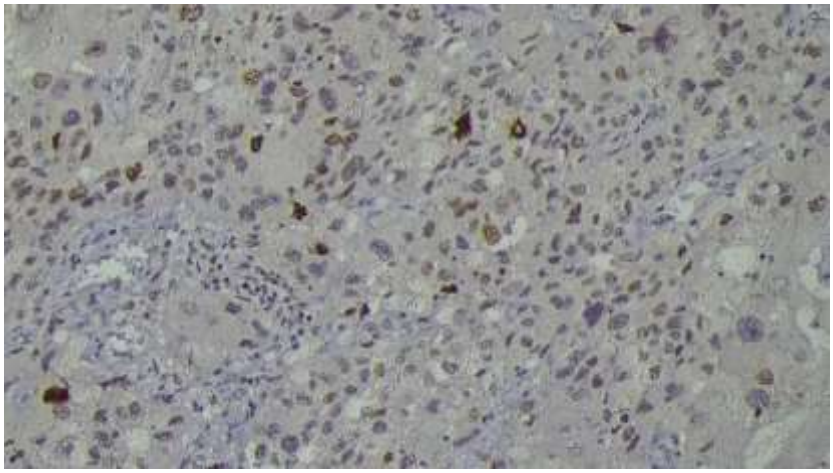

Figure 19: Microphotograph of IDC breast showing mitotic figures stained by PHH3 - Score 2 (8-14 mitotic figures) under 40x magnification

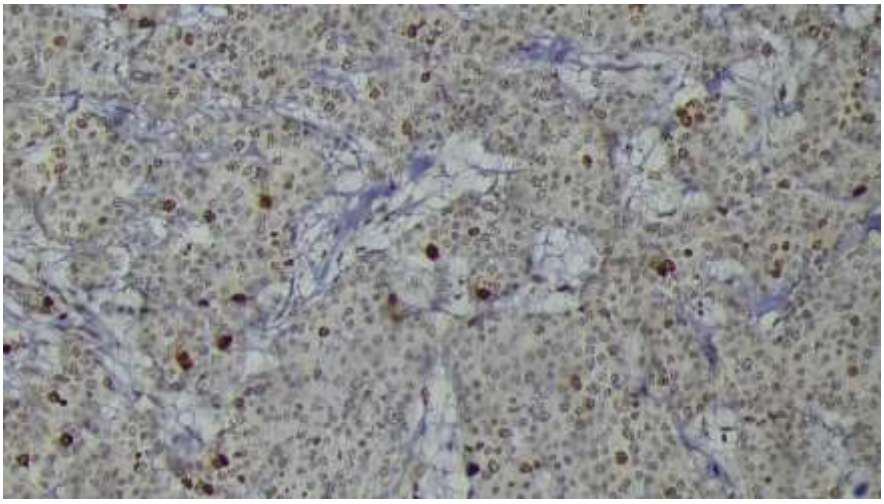

Figure 20: Microphotograph of IDC breast showing mitotic figures stained by PHH3 - Score 3 (≥15 mitotic figures) under 40x

Figure 21: Microphotograph showing Ki67 expression of <5% in IDC breast (4x magnification)

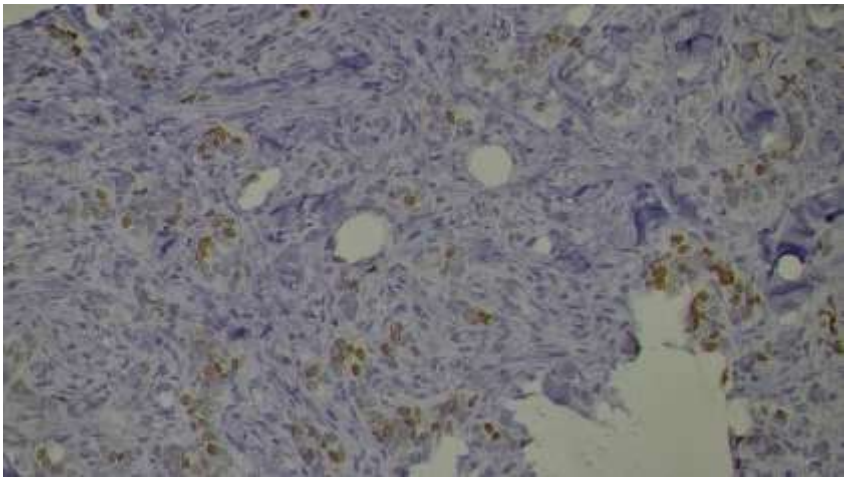

Figure 22: Microphotograph showing Ki67 expression of <6-29% in IDC breast (20x magnification)

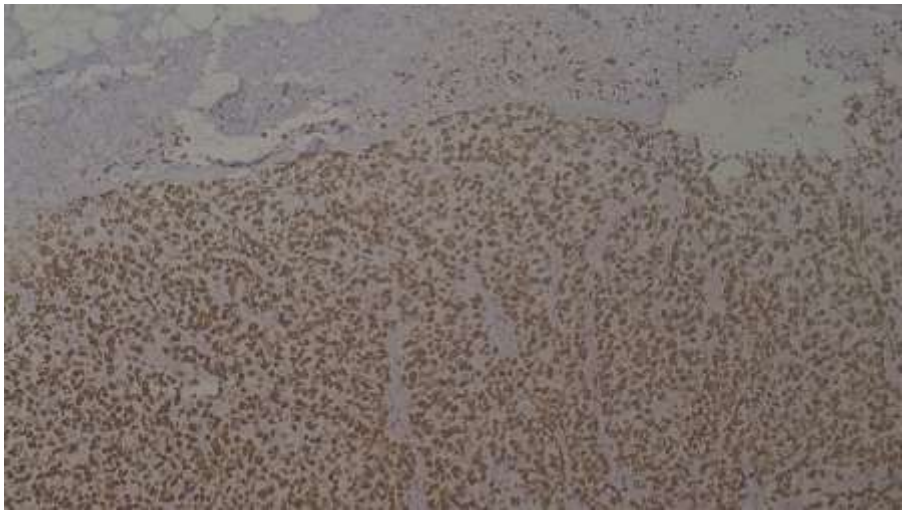

Figure 23 : Microphotograph showing Ki67 expression of >30% in IDC breast (20x magnification)

DISCUSSION

DISCUSSION

Breast cancer is the leading cause of cancer in women around the world, accounting for one-quarter of all female cancers. Breast cancer deaths in the Southeast Asia region are expected to increase to 61.7% by 2040.[104] Breast cancer is the most common cancer in India, accounting for 28.2% of all female cancers, with an estimated 216,108 cases by 2022.[105] The age□standardized incidence rate of female breast cancer has increased by 39.1% from 1990 to 2016, and this trend has been seen in every state of India over the past 26 years.[4] Population□based cancer survival is a key indicator for assessing the effectiveness of cancer control by a health care system in a specific geographic area. It acts as one of the surrogate indicators of the health system's efficiency for screening, early detection, and management of cases. It can be used as evidence by policymakers and stakeholders to monitor, validate, and scale up the current health system.[106]

There are multiple demographic, social, and biomedical risk factors of breast cancer. Age of the women, early age at menarche, delayed first birth and menopause, nulliparity, short duration lactation, use of birth control pills, obesity, excess consumption of fats, hormone replacements and more importantly women having family history are considered as significant risk factors of breast cancer by various epidemiological and clinical studies.[107–109] One of the meta-analysis by Vishwakarma et al[107] carried on 24 observational studies stated that highest odds ratio (OR) obtained for risk of breast cancer was among those who never had breastfeeding (pooled OR 3.69, 95% Confidence Interval = 1.70–8.01), never married women (pooled OR = 2.29, 95% CI = 1.65–3.17), and nulliparous women (pooled OR = 1.58, 95% CI = 1.21–2.06)[107]. One of the studies in South India found a higher risk of breast cancer in urban areas than in rural areas[108]. This study also reported that the odds of breast cancer among urban women increased with an increase in the proportion of overweight or obese (BMI-body mass Index > 25), size of the waist (> 85 cm), and size of the hip (> 100 cm) among both pre-menopausal and post-menopausal women.

Another study in rural Maharashtra found that most of the breast cancer cases were confined to women aged 40–49 years, homemakers, and upper economic strata groups. Further, this study found breast cancer risk was 8 times higher among unmarried women, 3 times more among nulliparous women, 2 times more likely among post-menopausal women, 10 times more among those who had never breastfed, 1.5 times higher among women who were exposed to hormonal contraceptives and 4.5 time more likely among women with a history of ovarian diseases than in comparison to married, non-nulliparous, premenopausal, women who ever breastfed, who have not been exposed to hormonal

contraceptives, and women without any ovarian diseases respectively.[109] Some studies found differences in exposure to different types of environmental pollutants as a risk factor to breast cancer.[110]

Several studies focused on different preventive and curative interventions which were carried both internationally and in India. Although breast cancer prevention remains a baffling task due to involvement of multiple cell types at multiple stages, most intervention literature on breast cancer suggests that modifiable risk factors may be prevented through the promotion of a healthy diet, regular physical activities, regulating alcohol consumption and controlling weight which is likely to reduce the incidence of breast cancer in longer time period.[111–117]

Early diagnosis of breast cancer is an important factor for the reduction of the mortality rate because its treatment plan is advised on the basis of the grade and prognosis of the cancer. To determine the grade of breast cancer, the Modified Bloom Richardson grading system has been widely used. According to this system, there are three biomarkers for the grading of breast cancer in histopathology images. These biomarkers are nuclear atypia, tubule formation, and the mitotic cell count. Among these biomarkers, the mitotic cell count is the most important biomarker as the mitosis cell division process is directly related to the prognosis of tumor[118,119]

The mitotic score is a key component of breast cancer (BC) grading and is a strong predictor of survival, reflecting the underlying biological behavior of the disease, but because of the poor reproducibility of mitotic count is mainly attributed to the challenges in detecting mitotically active regions in hematoxylin and eosin (H&E)-stained slides or the presence of mitotic mimickers such as hyperchromatic nuclei, karyorrhectic or apoptotic cells, even cells in prophase are usually not considered during routine scoring of mitotic figures. Additionally, the heterogeneity of mitotic activity in different regions, and cell density variations, might all be aggravating factors. Such factors can be avoided by use of better staining methods for mitotic counting like PHH3[28]

Proliferative index is a prognostic feature of invasive ductal carcinoma of the breast, and has more recently emerged as a predictor of IDC breast, used in combination with other predictive markers . Ki67 is the most commonly used immunohistochemical marker of proliferative index. However, high interobserver and interlaboratory variability has been reported, in part due to differences in staining

methodologies, positivity thresholds, and approaches to quantification. Phosphohistone-H3 (pHH3) is a marker of mitotic activity that has emerged as a more reliable indicator of proliferation in other neoplasms.[120–122]

Histone H3 is one of the five histone proteins that together form the major protein constituents of chromatin in eukaryotic cells.9,10 Antibodies directed against phosphorylated histone H3 (PHH3) are almost exclusively expressed in actively proliferating cells during the M phase and late G2 phase,11 and are not observed during apoptosis.12 The utility of PHH3 has been evaluated in various tumors, including melanoma, neuroendocrine tumors,2,17 colorectal and ovarian carcinomas, sarcomas, and central nervous system tumors, and revealed a correlation with outcome[54]

This study was done to assess the expression and association of PHH3, Ki67, and MAI in infiltrating ductal carcinoma breast with various parameters

Table 38 - Comparison of different studies based on Age

STUDIES	YEAR	TOTAL CASE	AGE						
			20-29	30-39	40-49	50-59	60-69	70-79	80-89
Shrisvasta et al[123]	2023	80	3	5	14	27	21	10	0
Manoharan et al[115]	2017	2744	66	338	704	750	542	182	162
Present study	2024	102	2	21	21	33	14	8	3

Table 38 shows - Shrisvasta et al had a total of 80 cases, with a relatively even but small number of cases in each age group. The majority of cases were in the 50-59 age group (27 cases), followed by the 60-69 age group (21 cases). Notably, there were no cases in the 80-89 age group. This study suggests that the incidence of the condition studied peaks in the 50-59 age range and then declines.

Manoharan et al, with a significantly larger sample size of 2744 cases, shows a different pattern. The largest number of cases is in the 50-59 age group (750 cases), followed closely by the 40-49 age group

(704 cases). There is a substantial number of cases in the 60-69 age group (542 cases) as well. The data indicate a high incidence in middle-aged and older adults, with a notable number of cases even in the 80-89 age group (162 cases). This extensive dataset provides a comprehensive view of age distribution, emphasizing the prevalence among middle-aged and elderly populations.

Our study, with 102 cases, shows a more uniform distribution across age groups compared to Shrisvasta et al. The highest number of cases is in the 50-59 age group (33 cases), followed by equal numbers in the 30-39 and 40-49 age groups (21 cases each). The 60-69 age group has 14 cases, and there are a few cases in the 70-79 (8 cases) and 80-89 (3 cases) age groups. This study's data suggest a significant incidence in the middle-aged group, particularly in the 50-59 range, similar to the patterns observed in the other studies but with a more balanced distribution in younger age groups.

Table 39 - Comparison of median age, mean and range of age in different studies

STUDIES	YEAR	TOTAL CASES	MEAN AGE	MEDIAN AGE	AGE RANGE
Kim et al[28]	2016	218	53.8	54	26-83
Steenhoven et al[95]	2020	159	51.5	57	33-70
Present study	**2024**	**102**	**51.45**	**52 years**	**28-82**

In comparing the three studies, there are variations in the total number of cases, mean age, median age, and age range. Kim et al had the highest total cases at 218, with a mean age of 53.8 and a median age of 54, ranging from 26 to 83. Steenhoven et al had 159 total cases, a mean age of 51.5, a median age of 57, and an age range of 33 to 70. The present study had 102 total cases, a mean age of 51.45, a median age of 52 years, and an age range of 28 to 82. Each study presents different demographic characteristics based on these factors. Our study showed a similar age distribution with a slightly younger median age compared to Kim et al and Steenhoven et al. The present study’s younger median age compared to Kim et al. and Steenhoven et al. suggests potential differences in disease onset, risk factors, and possibly even genetic predispositions.

Table 40 – Comparision of different studies based on Laterality of tumor

STUIDES	YEAR	TOTAL CASES	LEFT	RIGHT
Jitendra Singh Nigam[124]	2014	328	167 (50.9%)	161 (49.08%)
Ghosh et al[125]	2014	320	164 (51.25)	156 (48.75)
Gogia et al[126]	2018	550	280 (50.9)	270 (49.1)
Present study	102	102	55 (53.92%)	47 (46.08%)

The studies by Nigam et al, Ghosh et al, Gogia et al, and the present study reveal a consistent pattern in the distribution of tumor laterality.

Nigam et al's study, with 328 cases, found that 50.9% of tumors were left-sided (167 cases) and 49.08% were right-sided (161 cases), showing a nearly equal distribution. Similarly, Ghosh et al's study of 320 cases reported 51.25% left-sided tumors (164 cases) and 48.75% right-sided tumors (156 cases), again indicating a slight left-side predominance. Gogia et al, with a larger sample size of 550 cases, observed 50.9% left-sided tumors (280 cases) and 49.1% right-sided tumors (270 cases), which closely mirrors the findings of Nigam et al. Our study, consisting of 102 cases, showed 53.92% left-sided tumors (55 cases) and 46.08% right-sided tumors (47 cases), marking the highest percentage of left-sided tumors among the four studies but still maintaining a relatively balanced distribution. these studies demonstrate a consistent, though slight, left-sided predominance in tumor laterality, with approximately 50-51% of cases being left-sided and 48-49% being right-sided across all studies. This minor asymmetry may indicate a marginally higher tendency for tumors to develop on the left side, but the differences are minimal and unlikely to be clinically significant in isolation.

Table 41 - Comparison of different studies with respect to tumor size

STUDIES	YEAR	TOTAL CASES	≤5CM	>5CM
Srivastava et al[123]	2023	80	72.6%	27.4%
Ghosh et al[125]	2014	320	73.8%	26.2%
Present study	**2024**	**102**	**62.7%**	**37.25%**

The comparison of tumor size distribution across three studies reveals variations in the proportion of cases with tumors smaller and larger than 5 cm. Srivastava et al. (2023) reported that 72.6% of their 80 cases had tumors smaller than 5 cm, while 27.4% were larger. Similarly, Ghosh et al. (2014) found that 73.8% of their 320 cases had tumors under 5 cm, with 26.2% exceeding this size. In contrast, the present study from 2024 shows a lower percentage (62.7%) of cases with tumors smaller than 5 cm and a higher percentage (37.25%) with tumors larger than 5 cm among the 102 cases. This discrepancy may be attributed to differences in study populations, tumor biology, diagnostic criteria, or healthcare access and intervention timing, suggesting that the present study might be encountering more advanced-stage cases compared to the earlier studies.

Table 42- Comparison of different studies based pn distribution of TILS

STUDIES	YEAR	TOTAL CASES	<10 TILS	10-40 TILS	>40 TILS
Sayed et al.[127]	2021	226	60%	30%	10%
Agarwal et al.[128]	2023	229	40%	45%	14%
Present study	**2024**	**102**	**65.69%**	**26.47%**	**7.84%**

Table 42 shows he present study (2024) reported the highest percentage of cases with less than 10% TILs (65.69%), which is notably higher than the 60% observed in Sayed et al. (2021) and the 40% in Agarwal et al. (2023). This suggests a generally lower immune cell infiltration within the tumor microenvironment in the present study.

Conversely, the percentage of cases with 10-40% TILs in the present study (26.47%) is lower than both Sayed et al. (30%) and Agarwal et al. (45%). The same trend is observed in the >40% TILs

category, where the present study reports only 7.84% of cases, compared to 10% in Sayed et al. and 14% in Agarwal et al. These differences may be attributed to variations in patient demographics, tumor biology, or the methodologies used for TIL assessment across the studies. For instance, the higher proportion of cases with low TILs in the present study could reflect differences in the local population's immune response

Table 43- Comparison of different studies based on T:S ratio

STUDIES	YEAR	CASES	T:S ratio ≤50%	T:S ratio >50%
Chasma et al.[129]	2019	134	66.4%	
Karancsi et al.[130]	2023	178	29.78%	70.22%
Present study	**2024**	**102**	**30.39%**	**69.61%**

Table 43 shows that the study by Chasma et al. reported a significantly higher proportion of cases with T:S ratio ≤50% (66.4%) compared to our study's findings of 30.39%. On the other hand, Karancs et al. observed a distribution more closely aligned with our findings, with 70.22% of cases having T:S ratio >50% and 29.78% ≤50%, reflecting a pattern more akin to our study's results (69.61% >50%, 30.39% ≤50%). These similarities hint at potential consistency in findings across different cohorts or methodologies, though slight variations could still arise due to factors such as sample size differences, geographical variations clinical interpretations effectively.

Table 44- Comparison of the different studies with respect to Nodal status

STUDIES	YEAR	TOTAL CASES	NEGATIVE NODAL STATUS	POSITIVE NODAL STATUS
Reza et al[131]	2024	1832	38.0%	62.0%
Kim et al[28]	2016	218	76.6%	23.4%
Steenhoven et al[95]	2020	159	83%	13%
Present study	**2024**	**102**	**46.07%**	**53.92%**

Reza et al reported that out of 1832 cases, 38.0% had negative nodal status, while 62.0% had positive nodal status, indicating a higher proportion of cases with lymph node involvement. In Kim et al's study

with 218 cases, 76.6% had negative nodal status and 23.4% had positive nodal status, suggesting that most cases had no lymph node involvement. Steenhoven et al, with 159 cases, found 83% with negative nodal status and 13% with positive nodal status, indicating an even higher proportion of cases without lymph node involvement. The present study, out of 102 cases, reported 46.07% with negative nodal status and 53.92% with positive nodal status, suggesting a nearly balanced distribution with a slight predominance of positive nodal status. The present study's nodal status distribution (46.07% negative, 53.92% positive) lies between the extremes reported in the other studies. Compared to Reza et al, which had a higher proportion of positive nodal status (62%), the present study has fewer cases with lymph node involvement. On the other hand, the present study shows a significantly higher proportion of positive nodal status compared to Kim et al (23.4%) and Steenhoven et al (13%), where the majority of cases had negative nodal status.

Table 45- Comparison of the different studies with respect to Lymphovascular invasion

STUDIES	YEAR	TOTAL CASES	ABSENT	PRESENT
Jun Lee et al[132]	2023	381	91.6%	8.4%
Nishimura et al[133]	2022	4,652	70.8%	29.2%
Srivastava et al[123]	2023	80	55%	45%
Ghosh et al[125]	2014	320	86.9%	13.1%
Present study	**2024**	**102**	**65.57%**	**34.39%**

The present study has a higher percentage of lymphovascular invasion (34.39%) compared to Jun Lee et al (8.4%) and Ghosh et al (13.1%), but a lower percentage than Srivastava et al (45%). This suggests that the incidence of lymphovascular invasion in the present study is greater than in some other studies but not as high as in Srivastava et al. Nishimura et al reported a lymphovascular invasion rate of 29.2%, which is lower than that of the present study. The higher incidence of lymphovascular invasion in the present study indicates a potentially more aggressive or advanced stage of breast cancer, suggesting a higher risk of metastasis and a possibly worse prognosis

Table 46 - Comparison of different studies with respect to T status of TNM classification

STUDIES	YEAR	TOTAL CASES	T1	T2	T3	T4
Ghosh et al[125]	2014	320	10%	51.3%	25.6%	13.1%
Jitendra Singh et al[124]	2014	328	10.1%	46.9%	9. %5	5.5%
Present study	**2024**	**102**	**10.8%**	**52.0%**	**28.4%**	**8.8%**

The present study shows a similar distribution for T1 (10.8%) and T2 (52.0%) cases compared to Jitendra Singh et al (T1: 10.1%, T2: 46.9%). However, the present study reports a higher percentage of T3 cases (28.4%) compared to Jitendra Singh et al (9.5%) and Ghosh et al (25.6%). Additionally, the present study has a lower percentage of T4 cases (8.8%) compared to Ghosh et al (13.1%), though it is higher than the percentage reported by Jitendra Singh et al (5.5%). This comparison indicates that while the distribution for T1 and T2 cases is similar, the present study has a notably higher proportion of T3 cases and a lower proportion of T4 cases. The higher proportion of T3 cases in the present study suggests a greater incidence of more advanced tumors that have grown larger or invaded nearby tissues more extensively than in Jitendra Singh et al and Ghosh et al. Conversely, the lower proportion of T4 cases compared to Ghosh et al suggests fewer instances of the most advanced tumors that have invaded the chest wall or skin. These differences could be attributed to variations in the study populations, diagnostic practices, or healthcare access, which influence the stage at which breast cancer is detected and treated and since this study is being conducted in a rural setup, due to delayed presentation of the patients the detection of malignancy happens at and advanced stage

Table 47- Comparison of the different studies with respect to N status of TNM classification

STUDIES	YEAR	TOTAL CASES	N0	N1	N2	N3
Ghosh et al[125]	2014	320	50%	30.6%	8.1%	11.3%
Steenhoven et al[95]	2020	159	87%	8%	4%	0
Present study	**2024**	**102**	**46.1%**	**23.5%**	**15.7%**	**14.7%**

The present study shows a lower percentage of N0 cases (46.1%) compared to Steenhoven et al (87%) and Ghosh et al (50%), indicating fewer cases with no nodal involvement. Conversely, the present study has a higher distribution in the N1 (23.5%), N2 (15.7%), and N3 (14.7%) categories. This is higher than Steenhoven et al (N1: 8%, N2: 4%, N3: 0%) and Ghosh et al (N1: 30.6%, N2: 8.1%, N3: 11.3%). This comparison highlights that the present study has more advanced nodal involvement.

Table 48- Comparison of the different studies with respect to Lymph node status

STUIDES	YEAR	TOTAL CASES	Negative nodal status	Positive nodal status
Kancharla et al[134]	2024	76	48.09%	51.89%
Gnananmuttupulle et al[135]	2021	116	23.3%	76.7%
Present study	**2024**	**102**	**46.07%**	**53.92%**

In a study done by Kancharla et al which reported 48.09% of cases with negative nodal status and 51.89% with positive nodal status in their study of 76 cases. This distribution indicates a nearly equal representation of cases with and without lymph node involvement. In contrast, Gnananmuttupulle et al, with 116 cases, reported a significantly higher percentage of positive nodal status (76.7%) and a lower percentage of negative nodal status (23.3%). This suggests a higher prevalence of advanced-stage disease with lymph node metastasis in their study population compared to Kancharla et al and the present study. Our study, comprising 102 cases, shows a distribution more balanced between negative nodal status (46.07%) and positive nodal status (53.92%) compared to both Kancharla et al

and Gnananmuttupulle et al. These differences likely reflect variations in patient demographics, disease biology, and possibly differences in screening practices and access to healthcare among the study populations.

Table 49- Comparison of different studies with respect to TNM stage

STUDIES	YEAR	TOTAL CASES	STAGE I	STAGE II	STAGE III	STAGE 1V
Gogia et al[136]	2018	550	4%	33%	44.9%	18%
Ming Li et al-cohort[137]	2020	14759	46%	39%	12%	4%
Present study	**2024**	**102**	**6.9%**	**52.0%**	**40.2%**	**1.0%**

The comparison of TNM stage distribution among the studies reveals significant differences in disease staging across different populations. In our study, with 102 cases, shows a higher percentage of Stage II (52.0%) and Stage III (40.2%) cases compared to Gogia et al (Stage II: 33%, Stage III: 44.9%) and Ming Le et al (Stage II: 39%, Stage III: 12%). This suggests that the patients in the our study have presented with more advanced disease at diagnosis compared to the other studies maybe because this study is being conducted in a rural setup and there is delayed presentation pf patients detection happens at later stage. Conversely, the present study reports a lower percentage of Stage IV cases (1.0%) compared to Gogia et al (18%) and Ming Le et al (4%), indicating a smaller proportion of patients with metastatic disease.

Table 50 - Comparison of the different studies with respect to Ki67 (>14% and <14%)

STUDIES	YEAR	TOTAL CASES	<14%	>14%	Not availbale
Sabhari et al[138]	2020	100	14%	61%	25%
Ramkumar et al[139]	2017	160	58.1%	41.87%	-
Present study	**2024**	**102**	**35.3%**	**64.7%**	**-**

The present study reports a higher percentage of cases with Ki67 >14% (64.7%) compared to both Ramkumar et al (41.87%) and Sabhari et al (61%). In contrast, the present study shows a lower percentage of cases with Ki67 <14% (35.3%) compared to Ramkumar et al (58.1%) and Sabhari et al

(14%). Ramkumar et al did not provide data for cases where Ki67 was not available, while Sabhari et al reported 25% of cases in this category.

Table 51- Comparison of the different studies with respect to ER status

STUDIES	YEAR	TOTAL CASES	POSITIVE	NEGATIVE
Ghosh et al[125]	2014	320	56.25%	43.75%
Rao et al[140]	2014	126	36.5%	63.5%
Gogai et al[136]	2015	112	47.32%	52.62%
Present study	**2024**	**102**	**49.0%**	**51.0%**

The present study's distribution of ER status (49.0% positive, 51.0% negative) is similar to that reported by Gogai et al (47.32% positive, 52.62% negative) and falls between the ranges reported by Ghosh et al (56.25% positive, 43.75% negative) and Rao et al (36.5% positive, 63.5% negative). This indicates that the present study's ER status distribution aligns closely with Gogai et al, with a balance between ER-positive and ER-negative cases, and it falls within the range observed in the other referenced studies.

Table 52- Comparison of the different studies with respect to PR status

STUDIES	YEAR	TOTAL CASES	POSITIVE	NEGATIVE
Ghosh et al[125]	2014	320	53.1%	46.9%
Rao et al[140]	2014	126	31.7%	68.2%
Gogai et al[136]	2015	112	47.32%	52.62%
Present study	**2024**	**102**	**38.2%**	**61.8%**

The study by Ghosh et al., with a sample size of 320 cases, reported 53.1% positive and 46.9% negative cases. Rao et al.'s study on 126 cases found a lower positivity rate at 31.7% and a higher negativity rate at 68.2%. Gogai et al.'s study, which included 112 cases, had 47.32% positive and 52.62% negative cases. In contrast, the present study with 102 cases observed 38.2% positive and 61.8% negative cases. Comparing these results, Ghosh et al. had the highest positivity rate, followed by Gogai et al., the present study, and Rao et al. with the lowest positivity rate.

Table 53- Comparison of the different studies with respect to Her2 Neu status

STUDIES	YEAR	TOTAL CASES	POSITIVE	NEGATIVE
Gogai et al[136]	2015	112	57.14%	42.86%
Gogia et al[126]	2018	550	39%	61%
Present study	**2024**	**102**	**44.1%**	**55.9%**

The present study reports 44.1% of cases as Her2 Neu positive and 55.9% as negative. This falls between the percentages reported by Gogai et al (57.14% positive, 42.86% negative) and Gogia et al (39% positive, 61% negative) for Her2 Neu status. This indicates that the present study's distribution of Her2 Neu positive cases is closer to that of Gogai et al, with a higher proportion compared to Gogia et al.

Table 54- Comparison of the different studies with respect to mBR grade

STUDIES	YEAR	TOTAL CASES	mBR 1	mBR 2	mBR 3
Ghosh et al[125]	2014	320	6.3%	26.3%	67.5%
Steenhoven et al[141]	2020	159	16%	67%	27%
Gogai et al[136]	2015	112	16.96%	59.82%	23.21%
Present study- H & E	**2024**	**102**	31.4%	60.8%	7.8%

When comparing the current study's findings on mBR grading with those from Ghosh et al. (2014), Steenhoven et al. (2020), and Gogai et al. (2015), distinct differences and similarities emerge. The present study, conducted in 2024 and utilizing H&E staining, reports a significantly higher proportion of mBR 1 cases (31.4%) compared to the earlier studies. In contrast, Ghosh et al. (2014) found only 6.3% of cases to be mBR 1, Steenhoven et al. (2020) reported 16%, and Gogai et al. (2015) observed 16.96%. For mBR 2 cases, the present study shows a similar proportion (60.8%) to Gogai et al. (59.82%) and Steenhoven et al. (67%), but much higher than Ghosh et al. (26.3%). However, the present study reports a much lower percentage of mBR 3 cases (7.8%) compared to the other studies: Ghosh et al. (67.5%), Steenhoven et al. (27%), and Gogai et al. (23.21%). These discrepancies highlight variations in tumor grading across different cohorts and methodologies, underscoring the importance of context and criteria used in mBR grading.

For the counting of mitotic figures in our study we have followed the Latest WHO (2019) criteria using the $mm^{2.}$ We calculated the diameter of one field using Olympus CX 23 microscope which had a field number of +20 and objective magnification of 40 and got a field number of 0.196 which corresponds to mitotic Score 1 having <=7, Score 2 as 8-14 and score 3 as >=15 mitotic figures for 10 fields was 1.96 mm^2 area. The same scoring system was followed to score the mitotic figures using PHH3 IHC

Identifying mitotic figures in H&E-stained breast cancer tissue sections, such as infiltrating ductal carcinoma (IDC), is prone to several errors. These include mistaking apoptotic cells, pyknotic nuclei, and karyorrhectic debris for mitotic figures due to their similar appearance under the microscope. Additionally, poorly preserved tissue and overlapping cells can further complicate accurate identification. To address these issues, immunohistochemical (IHC) staining, specifically using markers like phosphohistone H3 (PHH3), provides a more reliable and objective method for identifying mitotic figures. PHH3 selectively stains mitotic chromatin, allowing for clear differentiation from other nuclear changes and improving the accuracy and consistency of mitotic counts in breast cancer assessments.

Table 55 : Comparison of mBR GRADE (PHH3) with mBR GRADE (MAI)

mBR Grade Change	**Woo et al. Grade Change (%)**[96]	**Grade Change in present study (%)**
Upgrade		
Grade 1 to Grade 2	49	56.3
Grade 2 to Grade 3	38	14.5
Grade 1 to Grade 3	7	3.1
Downgrade		
Grade 2 to Grade 1	2	3.2
Grade 3 to Grade 2	4	0

The table comparing mBR grade changes based on PHH3 staining (Woo et al.) and ,MAI (H &E) shows that in both studies, upgrading from lower to higher grades is more prevalent than downgrading,

reflecting a trend towards increased aggressiveness or proliferation in higher mBR grades identified through PHH3 staining. Woo et al. reported substantial upgrades from Grade 1 to Grade 2 (49%) and Grade 2 to Grade 3 (38%), paralleling the present study's findings of 56.3% and 14.5%, respectively. Conversely, downgrades were less frequent, particularly in moving from Grade 2 to Grade 1 (2% in Woo et al. versus 3.2% in the present study). Interestingly, the present study noted no downgrades from Grade 3 to Grade 2, whereas Woo et al. observed a small percentage (4%). **Overall, the data underscores PHH3's potential superiority in identifying higher-grade tumors with greater proliferative activity, influencing clinical decision-making and prognostic assessments in breast cancer management.**

Hematoxylin and Eosin (H&E) staining, while widely used in pathology, presents several challenges when identifying mitotic figures in tissue samples. These challenges include poor visibility due to the small size and subtle staining of mitotic cells, leading to potential underestimation or subjective interpretation by pathologists. In contrast, Phosphohistone H3 (PHH3) staining offers notable advantages for mitotic figure detection. PHH3 is a specific marker of cells in late stages of the cell cycle (G2 and M phases), providing a more intense and distinct signal at mitotic sites compared to H&E. This specificity enhances the accuracy and reproducibility of mitotic figure quantification, crucial for diagnostic and prognostic assessments in cancer pathology. Studies have demonstrated that PHH3 staining correlates closely with mitotic activity and clinical outcomes, underscoring its superiority over H&E in providing reliable data for treatment planning and patient management in oncology. Therefore, while H&E staining remains essential in histopathology, PHH3 staining represents a significant advancement in enhancing the precision and clinical utility of mitotic figure evaluation.

Table 56: Comparison of Ki67 with various studies according to IKWG

STUDIES	YEAR	CASES	<5%	6-29%	>30%
Shim et al.[142]	2024	307	8.8 - 16.0	61.5 - 79.8	11.4 - 22.5
Arora et al.[143]	2023	73	15.0%	28.8%	56.2%
Present study	**2024**	**102**	12.7	74.5	12.7

Table 57 shows the comparison of Ki67 expression levels according to the International Ki67 Working Group (IKWG) among different studies. Shim et al. (2024) reported a wide range for the 6-29% category (61.5 - 79.8%), with relatively balanced proportions in the ≤5% (8.8 - 16.0%) and ≥30% (11.4 - 22.5%) groups. Arora et al. (2023) found a higher proportion of cases in the >30% category (56.2%) and fewer in the 6-29% (28.8%) and <5% (15.0%) groups. In the present study (2024), 74.5% of cases were in the 6-29% category, 12.7% in the ≤5% group, and 12.7% in the ≥30% group. The higher percentage of cases in the intermediate range (6-29%) in the present study aligns closely with Shim et al., suggesting a potential consensus on the prevalence of this category, whereas Arora et al.'s findings highlight a significant deviation, possibly due to methodological differences or population-specific factors

Ki67 is a proliferation marker used to assess the growth fraction of a cell population in breast cancer. Its significance lies in its ability to provide prognostic information, as higher Ki67 levels are often associated with more aggressive tumor behavior and poorer outcomes. Ki67 staining helps in estimating the mitotic count and, consequently, the proliferation rate, which is crucial for determining treatment strategies and predicting patient prognosis. However, Ki67 has limitations, including inter-observer variability in scoring, differences in staining protocols, and the lack of standardized cut-off values for defining high versus low proliferation. Additionally, Ki67 can be expressed in non-mitotic phases of the cell cycle, potentially leading to overestimation of the true mitotic activity. Despite these limitations, Ki67 remains a valuable tool when used alongside other markers and clinical information.

In summary, while Ki67 is valuable for assessing overall proliferative activity, PHH3 offers more specific and reliable data for counting mitotic figures, making it a preferred marker for precise mitotic indexing in breast cancer.

Table 57: Pearson correlation showing correlation between MAI, PHH3, and KI67

		Mitotic count- PHH3	**Ki67**
Mitotic count- H&E	Pearson Correlation	.761**	.184
	p value	.000	.064

		Ki67
Mitotic count- H&E	Pearson Correlation	.184
	p value	.064
MITOTIC COUNT- PHH3	Pearson Correlation	.107
	p value	.283

To further support our hypothesis, Pearson correlation was used to correlate MAI, PHH3 and KI67

The analysis revealed a strong positive correlation between the mitotic count (H&E) and PHH3 expression, with a Pearson correlation coefficient of 0.761 and a p-value less than 0.001. This statistically significant result indicates that higher mitotic counts are associated with higher PHH3 expression in infiltrating ductal carcinoma of the breast.

In contrast, the correlation between the mitotic count (H&E) and Ki67 expression was weak and not statistically significant, with a Pearson correlation coefficient of 0.184 and a p-value of 0.064. This suggests there is no strong evidence of a linear relationship between the mitotic count and Ki67 expression in this sample.

Similarly, the correlation between the mitotic count (PHH3) and Ki67 expression was very weak and also not statistically significant, with a Pearson correlation coefficient of 0.107 and a p-value of 0.283. This indicates no strong linear relationship between PHH3 and Ki67 expression in the sample.

These findings suggest that PHH3 might be a more reliable marker for tumor proliferation in infiltrating ductal carcinoma of the breast compared to Ki67 and MAI (H&E)given the stronger and significant correlation with the mitotic count

SUMMARY

SUMMARY

- The present study was conducted in the Department of Pathology, Sri Devaraj Urs Medical College, Tamaka, Kolar spanning from July 2019 to June 2023
- This study investigated 102 cases of Infiltrating Ductal Carcinoma (IDC) of the breast, focusing on the immunohistochemical expression of Ki67 and PHH3 and comparing these markers with traditional Hematoxylin and Eosin (H&E) staining.
- The demographic data showed the majority of cases (56.9%) were in individuals above 50 years of age, with a mean age of 51.46 years.
- Tumor characteristics revealed that 55.9% of tumors were less than 5 cm, and 52% of cases were at T2 stage.
- Lymph node involvement was noted in 53.92% of cases, and lymph vascular invasion was present in 34.3%.
- Among the 102 cases, 65.7% showed no lymphovascular invasion, while 34.3% showed lymphovascular invasion.
- Based on the 8th AJCC criteria, the TNM staging distribution shows that 52% of cases were in Stage II, followed by Stage III. Stage I and Stage IV had 6.9% and 1% of cases, respectively.
- TILs were divided into three groups 0-10% as low TILs, 10-40% as intermediate TILs and >40% as High TILs majority were below 10% i.e Low TILs in 66.7% of cases.
- For Tumor Stromal Ratio, it was divided as $\leq$50% as a low T:S ratio and >50% as a High T:S ratio, and the majority of cases that is 72.5% had a high T:S ratio and only 27.5% had low T: ratio.
- The Mitotic Activity Index (MAI) was categorized based on the total number of mitotic figures in an area of 0.196 mm^2 following the latest WHO Breast(5^{th} edition). Specifically, a mitotic count of 0–7 was assigned a Score of 1, 8–14 a Score of 2, and $\geq$15 a Score of 3.
- Similarly, PHH3 (Phosphohistone H3) was categorized using the same scoring system
- The comparison of Mitotic Count (MC) scores using H&E and PHH3 staining in breast cancer studies shows that PHH3 consistently identifies higher mitotic activity, with significant upgrade rates (40.0% from score 1 to 2, 98.0% from score 2 to 3, and 32.0% from score 1 to 3) and no downgrades, indicating its potential for more accurate assessment.
- Based on molecular subtyping Ki67 was dived as <14% and $\geq$14% and showed that 64.7% of cases had Ki67 $\geq$14%, while 35.3% had Ki67 <14%, based on molecular subtyping

- Ki67 was also grouped according to the International Ki67 Working Group (IKWG), Ki67 scores were further categorized as low (≤ 5%), intermediate (6–29%), and high (≥ 30%). The majority of cases that is 71.6% of cases in the intermediate category.
- Statistical analysis highlighted a significant correlation between Ki67 expression and lymph node involvement, but not with the stage of disease
- The comparison of mBR grade assessments in 102 breast cancer cases reveals that PHH3 staining results in higher grade cases and more frequent upgrades than H&E staining. PHH3 identified 56.3% as Grade 1, 82.3% as Grade 2, and 100% as Grade 3, compared to H&E's 40.6% as Grade 1, 3.2% as Grade 2, and none as Grade 3. Upgrades were more common with PHH3 (56.3% from Grade 1 to 2 and 14.5% from Grade 2 to 3), with minimal downgrades noted in both methods.
- The comparison of PHH3 and Ki67 scores showed that PHH3 consistently identified higher mitotic activity with fewer discrepancies. Specifically, for PHH3 score 1, 57.1% corresponded to Ki67 score 3; for PHH3 score 2, 63.6% corresponded to Ki67 score 2; and for PHH3 score 3, 70.2% corresponded to Ki67 score 3. Upgrade rates from Ki67 to PHH3 were 57.1% from scores 1 to 3, and downgrade rates were 15.5% from scores 3 to 2, highlighting PHH3's sensitivity and specificity in detecting higher mitotic activity

CONCLUSION

CONCLUSION

The comparative study emphasizes the superiority of PHH3 staining over traditional H&E staining(MAI) in accurately assessing mitotic activity and MBR grades in IDC breast cancer cases. The high upgrade rates from H&E to PHH3 scores suggest that PHH3 is more sensitive in detecting higher mitotic counts, potentially leading to more precise grading and prognosis. Additionally, Ki67 expression showed a significant association with lymph node involvement, reinforcing its role as a prognostic marker. These findings advocate for the integration of PHH3 staining in routine diagnostic practices to enhance the accuracy of breast cancer grading and prognosis.

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ANNEXURES

PATIENT PROFORMA

Anonymized Sample No:

Chief complaint:

History of presenting illness:

Past history:

Personal history:

Local examination:

Biopsy Number:

Gross:

Tumour size:

Microscopy:

Metastatic Lymph Nodes:

Lymphovascular Invasion:

Tumor Infiltrating Lymphocytes: <10=low, 10-40=intermediate and >40=high TILs

Tumor Stromal Ratio: <50=LowT:S ratio, ≥50% = High T:S ratio

NPI prognostic score:

Histopathological diagnosis:

Modified Bloom Richardson grading: Grade I = 3-5

Grade I I= 6-7

Grade III = 8-9

Mitotic Activity Index(H&E): Score 1 = <7

Score 2= 8-14

Score 3= >15

Immunohistochemically Scoring:

PHH3 mitotic count score- Score 1 = <7

Score 2= 8-14

Score 3= >15

Estrogen Receptor:

Progesterone Receptor:

Her 2 Neu:

Ki 67: For molecular classification- <14% and ≥14%

Based on IKWG Ki67 is classified as <5 (low), 6-29(intermediate), >30(high)

INFORMED CONSENT FORM

STUDY TITLE: ASSESSMENT OF TUMOUR PROLIFERATION BY USE OF THE MITOTIC ACTIVITY INDEX, Ki67, AND PHOSPHOHISTONE H3 EXPRESSION IN INFILTRATING DUCTAL CARCINOMA OF BREAST

I, _____have read or have been read to me the patient information sheet and understand the purpose of the study, the procedure that will be used, the risks and benefits associated with my involvement in the study, and the nature of informationthat will be collected and disclosed during the study.

I have had my opportunity to ask my questions regarding various aspects of the study and my questions are answered to my satisfaction.

I, the undersigned, agree to participate in this study and authorize the collection and disclosure of my personal information for the dissertation.

Name and signature / thumb impression	Date:
(subject)	Place:
Name and signature / thumb impression	Date:
	Place:
(Witness/Parent/ Guardian/ Husband)	

PATIENT INFORMATION SHEET:

STUDY TITLE: Assessment of tumor proliferation by use of the mitotic activity index, and Ki67 and phosphohistone H3 expression, in infiltrating ductal carcinoma of Breast

PLACE OF STUDY: Department of Pathology, Sri Devaraj Urs Medical College, Kolar.

The main aim of the study is to assess tumour proliferation by use of the mitotic activity index, and Ki67 and phosphohistone H3 expression, in infiltrating ductal carcinoma of Breast. The specimens will be collected from the department of pathology, SDUMC,Kolar. This study will be approved by the institutional ethical committee. The information collected will be used only for dissertation and publication. There is no compulsion to agree to participate. You are requested to sign / provide thumb impression only if you voluntarily agree to participate in the study. All information collected from you will be kept confidential and will not be disclosed to any outsider. Your identity will not be revealed. You will not receive any monetary benefits to participate in this research. This informed consent document is intended to give you a general background of study. Please read the following information carefully and discuss with your family members. You can ask your queries related to study at any time during the study. If you are willing to participate in the study you will be asked to sign an informed consent form by which you are acknowledging that you wish to participate in the study and entire procedure will be explained to you by the study doctor. You are free to withdraw your consent to participate in the study any time without explanation and this will not change your future care.

For any clarification you are free to contact the investigator.

PRINCIPAL INVESTIGATOR: Dr Zubiya Suha Fathima
Phone number: 9742140924

ರೋಗಿಯ ಮಾಹಿತಿ ಹಾಳೆ

ಅಧ್ಯಯನದ ಶೀರ್ಷಿಕೆ:– ಸ್ತನದ ಡಕ್ಟಲ್ ಕಾರ್ಸಿನೋಮಾದ ಒಳನುಸುಳುವಲ್ಲಿ ಮೈಟಾಟಿಕ್ ಚಟುವಟಿಕೆಯ ಸೂಚ್ಯಂಕದಂತೆ ಕೆ.ಐ.67 ರಂತೆ ಫಾಸ್ಫೋಹಿಸ್ಟೋನ್ ಎಚ್.3 ಅಭಿವಯಕ್ತತೆಯ ಬಳಕೆಯಿಂದ ಗಡ್ಡೆಯ ಪ್ರಸರಣದ ಮೌಲ್ಯಮಾಪನದ ಅಧ್ಯಯನ.

ಅಧ್ಯಯನದ ಸ್ಥಳ: ರೋಗ ಶಾಸ್ತ್ರ ವಿಭಾಗ, ಶ್ರೀ ದೇವರಾಜ ಅರಸು ವೈದ್ಯಕೀಯ ಕಾಲೇಜು, ಕೋಲಾರ.

ಸ್ತನದ ಡಕ್ಟಲ್ ಕಾರ್ಸಿನೋಮಾದ ಒಳನುಸುಳುವಲ್ಲಿ ಮೈಟಾಟಿಕ್ ಚಟುವಟಿಕೆಯ ಸೂಚ್ಯಂಕದಂತೆ ಕೆ.ಐ.67 ರಂತೆ ಫಾಸ್ಫೋಹಿಸ್ಟೋನ್ ಎಚ್.3 ಅಭಿವಯಕ್ತತೆಯ ಬಳಕೆಯಿಂದ ಗಡ್ಡೆಯ ಪ್ರಸರಣದ ಮೌಲ್ಯಮಾಪನದ ಅಧ್ಯಯನ.

ರೋಗಶಾಸ್ತ್ರ ವಿಭಾಗ ಎಸ್.ಡಿ.ಯು.ಎಂ.ಸಿ ಕೋಲಾರದಿಂದ ಮಾದರಿಯನ್ನು ಸಂಗ್ರಹಿಸಿ ಈ ಅಧ್ಯಯನವನ್ನು ನೈತಿಕ ಸಮೀತಿಯು ಅನುಮೋದಿಸಲಾಗುವುದು.

ಈ ಮಾಹಿತಿಯನ್ನು ಪ್ರಬಂಧ ಮತ್ತು ಪ್ರಕಟನೆಗಳಿಗೆ ಮಾತ್ರ ಬಳಸಲಾಗುತ್ತದೆ. ಭಾಗವಹಿಸಲು ಒಪ್ಪಿಕೊಳ್ಳಲು ಯಾವುದೇ ಒತ್ತಾಯವಿಲ್ಲ. ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನೀವು ಸ್ವಯಂ ಪ್ರೇರಣೆಯಿಂದ ಒಪ್ಪಿದರೆ ಮಾತ್ರ, ಹೆಬ್ಬರಳಿನ ಗುರುತನ್ನು/ಸಹಿಮಾಡಲು/ಒದಗಿಸಲು ನಿಮ್ಮನ್ನು ವಿನಂತಿಸಲಾಗಿದೆ. ನಿಮ್ಮಿಂದ ಸಂಗ್ರಹಿಸಿದ ಮಾಹಿತಿಯನ್ನು ಮತ್ತು ನಿಮ್ಮ ಗುರುತನ್ನು ಗೌಪ್ಯವಾಗಿ ಇರಿಸಲಾಗುತ್ತದೆ. ಮತ್ತು ಹೊರಗಿನವರಿಗೆ ಭಹಿರಂಗ ಪಡಿಸುವುದಿಲ್ಲ ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಭಾಗವಹಿಸಲು ನೀವು ಯಾವುದೇ ಹಣಕಾಸಿನ ಸೌಲಭ್ಯವನ್ನು ಪಡೆಯಲಾಗುವುದಿಲ್ಲ.

ಈ ಮಾಹಿತಿಯು ಸನ್ಮತಿ ದಾಖಲೆಯು ನಿಮಗೆ ಸಾಮಾನ್ಯ ಅಧ್ಯಯನದ ಹಿನ್ನಲೆಯನ್ನು ನೀಡಲು ಉದ್ದೇಶಿಸಿದೆ. ದಯವಿಟ್ಟು ಕೆಳಗಿನ ಮಾಹಿತಿಯನ್ನು ಎಚ್ಚರಿಕೆಯಿಂದ ಓದಿ ಮತ್ತು ನಿಮ್ಮ ಕುಟುಂಬ ಸದಸ್ಯರೊಂದಿಗೆ ಚರ್ಚಿಸಿ, ಅಧ್ಯಯನದ ಸಮಯದಲ್ಲಿ ನೀವು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಅಧ್ಯಯನಕ್ಕೆ ಸಂಬಂಧಿಸಿದ ನಿಮ್ಮ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಬಹುದು. ನೀವು ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಸಿದ್ದರಿದ್ದರೆ ತಿಳಿವಳಿಕೆಯುಳ್ಳ ಸಮ್ಮತಿಯ ನಮೂನೆಗೆ ಸಹಿ ಹಾಕಲು ನಿಮ್ಮನ್ನು ಕೇಳಲಾಗುತ್ತದೆ. ಮತ್ತು ಅದರ ಮೂಲಕ ನೀವು ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಬಯಸುತ್ತೀರಿ ಎಂದು ಒಪ್ಪಿಕೊಳ್ಳುತ್ತೀರಿ. ಮತ್ತು ಸಂಪೂರ್ಣ ಕಾರ್ಯವಿಧಾನವನ್ನು ಅಧ್ಯಯನ ವೈದ್ಯರು ನಿಮಗೆ ವಿವರಿಸುತ್ತಾರೆ. ವಿವರಣೆಯಿಲ್ಲದೆ ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನಿಮ್ಮ ಸಮ್ಮತಿಯನ್ನು ಹಿಂಪಡೆಯಲು ನೀವು ಸ್ವತಂತ್ರರಾಗಿದ್ದೀರಿ. ಮತ್ತು ಇದು ನಿಮ್ಮ ಭವಿಷ್ಯದ ಚಿಕೆತ್ಸೆಯನ್ನು ಬದಲಾಯಿಸುವುದಿಲ್ಲ.ಯಾವುದೇ ಸ್ಪಷ್ಟೀಕರಣಕ್ಕಾಗಿ ನೀವು ತನಿಖಾಧಿಕಾರಿಯನ್ನು ಸಂಪರ್ಕಿಸಲು ಮುಕ್ತರಾಗಿದ್ದೀರಿ.

ಪ್ರಮುಖ ಸಂಶೋಧಕರ ಹೆಸರು ಮತ್ತು ರುಜು:–

ಹೆಸರು ಮತ್ತು ಸಹಿ/ಹೆಬ್ಬರಳಿನ ಗುರುತು
(ವಿಷಯ)

ಹೆಸರು ಮತ್ತು ಸಹಿ/ಹೆಬ್ಬರಳಿನ ಗುರುತು
(ಸಾಕ್ಷಿ/ಪೋಷಕ/ಗುರು/ಪತಿ)

ಮತ್ತಷ್ಟು ಸ್ಪಷ್ಟೀಕರಣಕ್ಕಾಗಿ ನೀವು ಅಧ್ಯಯನ ಶೋಧಕವನ್ನು ಸಂಪರ್ಕಿಸಬಹುದು.
ಡಾ॥ ಜುಬಿಯಾ ಸುಹಾ ಫಾತಿಮಾ

ತಿಳಿಸಲಾದ ಒಪ್ಪಿಗೆ ನಮೂನೆ

ಅಧ್ಯಯನದ ಶೀರ್ಷಿಕೆ:– ಸ್ತನದ ಡಕ್ಟಲ್ ಕಾರ್ಸಿನೋಮಾದ ಒಳನುಸುಳುವಲ್ಲಿ ಮೈಟಾಟಿಕ್ ಚಟುವಟಿಕೆಯ ಸೂಚ್ಯಂಕದಂತೆ ಕೆ.ಐ.67 ರಂತೆ ಫಾಸ್ಫೋಹಿಸ್ಟೋನ್ ಎಚ್.3 ಅಭಿವ್ಯಕ್ತಿಯ ಬಳಕೆಯಿಂದ ಗಡ್ಡೆಯ ಪ್ರಸರಣದ ಮೌಲ್ಯಮಾಪನದ ಅಧ್ಯಯನ.

ನಾನು_____ ಮಾಹಿತಿಯನ್ನು ಓದಿದ್ದೇನೆ ಅಥವಾ ನನಗೆ ಓದಿ ತಿಳಿಸಿದ್ದಾರೆ ಮತ್ತು ಅಧ್ಯಯನದ ಉದ್ದೇಶ, ಬಳಸಲಾಗುವ ವಿಧಾನ, ಅಧ್ಯಯನದಲ್ಲಿ ನನ್ನ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಗೆ ಸಂಬಂಧಿಸಿದಂತೆ ಅಪಾಯ ಮತ್ತು ಪ್ರಯೋಜನಗಳು ಮತ್ತು ಮಾಹಿತಿಯ ಸ್ವರೂಪವನ್ನು ಸಂಗ್ರಹಿಸಲಾಗುತ್ತದೆ ಮತ್ತು ಅಧ್ಯಯನದ ಸಮಯದಲ್ಲಿ ಬಹಿರಂಗಪಡಿಸಲಾಗುತ್ತದೆ ಅಧ್ಯಯನದ ವಿವಿಧ ಅಂಶಗಳಿಗೆ ಸಂಬಂಧಿಸಿದಂತೆ ನನ್ನ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ಅವಕಾಶವನ್ನು ನಾನು ಹೊಂದಿದ್ದೇನೆ ಮತ್ತು ನನ್ನ ಪ್ರಶ್ನೆಗಳಿಗೆ ಸಮಾದಾನಕರ ಉತ್ತರವನ್ನು ಪಡೆದಿದ್ದೇನೆ. ಈ ಕೆಳಗೆ ಸಹಿಮಾಡಿರುವ ನಾನು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಒಪ್ಪಿರುತ್ತೇನೆ ಮತ್ತು ಪ್ರಬಂಧಕ್ಕಾಗಿ ನನ್ನ ವೈಯಕ್ತಿಕ ಮಾಹಿತಿಯ ಸಂಗ್ರಹಣೆ ಮತ್ತು ಬಹಿರಂಗ ಪಡಿಸುವುದಕ್ಕೆ ನಾನು ಒಪ್ಪಿಕೊಳ್ಳುತ್ತೇನೆ.

ಹೆಸರು ಮತ್ತು ಸಹಿ/ಹೆಬ್ಬರಳಿನ ಗುರುತು
(ವಿಷಯ)

ಹೆಸರು ಮತ್ತು ಸಹಿ/ಹೆಬ್ಬರಳಿನ ಗುರುತು
(ಸಾಕ್ಷಿ/ಪೋಷಕ/ಗುರು/ಪತಿ)

ಮತ್ತಷ್ಟು ಸೃಷ್ಟೀಕರಣಕ್ಕಾಗಿ ನೀವು ಅಧ್ಯಯನ ಸಂಶೋಧಕರನ್ನು ಸಂಪರ್ಕಿಸಬಹುದು.
ಡಾ॥ ಜುಬಿಯಾ ಸುಹಾ ಫಾತಿಮಾ

MASTER CHART

Sl No	YEAR	Biopsy no	Age	Laterality	Hosp N	Tumor size(cm)	Lymph node Examn	Lymph node pos	Lymph node score	LV invasion	T	N	M	Stage	NPI	Tubule	Nuclear size	MITOTIC COUNT score - H&E	MITOTIC COUNT- H&E	MBR Grade-H&E	MITOTIC COUNT score- PHH3	MITOTIC COUNT- PHH3	MBR Grade-PHH3	Ki67	Ki67	Ki67 EXACT value	ER	PR	Her2neu	TILS	T:S RATIO
1	2019	31	48	L	664790	8	15	11	1	0	3	3	x	III	6.6	2	2	2	13	II	3	53	II	>14%	6-29	25	0	0	0	<10	<50
2	2019	347	55	R	678651	4	13	6	1	0	2	2	x	III	4.8	1	2	2	12	I	3	40	II	>14%	6-29	25	0	0	0	<10	>50
3	2019	369	82	L	681338	3	9	0	0	0	2	0	x	II	2.5	1	1	1	5	I	2	8	I	>14%	6-29	8	1	1	0	<10	>50
4	2019	371	58	R	681638	4	14	1	1	1	2	1	x	II	4.8	3	2	2	8	II	3	29	III	<14 %	<5	2	0	0	0	<10	>50
5	2019	386	80	L	682643	2	25	25	1	0	1	3	x	III	5.4	3	2	1	5	II	3	18	III	<14 %	<5	3	1	1	0	<10	>50
6	2019	550	49	R	694955	12	13	0	0	1	4	0	x	III	4	3	2	2	12	II	3	56	III	>14%	6-29	15	0	0	1	<10	<50
7	2019	641	45	L	690144	3	6	0	0	0	2	0	x	II	4	2	1	1	2	II	1	2	I	<14 %	<5	2	1	1	0	<10	>50
8	2019	921	44	R	706401	2.5	5	0	0	0	2	0	x	II	2.5	2	1	1	5	I	2	14	I	<14 %	6-29	12	1	1	0	<10	<50
9	2019	984	53	L	708238	3.2	13	0	0	1	2	0	x	II	2.64	2	2	2	8	II	3	20	II	<14 %	6-29	13	1	0	0	<10	<50
10	2019	1108	52	L	407606	3.5	4	0	0	0	2	0	x	II	2.7	2	2	2	8	II	2	8	II	<14 %	6-29	12	1	1	0	<10	>50
11	2019	1252	52	L	717035	2.2	5	1	1	0	2	1	x	II	3.44	2	2	1	4	I	2	8	II	<14 %	<5	3	1	1	1	<10	>50
12	2019	1373	75	L	726027	4	1	1	1	0	2	1	x	II	2.8	2	1	1	5	I	2	11	I	<14 %	6-29	10	1	1	0	<10	>50
13	2019	1392	40	R	728557	4	5	1	1	1	2	1	x	II	4.8	3	2	1	2	II	1	5	II	<14 %	6-29	13	0	0	1	<10	>50
14	2019	1410	75	L	726902	6	6	0	0	0	3	0	x	II	5.2	3	3	3	15	III	3	32	III	>14%	6-29	20	1	1	1	<10	>50
15	2019	1454	50	L	713964	3.5	14	0	0	0	2	0	x	II	2.7	2	2	2	10	II	3	40	II	>14%	6-29	20	0	0	0	10-40	>50
16	2019	1490	55	L	730817	3	25	21	1	0	2	3	x	III	5.6	3	2	2	11	II	3	28	III	>14%	6-29	25	0	0	0	10-40	>50
17	2019	1599	80	R	736905	9	7	1	1	1	3	1	x	III	6.8	3	3	2	10	III	3	25	III	<14 %	<5	2	1	1	0	<10	>50
18	2019	1643	53	R	735987	2.5	5	1	1	0	2	1	x	II	5.5	3	2	3	17	III	3	41	III	>14%	6-29	25	1	1	0	<10	>50
19	2019	1744	40	L	734649	9	6	0	0	1	3	0	x	II	4.8	2	2	3	16	II	3	48	II	>14%	>30	30	0	0	0	<10	<50
20	2019	2257	41	R	761094	2.2	10	7	1	0	2	2	x	IIIA	6.4	2	3	2	12	II	3	37	III	<14%	6-29	13	1	1	1	<10	>50
21	2019	2275	55	R	684653	1.5	3	0	0	1	1	0	x	I	2.3	2	1	3	16	II	3	47	II	>14%	6-29	25	1	1	1	<10	>50
22	2019	2390	54	L	767363	7	20	14	1	0	3	3	x	III	6.4	2	2	3	15	II	3	67	II	>14%	6-29	20	0	0	0	<10	<50
23	2019	2476	36	L	742083	13	7	4	1	0	4	2	x	III	8	2	2	1	5	I	2	13	II	<14 %	6-29	12	0	0	0	<10	>50
24	2019	2523	33	L	757437	4	5	4	1	1	2	2	x	III	6.8	3	2	3	17	III	3	43	III	>14%	6-29	20	1	1	1	10-40	>50
25	2019	2549	55	L	746844	11	11	4	1	0	3	2	x	III	8.2	3	3	2	10	III	3	51	III	<14 %	6-29	11	0	0	1	10-40	<50
26	2019	2726	38	L	759213	2	4	1	1	0	2	1	x	II	2.4	2	1	1	2	I	1	2	I	>14%	6-29	20	0	0	1	<10	>50
27	2019	2830	42	R	786531	5	13	2	1	0	3	2	x	I	4	2	1	3	15	II	3	31	II	>14%	>30	30	1	1	1	10-40	>50
28	2019	1002	39	R	820441	4.5	35	0	0	0	2	0	x	II	1.9	2	1	3	17	II	3	30	II	>14%	6-29	22	1	1	0	>40	>50
29	2020	228	33	L	812928	7	9	0	0	0	3	0	x	III	5.4	1	2	2	8	I	3	17	II	>14%	6-29	22	0	0	0	<10	>50
30	2020	397	42	L	797271	3	9	0	0	0	2	0	x	I	1.6	1	1	2	8	I	3	25	I	<14 %	<5	3	1	1	0	<10	<50
31	2020	480	65	R	825541	2.4	12	0	0	1	2	0	x	II	3.4	2	2	2	10	II	3	42	II	<14 %	<5	2	1	1	1	<10	>50
32	2020	621	53	L	815423	2	15	9	1	0	1	2	x	II	5.8	3	1	1	5	I	1	5	I	>14%	6-29	25	0	0	1	>40	>50
33	2020	910	70	L	844590	2.5	15	6	1	0	2	3	x	II	4.5	2	2	2	10	II	3	48	II	>14%	6-29	20	1	1	0	<10	>50
34	2020	914	65	L	837500	4.5	8	0	0	1	2	0	x	II	1.6	2	2	2	14	II	3	29	II	<14 %	<5	3	0	0	0	10-40	>50
35	2020	935	65	L	845622	4	9	7	1	0	4	2	x	III	4.8	2	2	2	13	II	3	30	II	>14%	6-29	25	0	0	1	>40	>50
36	2020	1002	39	R	820441	4.5	35	0	0	0	2	0	x	II	1.9	2	1	3	17	II	3	30	II	>14%	6-29	22	1	1	0	>40	>50
37	2020	1044	56	R	839107	2	9	3	1	0	2	1	x	II	3.4	2	2	2	13	II	3	36	II	>14%	6-29	24	0	0	0	10-40	>50
38	2020	1354	59	L	857344	3.5	11	0	0	1	2	0	x	II	2.7	1	2	3	15	II	3	30	II	<14 %	6-29	11	1	1	0	10-40	>50
39	2020	1407	72	L	863435	3.5	13	0	0	0	2	0	x	II	2.7	3	3	1	5	II	2	14	III	<14 %	6-29	13	0	0	0	<10	>50
40	2020	1744	31	R	852475	3	9	1	1	0	2	1	x	II	3.6	2	2	2	11	II	3	22	II	>14%	6-29	20	0	1	1	<10	>50
41	2020	1476	60	L	865757	3.3	9	7	1	0	2	1	x	III	4.6	1	1	2	11	I	3	15	I	<14 %	6-29	12	1	1	0	<10	>50
42	2020	1648	45	L	873038	2.5	0	0	0	1	2	0	x	II	1.5	2	2	2	13	II	3	38	II	<14 %	6-29	13	1	1	0	10-40	>50
43	2020	1913	67	R	879823	6.5	1	1	1	1	3	1	x	III	4.26	3	2	1	4	II	3	21	III	<14 %	<5	2	1	1	0	<10	>50
44	2020	1920	45	L	882814	8	22	6	1	1	3	1	x	III	5.4	3	2	2	10	II	3	46	III	>14%	6-29	25	1	1	0	10-40	>50
45	2020	2017	50	L	876387	2	14	0	0	1	1	0	x	II	2.89	2	2	2	11	II	3	53	II	>14%	>30	30	0	0	1	10-40	>50
46	2021	33	76	R	885577	6	11	0	0	0	3	0	x	II	4.2	2	2	1	5	I	3	18	II	>14%	6-29	25	1	1	1	<10	>50
47	2021	256	48	R	892991	7	33	2	1	0	4	1	x	III	6.6	3	2	3	28	III	3	72	III	<14 %	<5	2	1	1	1	<10	<50
48	2021	410	35	L	897214	4	12	5	1	0	3	2	x	III	6	1	2	2	9	I	3	18	II	>14%	6-29	20	0	0	1	<10	>50
49	2021	544	37	R	905154	9	8	0	0	0	2	0	x	IIB	3.8	2	2	3	18	II	3	26	II	>14%	6-29	25	1	1	0	10-40	>50
50	2021	582	57	R	903629	4	10	0	0	1	2	0	x	IIA	3.8	2	2	1	3	I	2	12	II	>14%	>30	30	0	0	1	<10	>50

51	2021	893	50	R	923478	1.7	2	0	0	0	1	0	x	II	2.24	2	2	1	2	I	2	8	II	>14%	6-29	25	0	0	0	>40	>50
52	2021	910	52	L	923327	3	16	0	0	1	2	0	x	IIA	2.6	2	2	2	8	II	3	22	II	>14%	6-29	25	0	0	0	>40	>50
53	2021	1408	71	R	938765	6.2	25	0	0	0	3	0	x	IIB	2.24	2	2	2	11	II	3	37	II	>14	>30	30	1	0	0	10-40	>50
54	2021	1452	53	R	933465	3.5	7	0	0	1	2	0	x	IIA	2.62	2	2	1	2	I	1	2	I	>14%	6-29	25	1	0	0	<10	>50
55	2021	1477	54	L	936249	10.5	10	2	1	1	2	1	x	IIIA	3.8	2	2	1	5	I	1	5	I	>14%	6-29	25	1	0	0	<10	<50
56	2021	1540	70	L	940132	2.5	10	1	1	0	2	1	x	IIIA	3.5	2	2	2	10	II	3	48	II	>14%	6-29	29	1	1	0	10-40	>50
57	2021	1570	54	L	936249	5	21	13	1	0	3	3	x	IIIC	6	2	1	2	12	I	3	44	II	>14%	6-29	29	1	1	0	10-40	>50
58	2021	1586	51	R	941346	1.5	10	0	0	0	1	0	x	IA	2.3	2	2	2	14	II	3	62	II	>14	>30	40	0	0	0	10-40	<50
59	2021	1678	65	R	946403	3.5	4	0	0	0	2	0	x	IIA	2.7	2	2	2	13	II	3	25	II	>14%	6-29	28	0	0	1	>40	>50
60	2021	1705	65	R	945963	6	19	1	1	0	4	1A	x	IIIB	3.7	2	3	1	8	I	3	22	III	>14%	6-29	28	0	0	0	<10	<50
61	2021	1852	42	L	39485	3.5	21	5	1	1	3	1	x	IIIA	4	2	2	3	18	II	3	50	II	>14%	6-29	28	0	0	0	<10	>50
62	2021	1970	65	L	39318	14	21	18	1	0	3	3		IV	11	2	2	2	10	II	3	45	II	>14%	6-29	27	0	0	1	<10	>50
63	2021	2155	55	R	39217	8	10	0	0	1	2	0	x	IIB	3.6	2	2	1	3	I	2	12	II	<14%	6-29	13	1	0	0	<10	>50
64	2022	321	57	L	63084	3	17	12	1	0	2	3a	x	IIIC	3	3	2	1	2	II	2	8	II	>14%	6-29	27	0	0	0	>40	>50
65	2022	493	41	R	65320	3	11	0	0	0	2	0	x	II A	2.6	2	2	2	8	II	3	32	II	<14%	<5	2	1	1	0	<10	>50
66	2022	551	41	R	68097	3	14	9	1	0	4	2A	x	III B	6.5	2	2	3	18	II	3	38	II	>14%	>30	40	1	1	0	<10	>50
67	2022	618	45	L	67214	3	5	0	0	0	2	0	x	II A	2.6	1	1	3	22	I	3	66	I	>14%	>30	40	0	0	0	<10	<50
68	2022	645	37	L	69623	5.2	5	5	1	1	4	3	x	III C	6.04	2	2	1	7	I	3	18	II	>14%	6-29	20	0	0	1	<10	<50
69	2022	690	61	R	71018	5.5	17	9	1	0	2	2A	x	IIA	3	2	2	3	17	II	3	50	II	>14%	6-29	25	1	1	0	10-40	>50
70	2022	717	37	L	71915	4.5	3	0	0	1	2	0	x	IIA	5	2	2	2	11	II	3	42	II	>14%	>30	30	0	0	0	<10	<50
71	2022	857	39	R	75439	1.8	6	0	0	1	1	0	x	IA	2.8	1	1	2	14	I	3	58	I	>14%	6-29	25	1	0	1	10-40	>50
72	2022	955	38	L	34745	3.5	24	0	0	0	2	0	x	IIA	4.7	3	3	2	10	III	3	45	III	<14%	6-29	10	0	0	0	<10	>50
73	2022	1167	30	L	83399	6	6	0	0	0	3	0	x	II B	5	2	2	2	10	II	3	60	II	<14%	6-29	8	0	0	0	<10	>50
74	2022	1097	68	R	81315	7	14	7	1	0	3	2A	x	III A	4.4	2	1	2	12	I	3	37	II	>14%	6-29	25	0	0	0	<10	>50
75	2022	1472	60	L	73975	2	14	0	0	0	1	0	x	I B	2.4	2	1	3	15	II	3	55	II	>14%	6-29	26	0	0	0	10-40	>50
76	2022	1817	40	L	114769	4.2	41	3	1	0	2	1	x	II B	3.84	1	1	2	13	I	3	18	I	<14%	6-29	11	0	0	1	10-40	>50
77	2022	2204	74	L	130799	6	21	17	1	1	4	2A	x	III B	6.2	3	2	2	13	II	3	50	III	<14%	6-29	12	0	0	1	10-40	>50
78	2022	2717	49	R	105872	5.2	14	7	1	1	3	2A	x	II A	5	2	2	3	15	II	3	50	II	>14%	>30	30	1	0	1	10-40	<50
79	2022	2820	55	L	143627	4	19	17	1	0	2	3A	x	IIIC	6	2	2	3	16	II	3	58	II	>14%	6-29	25	0	0	1	<10	<50
80	2022	2935	53	L	155702	4	1	0	0	0	2	0	x	II B	3.8	2	2	2	12	II	3	36	II	>14%	6-29	20	1	0	1	<10	>50
81	2022	2975	55	L	144626	5.3	9	0	0	1	3	0	x	II B	4.06	2	2	2	9	II	3	29	II	<14%	6-29	14	0	0	1	<10	>50
82	2022	3061	55	L	155400	3	0	0	0	0	2	0	x	II A	7	2	2	3	16	II	3	25	II	<14%	6-29	14	0	0	1	<10	>50
83	2023	17	31	R	176388	6.5	18	8	1	1	3	2A	x	III A	5.3	2	1	1	6	I	3	17	II	>14%	6-29	26	1	1	0	10-40	>50
84	2023	103	33	L	177470	4.5	19	0	0	0	2	0	x	II A	1.9	1	2	2	10	I	3	20	II	>14%	6-29	25	0	0	1	10-40	<50
85	2023	114	46	L	185191	4	2	0	0	0	2	0	x	II A	1.8	2	2	1	4	I	3	16	II	>14%	>30	30	0	0	1	10-40	>50
86	2023	252	48	L	185625	4.2	13	10	1	1	2	3A	x	III C	4.84	2	2	3	16	II	3	25	II	>14%	6-29	25	1	0	1	<10	>50
87	2023	272	61	L	92482	7.5	27	2	1	0	3	1	x	III B	4.5	1	2	3	15	II	3	58	II	>14%	6-29	20	0	0	0	<10	<50
88	2023	513	36	R	134051	2	20	0	1	0	1	1	x	I A	3	1	2	2	10	I	3	38	II	<14%	<5	2	0	0	1	<10	>50
89	2023	625	39	R	197794	6	6	2	1	0	3	1	x	IIIA	5.2	3	2	1	4	II	3	17	II	>14%	6-29	20	1	1	0	<10	>50
90	2023	1082	55	R	209821	8	13	10	1	1	3	2	x	IIIA	2.2	2	2	3	20	II	3	54	II	>14%	>30	20	1	0	1	<10	<50
91	2023	1150	52	R	211098	10	17	1	1	0	4	1	x	IIIB	4	2	2	3	15	II	3	67	II	>14%	>30	20	1	1	1	<10	<50
92	2023	1232	38	L	215963	52	25	14	1	1	1	3	x	III	5	2	2	2	12	II	3	50	II	>14%	6-29	20	1	1	1	<10	<50
93	2023	1281	57	L	56961	6	14	0	0	0	3	0	x	IIB	3.2	2	2	2	10	II	3	25	II	>14%	6-29	25	1	1	1	10-40	>50
94	2023	1287	63	R	218496	4	16	3	1	1	2	1	x	IIB	2.8	1	2	2	14	I	3	65	II	>14%	6-29	25	1	0	1	<10	<50
95	2023	1686	28	R	270102	5.5	14	0	0	0	2	0	x	IIB	2.86	3	3	2	11	III	3	51	III	>14%	6-29	20	0	0	0	<10	>50
96	2023	1934	40	R	241407	4.5	29	23	1	1	2	3A	x	IIIC	6.25	2	2	2	13	II	3	52	II	>14%	6-29	22	1	0	1	<10	<50
97	2023	1971	28	L	270102	5.5	14	0	0	0	2	0	x	IIB	2.86	3	3	1	3	II	1	3	I	<14%	<5	5	0	0	0	<10	>50
98	2023	1232	38	L	215963	1.5	25	14	1	0	1	3	x	III	5	2	2	2	12	II	3	50	II	>14%	6-29	20	1	1	1	<10	<50
99	2023	2605	57	R	224952	7	27	0	0	1	3	0	x	IIB	3.4	1	2	3	15	II	3	58	II	<14%	<5	3	0	0	1	<10	<50
100	2023	2640	52	R	257482	6	21	1	1	0	3	1	x	III	4	1	2	3	15	II	3	57	II	<14%	6-29	8	0	0	1	<10	<50
101	2023	2748	65	L	267791	3.5	9	0	0	0	2	0	x	IIA	2.64	1	1	2	11	I	3	58	I	<14%	<5	1	0	0	1	<10	>50
102	2023	2832	38	L	215963	2	25	14	1	1	2	3	x	III	5	2	2	3	18	II	3	53	II	>14%	6-29	25	1	1	1	10-40	<50