"IMMUNOHISTOCHEMICAL EXPRESSION OF MDM2 IN INVASIVE DUCTAL CARCINOMA OF BREAST AND ITS ASSOCIATION WITH HISTOPATHOLOGICAL PARAMETERS AND HORMONAL STATUS"

BY

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DISSERTATION SUBMITTED TO

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TAMAKA, KOLAR, KARNATAKA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF MEDICINE

IN

PATHOLOGY

UNDER THE GUIDANCE OF

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This study is carried out by **Dr. Damarasingu V Sai Priyanka[1] Dr. Kalyani R[1]** and **Dr. P.N. Sreeramulu[2]** in the Departments of Pathology[1] and Surgery[2] at Sri Devaraj Urs Academy of Higher Education and Research Tamaka, Kolar.

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Place: Kolar ***Dr. DAMARASINGU.V.SAI.PRIYANKA***

LIST OF ABBREVATIONS

BC- Breast Cancer

IHC – Immunohistochemistry

MDM2- Mouse Double Minute 2

IDC – Infiltrating Ductal Carcinoma

TDLU – Terminal duct lobular unit

WHO – World Health Organisation

DCIS – Ductal carcinoma in situ

AJCC – American Joint Committee on Cancer

MBR – Modified Bloom Richardson

H&E – Haematoxylin and eosin

NPI – Nottingham Prognostic index

TBS – Tris buffer Solution

ASCO – American Society of Clinical Oncology

ABSTRACT

BACKGROUND:

Worldwide, breast cancer ranks first with regards to incidence as well as mortality rates among females. Out of all the breast cancer types, ductal carcinoma is by far the most prevalent one. MDM2 exerts a key negative control on p53 by inhibiting its expression. Tumors with overexpression of MDM2 are linked to increased invasiveness, increased potential for metastasis, and resistance to radiation and chemotherapy. Death rates and recurrence rates for BC remain high despite individualized treatment protocols. In order to ascertain the elucidation of MDM2 IHC status in invasive ductal carcinoma and its relationship with tumor metastasis, tumor staging, lesion dimensions, lymph nodal staging, as well as hormone expression, this study has been undertaken.

OBJECTIVES:

1)To determine the expression of MDM2 in invasive ductal carcinoma.

2)To determine association of MDM2 with histopathological parameters and hormonal expression in invasive ductal carcinoma.

MATERIALS AND METHODS:

This research has assessed ninety-three cases of infiltrating ductal carcinoma that were surgically resected. All patient H&E slides were examined, and MDM2 immunohistochemistry was conducted. Clinicopathological information about the cases, including age, tumor staging, histological tumor grading, nodal staging, extranodal extension, TNM staging, and hormonal expression, was assessed and correlated with MDM2 expression.

RESULTS:

Patients in this study had an average age of about 53.7 years. Significant association was found between MDM2 and histological grading of tumor (MBR), tumor staging, nodal staging and TNM staging with a p-value of <0.001, <0.001, <0.001 and <0.028. There was no correlation that was statistically important reported with MDM2 & age group, size of the tumor, extra nodal staging, and NPI score. Majority of the tumors with Ki67 >14% showed an MDM2 score of 2 (39.7%) and 3 (29.3%) which implies that proliferative index was more in MDM2 positive cases and is statistically noteworthy with a p-value of 0.012. Among the four molecular types Luminal A and TNBC were determined to have a noteworthy statistical correlation having a probability value with 0.004 & 0.001 correspondingly, whereas no significant association was seen between Luminal-B and Her-2Neu Enriched. Most of the Luminal-A cases showed score of zero and 1 that is 21.9% and 46.9% respectively. Almost 60% of patients with TNBC showed a score of 3.

CONCLUSION:

MDM2 score reached its peak at 3 in TNM stage III and stage IV, indicating the enhanced aggressiveness of the tumor as the score increases. Prior treatment with MDM2 could potentially reduce the tumor burden and metastasis as most of the cases with MDM2 over expression was noted in subjects with extra nodal extension and higher TNM staging.

KEYWORDS: Mouse Double Minute 2 (MDM2), Infiltrating ductal carcinoma and Breast Carcinoma (BC).

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INTRODUCTION

INTRODUCTION:

When it comes to cancers affecting women, the most common and deadly is carcinoma of the breast, which ranks second globally. The fatality rate from breast carcinoma is 15%. Around 23 lakh new carcinoma cases are identified annually, with BC accounting one among every eight cases. Amongst Indian females, carcinoma of breast ranks highest with regard to cancer-related deaths. Nevertheless, it accounts for a negligible 11.1% of all cancer deaths in India. Globally, there were 34,65,951 newly diagnosed BC cases during the year 2020, with 11,21,413 deaths reported; in India, recently detected cases were 1,204,532, and there were 436,417 deaths. These figures are sourced from GLOBOCAN 2020. [1,2]

Multiple variables, such as exposure to certain environmental agents, hormonal imbalances, changes in genetic makeup, and other alterations, contributing for evaluation of BC. It kills more women than any other cancer in the world and is very common among women. [3-5] Epidemiological studies predict that approximately 2 million cases of BC will be reported by 2030. [6]

India witnessed a 50% increase in frequency during 1965 to 1985. [6] New cases reported within India during 2016 was 1,18,000 (95% CI: 1,07,000 to 1,30,000), with females accounting for 98.1% of those cases. Approximately 5,26,000 cases were involved (ranging from 4,74,000 to 5,74,000). With a 95% confidence interval ranging from 5.1% to 85.5%, the age-standardized incidence rate of BC grew in every state in the country between 1990 and 2016. [7]

Based on data from Globocan for 2020, BC patients made up 10.6% (9,408) among cancer deaths & 13.5% (17,361) of every case diagnosed with cancer across India, for a total risk of 2.81. [8] Except for the Nagpur PBCR, every PBCR that keeps track of cancer cases

has seen a huge rise in BC over the years. Cities had a higher rate of BC. Localized metastatic breast cancer was detected in a large proportion of patients. [9]

The cervix was where most cancers were found in India in the year of 1990. Mumbai had more cases of cancer than any other city (16.0% vs. 24.1%). In Bangalore (23.0% vs. 15.9%), Bhopal (23.2% vs. 21.4%), Chennai (28.9% vs. 17.7%), and Delhi (21.6% vs. 20.3%), BC had been found to be most common cancer type. However, the locations of cancer cases changed between 2000 and 2003. In most cases, the breast was the most common site, except in the Barshi (16.9% vs. 36.8%). The registries of Bhopal, Chennai, and Delhi showed a notable rise in BC cases. [10]

Among the BC cases analysed over a span of 5 years, the survival rate for individuals in the first stage was 95%. However, the survival rates dropped to 92%, 70%, and a mere 21% for those in 2^{nd}, 3^{rd} as well as 4^{th} stages, respectively. [11]

In Kolar, BC incidence among women constitutes 10.8%, with ductal carcinoma being the predominant type, accounting for 70-80% of cases. Lobular carcinoma and DCIS make up remaining 20% of cases. [12]

India's survival rate is low compared to Western nations can be attributed to factors like early disease onset at a younger age, advanced disease presentation leading to delayed definitive care, and inadequate or fragmented treatment. Among females worldwide, carcinoma of the breast is a leading multifactorial disease with regard to frequency & even mortality. It evolves by a combination of genetic, hormonal, as well as environmental changes. World Cancer Report 2020 states that timely and prompt detection & treatment are the finest efficacious measures to combat BC. [13]

In accordance with 2018 systematic review of 20 studies, expenditure for BC chemotherapy has significantly risen substantially with an increase in the stage of cancer during diagnosis. Expenses for BC treatment has decreased with early detection. [14]

Researchers have developed drugs that prevent MDM2 and p53 from interacting directly, increasing p53 levels and there by killing cancer cells. Chalcones are among the compounds that bind to MDM2 and increase p53 phosphorylation. [15] Because these drugs stop cancer cells from growing and cause apoptosis, the experimental results show that medications that target the relationship between MDM2 and p53 are effective at activating p53. [16]

Tumors with overexpression of MDM2 are linked to increased invasiveness, increased potential for metastasis, and resistance to radiation and chemotherapy. [17,18]

According to recent research, treating cancer patients with overexpressed MDM2 with MDM2 inhibitors in addition to chemotherapy or other targeted therapy may improve efficacy and lessen the development of drug resistance in the illness. [16]

Death rates and recurrence rates for BC remain high despite individualized treatment protocols. In order to ascertain MDM2 expression in invasive ductal carcinoma and its relationship with tumor metastasis, tumor staging, lesion size, nodal positivity, as well as hormonal expression, this study has been undertaken.

AIMS & OBJECTIVES

OBJECTIVES OF THIS STUDY:

1. To determine the expression of MDM2 in invasive ductal carcinoma
2. To determine the association of MDM2 with histopathological parameters and hormonal expression in invasive ductal carcinoma

REVIEW OF LITERATURE

REVIEW OF LITERATURE:

Using a gene probe for MDM2, Olineret et al. made the initial discovery of MDM2 in 1992. [19]

The MDM2 gene is an oncogene on chromosome 12 that codes for an E3 ligase. It tags p53 with ubiquitin and, after proteasome breakdown, keeps p53 at a low quantity in cells that are not under stress. When p53 is activated in response to cellular stressors, MDM2's inhibitory impact on p53 ends. Mice with MDM2 deficiency had embryonic mortality, while mice lacking both MDM2 and p53 developed normally, showing that MDM2 exerts a key negative control on p53. [20]

Although normal cells have low levels of MDM2, its significance in cancer cells devoid of functional p53 is highlighted by the fact that cancer cells display higher levels of MDM2 expression and inactivated p53. [21]

Tumor cells can develop resistance to chemotherapy and radiation treatment when MDM2 is overexpressed and amplified. There are two primary pathways that contribute to chemotherapeutic resistance caused by MDM2 overexpression: one is dependent on the p53-MDM2 loop, and the other is independent of it. Both cancer cells and individuals who have developed a primary resistance to EGFR inhibitors have been found to overexpress MDM2. In order to make tumor cells resistant to doxorubicin, MDM2 primarily downregulates Wtp53 expression. [18]

In addition to overexpression of Her-2 Neu and Ki67, Opoku F et al. observed that 66.7% of BC patients had MDM2, along with overexpression of Her-2 Neu and Ki67.[20]

An amplified version of the MDM2 gene promotes the development and advancement of ER+ BC with NSG humanized mouse model used for preclinical research. In

vitro, blocking MDM2 decreased the migration and cancer cells propagation caused tumor cell apoptosis. A negative outcome of Luminal Breast Cancer illness is also closely linked to an MDM2 increase. Therefore, a potential method for treating Luminal Breast Cancer with an increased MDM2 expression is by targeting MDM2 in conjunction with other treatments. [22]

The genetic study of the MDM2 polymorphism (rs2279744) was investigated in 136 patients by Floris.M. et al. All individuals who took part in this study were asked to fill out a questionnaire that sought to determine the causes of BC. Blood samples were used to extract DNA from the genome and detected by polymerase chain reaction (PCR). Out of 136 breast cancer patients, 53 had MDM2 over expression and were on oral contraceptives for more than 10 years. [23]

In their analysis of 182 breast cancer cases, Han M et al. examined differences in the copy number (CN) of MDM2. In samples where MDM2 CN amplification was present, luminal B type features were more abundant, and TP53-signature score was high. MDM2 mRNA upregulation was associated with a poorer outcome and showed no correlation with endocrine therapy. Despite the lack of association with endocrine treatment, MDM2 mRNA expression was linked to a worse prognosis. These copy number alterations enhanced classification of subtypes and prediction of prognosis in initial stage but with luminal type BC patients or TP53 wild-type. [24]

Research of Bianco G et al. indicate that GATA 3 and MDM2 are synthetically lethal in 2,379 patients with ER-positive BC. An increased fraction of apoptotic cells of 15-20% was seen after dual-GATA3/MDM2 silencing. Cases where GATA3 was lacking showed a marked decrease in tumor growth when MDM2 was either depleted or pharmaceutically inhibited. When GATA3 expression is down, cells become more

susceptible to drugs that block MDM2. Clinical trials are now investigating the effectiveness of targeting MDM2 in patients with ER-positive and GATA3-mutant BC. [25]

Researchers Qi M et al. looked at 107 EC-T neoadjuvant breast cancer patients. Tumor growth was detected in mice treated with intraperitoneal injections of paclitaxel. S100A6 inhibited MDM2, which in turn reduced tumor growth and enhanced paclitaxel sensitivity; 55 of 107 cases with high S100A6 expression and 47 of 107 cases with low MDM2 expression were involved in this study. A p-value of less than 0.0001 was seen in 31 cases, or 59.6% of the total, indicating high MDM2 expression, while the remaining 52 cases demonstrated low S100A6 expression. The researchers discovered that S100A6 facilitated the MDM2 translocation into cytoplasm from nucleus, where it is bound to Herpesvirus Associated Ubiquitin Specific Protease (HAUSP) binding site on MDM2. This resulted in MDM2 self-ubiquitination and degradation by interfering with its interactions with HAUSP-DAXX. It allowed the researchers to slow the BC progression and increase paclitaxel sensitivity in animal models. There was a negative relationship between S100A6 and MDM2, and a complete pathologic response was more likely at higher expression levels. [26]

Using 128 BC cells and 12 normal cells taken from Xi'an Alina Biotechnology Co., Ltd., Tang Y et al. investigated GSG2 expression by immunohistochemistry in human surviving BC patients who did not undergo chemotherapy or radiation treatment. MDM2 acts like an E3 ubiquitin ligase that GSG2 employs for controlling the process of E2F1 ubiquitination. Low GSG2 expression was observed in 55 (43%) of 128 tumor tissues, while high GSG2 expression was observed in 73 (57%) cases. The expression of GSG2 was shown to be low in all twelve normal tissues, with a p-value of less than 0.001. The malignant transformation of BC was decreased by GSG2 knockdown through apoptosis while reducing proliferation. Additionally, tumor growth was attenuated by speeding the ubiquitination of

the E2F1 protein. Overexpression of GSG2, which interacts with MDM2 to inhibit E2F1 degradation, has been linked to a poorer prognosis in BC cases. They found that GSG2 promoted BC formation and progression by ubiquitinating E2F1 through MDM2, suggesting that this protein may be a therapeutic target. [27]

Ayoup MS et al. suggested using Optimized Passerini caspase activators to alter the signaling axis of P53 MDM2 pathway, which has been examined using flow cytometry, in order to induce P53-dependent apoptosis in a synergistic fashion. MDM2 regulates P53 transcriptional activity; nevertheless, overexpression of MDM2 causes it to behave like an oncogene by hiding the P53 N-terminal transactivation domain and triggering its proteasomal destruction. Because MDM2 inhibitors include numerous aromatic rings, they effectively sensitized tumors and induced apoptosis through direct caspase activation and P53-MDM2 axis blockage. Some cyclized imidazolidine derivatives showed 2-4 times greater potential than doxorubicin for selectively killing breast cancer cells by activating caspase 3/7. These compounds were 3, 4, 8, and 12. The antiapoptotic oncogene Bcl-2 was downregulated, and the apoptosis regulator BAX was overexpressed as a result of these chemicals. Spiro oxindoles and other MDM2 inhibitors were being evaluated in human clinical trials. [28]

Singh et al's research using RNA-sequence to identify a series of important proteins implicated in the apoptotic pathway, with a specific focus on TNBC cell line (MDA-MB-231). Among 15658 genes that showed differential expression, An overall 808 genes were identified as being up-regulated in this specific cell line. Subsequently, these genes were classified into 35 clusters according to their analogous cellular and molecular functions. A single cluster, comprising of 18 genes linked to the function of repairing DNA damage, was chosen for additional analysis. The cluster contained the topoisomerase IIα gene (5GWK) & the p53-MDM2 gene (4OQ3). Ligand-based screening techniques revealed that resveratrol, a bioactive molecule found in plants, had a stronger binding affinity to Topo IIα compared to

the control drugs doxorubicin and etoposide, which are commonly used in treatment. In addition, resveratrol demonstrated the ability to target multiple aspects of TNBC thereby specifically attacking the p53-MDM2 complex. [29]

MDM2 amplification in BC cases are susceptible for development of hyper progressive disease (HPD) after undergoing immunotherapy for cancer by decreasing effector-cells in the peripheral blood, which significantly impacts survival rates in a negative way with an overall survival of 11months. 0-63.6% BC patients were found to have MDM2 overexpression. The overexpression of this gene stimulates the angiogenesis and affects the release of cytokines, which creates an environment conducive to angiogenesis. As a result, tumor cells have enhanced ability to travel and infiltrate nearby tissues, ultimately boosting immune evasion and tolerance. Inhibiting MDM2 can stimulate production of TNF-α, Interferon-γ, & Interleukin-15 through activation of CD8+T and even natural killer cells. Angiogenesis and tumor growth can be effectively inhibited by combining bevacizumab, an inhibitor of vascular endothelial growth factor (VEFG), with Nutlin-3/APG-135 an inhibitor of mitogen-activated protein 2. [30]

The study by Ma X et al, included a group of 157 individuals with BC. Interactions among ZNF500, MDM2, and P53 were investigated using a Co-Immunoprecipitation assay. After overexpressing ZNF500 in MCF-7 cells, the distribution of MDM2 subcellularly was examined by means of Western blot & immunofluorescence (IF) experiments. There was a dose-dependent decrease in MDM2–p53 binding observed when ZNF500 expression was upregulated in MCF-7 cells. Comparable outcomes were noted when there was an excessive expression of MDM2 in the presence of ZNF500. ZNF500 overexpression was observed to completely suppress the growth of BC cells both in laboratory settings and as well as in living organisms. This was achieved by blocking MDM2's ability to degrade p53 via direct interaction with p53 via its C2H2 domain, which activated the p53-p21-E2F4 signalling

pathway. Consequently, p53 is stabilized. A difference between ZNF500-ΔC2H2 and ZNF500-FL is that the former can disrupt MDM2's interaction with p53, leading to p53 degradation through ubiquitin-mediated breakdown by MDM2. This disturbance impedes the multiplication of BC cells, amplifies DNA harm, thereby heightens the vulnerability of BC patients to chemotherapy. [31]

In a Research by Li Y et al, they have utilized molecular docking, Surface Plasmon Resonance, Cellular Thermal Shift Assay, as well as Western blot techniques which has demonstrated that both GAA-PROTAC's (Ganoderic Acid A-Proteolysis targeting chimera) V9 and V10 have the ability to connect with MDM2 and induce protein degradation via Ubiquitin-Proteasome System. At 50 μg/mL, V9 & V10 inhibited MDM2 degradation by employing a ubiquitin-proteasome system-dependent mechanism, leading to a 27.2% inhibition rate, making them more effective against breast cancer. Additionally, V10 has demonstrated the greatest selectivity in the treatment of TNBC. [32]

BREAST ANATOMY: [33,34]

Essentially, breast tissue is just an altered sweat gland. Although it is fully formed in girls following puberty, in males it is still in its early stages. It is an accessory organ essential for nursing and a vital part of the reproductive system in women.

DEVELOPMENT: [33,34]

In the fourth week of fetal development, an epidermal thickening known as mammary ridge forms within the ventral region of the body, and a milk line, or Schultz line, which runs from the axilla to the groin forms later. From this ridge, the breast tissue develops. The milk line could be adorned with accessory breast tissue. Both the right and left mammary glands are produced from the pectoral portion of the milk ridge. A mammary pit replaces the mammary ridge remnant. From the floor of the mammary pit, 15-20 secondary

buds emerge, branch out, and eventually become the canalized gland lobes. Tanner explains that the hypophysis cerebri secretes the lactogenic hormone prolactin, which induces the development of breast tissue throughout puberty (Fig. 1). The glandular element, including ducts and alveoli, is formed by the ectoderm, while the stromal component, including connective tissue and vessels, is born from the mesenchyme.

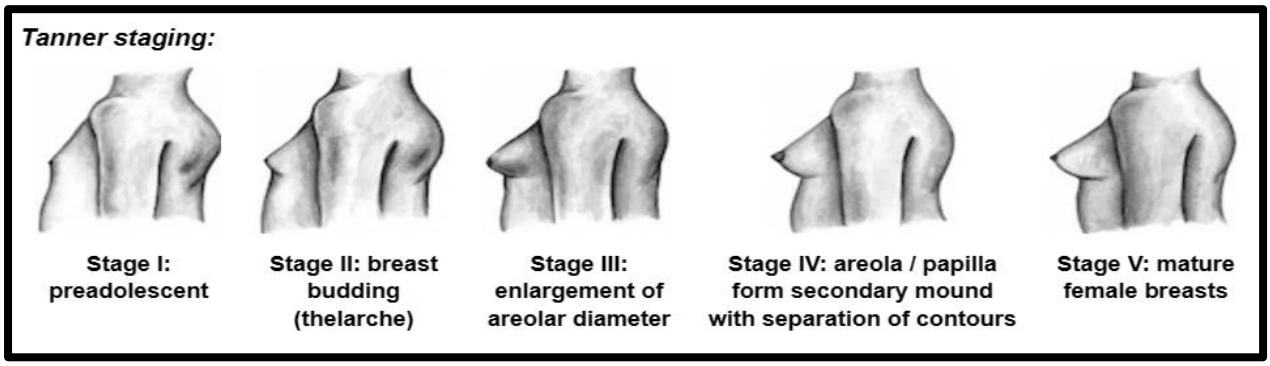


FIGURE-1: Tanner's Developmental Staging Of Breast [35]

STRUCTURE OF THE BREAST: [33-35]

Superficial pectoral fascia encircles breast tissue and even runs along chest wall, while the deep pectoral fascia provides support. It begins at the 2nd rib and continues to the 6th rib, along the midline between the sternum's medial border and midaxillary line. The mammary gland extends laterally as the axillary tail of Spence and terminates at the mid-axillary line. It traverses deep fascia through foramen of Langer, originating from the upper lateral portion of the gland. Due to its location in the retromammary region, which consists of loose areolar tissue, it is able to smoothly move over the pectoralis major muscle without being restricted by the pectoralis fascia. Cooper's ligament is a suspensory ligament that connects the nipple and areola to the supporting structure of the breast. It is located at the 4th intercostal gap & can be demonstrated in Figures 2 and 3. Surface sebaceous glands are connected to collecting ducts that open into the nipple; these glands are identified as Montgomery's glands. On top of the areola, you can see little bumps called Morgagni's tubercles. Mammary gland is

comprised of 15-25 lobes known as alveoli, each of which contains a lactiferous sinus and a branching duct system referred to as a lactiferous duct. From the collecting ducts, these lactiferous ducts branch out to the TDLUs. At TDLUs, which change with age, lactation, parity, and hormonal condition, the lactating breast produces milk. There is a decrease in the lobular unit and an increase in adipose tissue after reproductive age, whereas the main duct system is preserved. The gradual replacement of glandular tissue by fat causes a denser backdrop in older women as opposed to younger ones. The gland is mostly composed of fatty stroma, with the exception of the areola and nipple, which do not contain any stroma. An individual's chance of getting breast cancer, particularly aggressive breast cancer, increases four to six times as the density of their breasts rises. Although they are more prevalent in women with dense breasts than fatty ones, stromal cells nonetheless play an important role in accelerating carcinogenesis. BI-RADS Score 1 indicates very dense breast tissue, whereas BI-RADS score 4 indicates very adipose tissue. These four patterns of density are primarily defined by the BI-RADS vocabulary, which is maintained by the American College of Radiology (ACR).

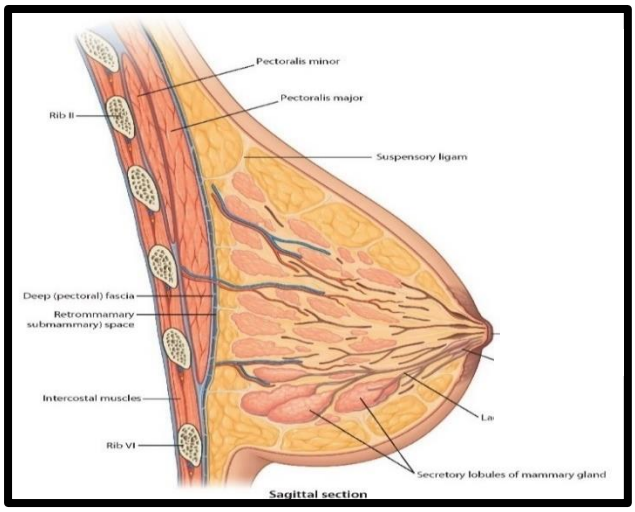


FIGURE-2: Normal Anatomy of The Breast [33]

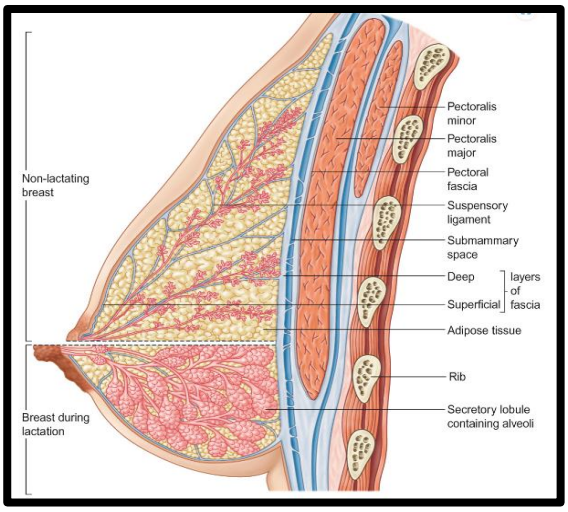


FIGURE-3: Normal Vs Lactating Breast [33]

VASCULAR AND NERVE SUPPLY: [33]

A number of arteries, including those in the axilla, posterior intercostal, and internal thoracic regions, supply blood to the mammary gland. A system of superficial veins and deep veins, which are paired with arteries. Superficial veins empty through internal thoracic vein neck's lower region, while the deep veins empty into the same veins in the chest, the arms, and the back (Fig. 4). Cancer of the breast that has spread to the blood vessels can metastasize to the spine. The breast receives sensory input from the anterior and lateral cutaneous branches of the 4th–6th intercostal nerves. These neurons serve the dual function of providing sensory innervation to the skin and autonomic to smooth muscles and blood vessels. Anterior portion of the pituitary gland, known as the pars anterior, releases the hormone prolactin. Prolactin is responsible for regulating the production of milk, rather than transmitting nerve signals (Fig. 4).

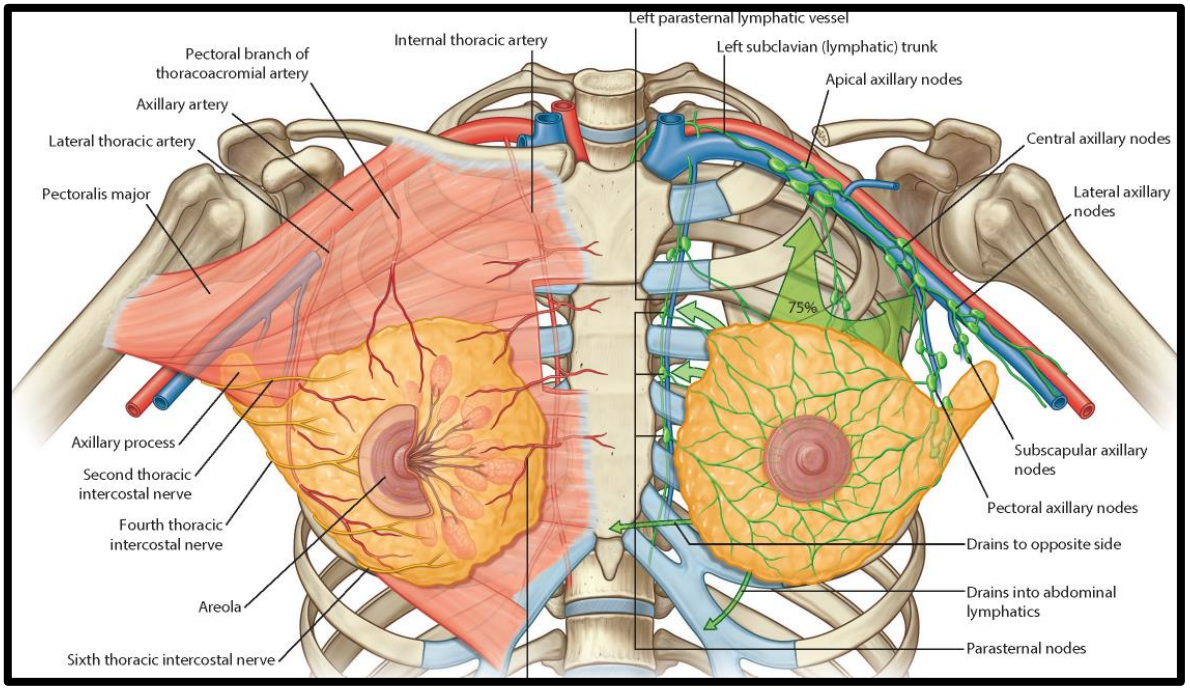


FIGURE-4: One Half of The Picture Depicts the Vascular and Nerve Supply of the Breast, and the Other Half Illustrates Lymphatic Drainage [33]

LYMPHATIC FLOW-BREAST: [36]

Lymphatic system of mammary gland is organized as follows (Fig. 4).

a. The lymphatic vessels that supply the parenchyma and skin around the areola and nipple pass through a complex system of subareolar plexus. These vessels primarily empty laterally into the group of axillary nodes located anteriorly, where they come into direct contact with the axillary tail of Spence. A small number of lymphatic nodes flow via the posterior group. Both groups of lymphatic nodes eventually make their way to the central nodes, which in turn send their lymph to the apical nodes in the axillae (Fig. 5).

b. Some of them drain to the supraclavicular nodes, the lowest nodes in the deep cervical chain, while others drain to the areola and nipple, which cover the breasts but do not. In this manner, cancer can spread to the opposite breast by crossing the midline (Fig. 6). Edema, or skin discoloration, can occur when superficial lymphatics get blocked, giving the patient a peau d'orange like appearance. Seventy-five percent of the lymphatic flow is delivered to the axillary nodes, while twenty percent to internal mammary nodes inside as well as outside of breast, and rest of five percent to posterior intercostal nodes. Through clavipectoral fascia & pectoralis major muscle, deep surface of breast travels to mammary nodes, which are located inside the breast. Spread from inferomedial region of breast tissue to the peritoneum, liver, and pelvic organs is possible via lymphatics communicating with the sub peritoneal plexus in the abdominal cavity.

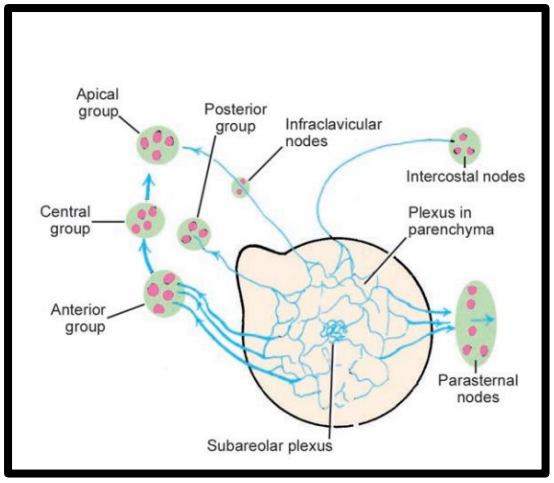


FIGURE-5: Flow of Lymph from Breast Tissue along with Skin of Nipple & Areola [36]

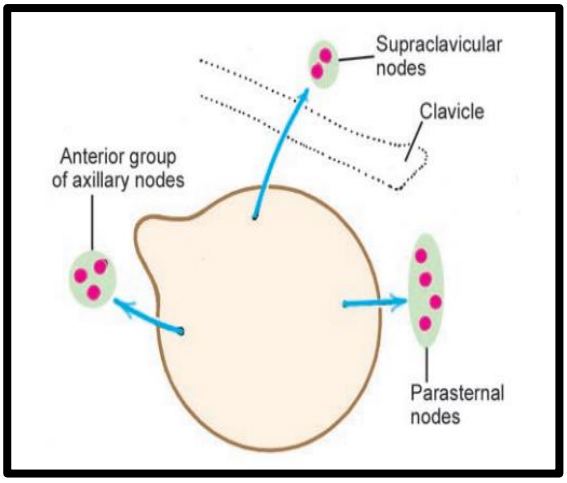
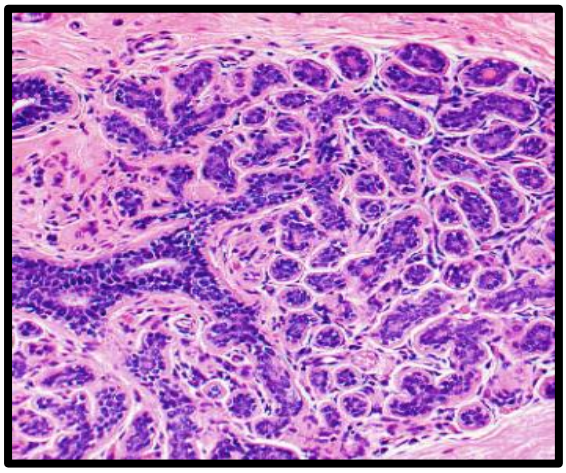


FIGURE-6: Lymphatic Drainage of Breast Tissue Skin Except Nipple & Areola [36]

NORMAL HISTOLOGY - BREAST: [37,38]

Breast tissue is an example of a cutaneous adnexal structure that has been changed. It is made up of central lactiferous channels that start at the nipple and branch out until they induce clusters of secretory glands that look like grapes, called lobules. In the fifth week of gestation, the embryo begins to form breasts in the shape of ectoderm thickenings that run from the axilla to the groin and are known as mammary ridges or milk lines. Aside from a small patch in the pectoral area, most of this thickening goes away. A fibro-adipose stroma encases ductless and lobulated acinar units, as well as branching ducts, in an adult female's breast. The structure that makes up TDLUs are the alveolar glands clusters called "lobules," which are linked to one terminal ductule by loose intralobular connective tissue. Pathologic diseases affecting the breast often begin in these specific functional and anatomical units. Ducts and lobules consist of two distinct layers: the myoepithelial basal layer and the cuboidal to columnar luminal layer. The morphologies of myoepithelial cells vary greatly, ranging from flat to epithelioid with transparent cytoplasm.

Typically, there is a clear demarcation between the intralobular stroma and the thicker, collagenized, paracellular stroma that lies between the lobules. Younger women tend to have denser connective tissue; however, the ratio of dense stroma to adipose tissue can vary. Based on their outward appearance, there are primarily three distinct kinds of breast lobules. Prepubescent and lactating women typically have type 1 lobules, which are the most basic. Parous and postmenopausal women typically have the most developed lobules, type 3. Increased alveolar buds and more branching characterize the transition from type 1 to type 3.



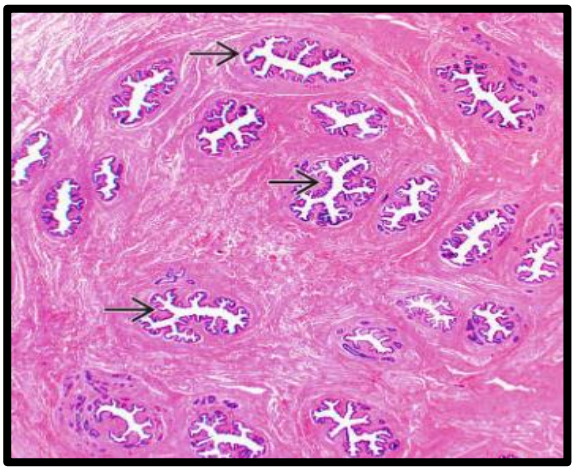


FIGURE-7&8: Lactiferous Sinuses & Terminal Ductal Lobular Unit [38]

BREAST CARCINOMA:

WHO BREAST TUMOURS CLASSIFICATION (5th EDITION 2019): [39]

I. Tumors with Epithelial Origin:

A) Invasive Breast Carcinoma:

1. Infiltrating ductal carcinoma (NOS)
2. Oncocytic carcinoma
3. Lipid rich carcinoma
4. Glycogen rich carcinoma
5. Sebaceous carcinoma
6. Lobular carcinoma NOS

7. Tubular carcinoma
8. Cribriform carcinoma
9. Mucinous adenocarcinoma
10. Mucinous cystadenocarcinoma
11. Invasive micropapillary carcinoma of breast
12. Metaplastic carcinoma

B) Rare salivary gland type tumors:

1. Secretory carcinoma
2. Acinar cell carcinoma
3. Mucoepidermoid carcinoma
4. Polymorphous adenocarcinoma
5. Adenoid cystic carcinoma
    (i) Classical adenoid cystic carcinoma
    (ii) Solid basaloid adenoid cystic carcinoma
    (iii)Adenoid cystic carcinoma with high-grade transformation
6. Tall cell carcinoma with reversed polarity

C) Neuroendocrine neoplasms:

1. Neuroendocrine tumor
2. Grade 1 Neuroendocrine tumor
3. Grade 2 Neuroendocrine tumor
4. Neuroendocrine carcinoma NOS
5. Small cell Neuroendocrine carcinoma
6. Large cell Neuroendocrine carcinoma

D) Tumors with Epithelial and Myoepithelial component:

1. Pleomorphic adenoma

2. Adenomyoepithelioma
3. Adenomyoepithelioma with carcinoma
4. Epithelial-myoepithelial carcinoma

E) Non-Invasive lobular neoplasia:

1. Atypical lobular hyperplasia
2. Lobular carcinoma in situ
    (i) Lobular carcinoma in situ classical variant
    (ii) Lobular carcinoma in situ florid type
    (iii) Lobular carcinoma in situ pleomorphic type

F) Ductal carcinoma in situ (DCIS):

Non-infiltrating type of Ductal carcinoma

(i) Low Grade DCIS
(ii) Intermediate Grade DCIS
(iii) High Grade DCIS

G) Benign proliferation of epithelium and its precursors:

1. Usual ductal hyperplasia
2. Columnar cell lesion along with flat epithelial atypia are taken into account
3. Atypical ductal hyperplasia

H) Adenosis and benign sclerosing lesions:

1. Sclerosing adenosis
2. Apocrine adenoma
3. Micro glandular adenosis
4. Radial scar / complex sclerosing lesion

I) Papillary neoplasms:

1. Intraductal papilloma

2. Papillary Ductal carcinoma in situ
3. Encapsulated papillary carcinoma
4. Encapsulated papillary carcinoma with invasion
5. Solid papillary carcinoma in situ
6. Solid papillary carcinoma with invasion
7. Intraductal papillary adenocarcinoma with invasion

J) Adenomas:

1. Tubular adenoma
2. Lactating adenoma
3. Duct adenoma

II. Mesenchymal tumors:

A) Vascular tumors:

1. Hemangioma:
    i. Perilobular hemangioma
    ii. Venous hemangioma
    iii. Cavernous hemangioma
    iv. Capillary hemangioma
2. Angiomatosis:
    i. Atypical vascular lesion
    ii. Lymphatic atypical vascular lesion similar to lymphangioma
    iii. Vascular atypical vascular lesion similar to hemangioma
    iv. Post radiation angiosarcoma
3. Angiosarcoma
    i. Epithelioid angiosarcoma

B) Tumors of Fibroblasts and Myofibroblasts:

1. Nodular fasciitis
2. Myofibroblastoma
3. Desmoid type fibromatosis
4. Inflammatory Myofibroblastic tumor

C) Peripheral nerve sheath tumors:

1. Schwannoma
2. Neurofibroma
3. Granular cell tumor
4. Malignant Granular cell tumor

D) Smooth muscle tumors:

1. Leiomyoma
    i. Cutaneous leiomyoma
    ii. Leiomyoma of the nipple and areola
2. Leiomyosarcoma

E) Adipocytic tumors:

1. Lipoma
2. Angiolipoma
3. Liposarcoma

F) Other mesenchymal tumors and tumor-like conditions:

1. Pseudo Angiomatous Stromal Hyperplasia (PASH)

III. Fibroepithelial tumors:

1. Fibroadenoma
2. Phyllodes tumor

i. Periductal stromal tumor

3. Benign Phyllodes tumor
4. Borderline Phyllodes tumor
5. Malignant Phyllodes tumor
6. Hamartoma

IV. Tumors of the nipple:

1. Nipple adenoma
2. Syringoma
3. Paget's disease of the nipple

V. Malignant lymphoma:

1. Diffuse large B cell lymphoma
2. Burkitt lymphoma /Acute leukaemia, Burkitt type
    i. Endemic Burkitt lymphoma
    ii. Sporadic Burkitt lymphoma
    iii. Immunodeficiency associated Burkitt lymphoma
3. Breast implant associated anaplastic large cell lymphoma
4. Mucosa associated lymphoid tissue lymphoma
5. Follicular lymphoma

VI. Metastatic tumors

VII. Tumors of the male breast:

1. Gynecomastia
2. Carcinoma

iv. Invasive carcinoma

v. In situ carcinoma

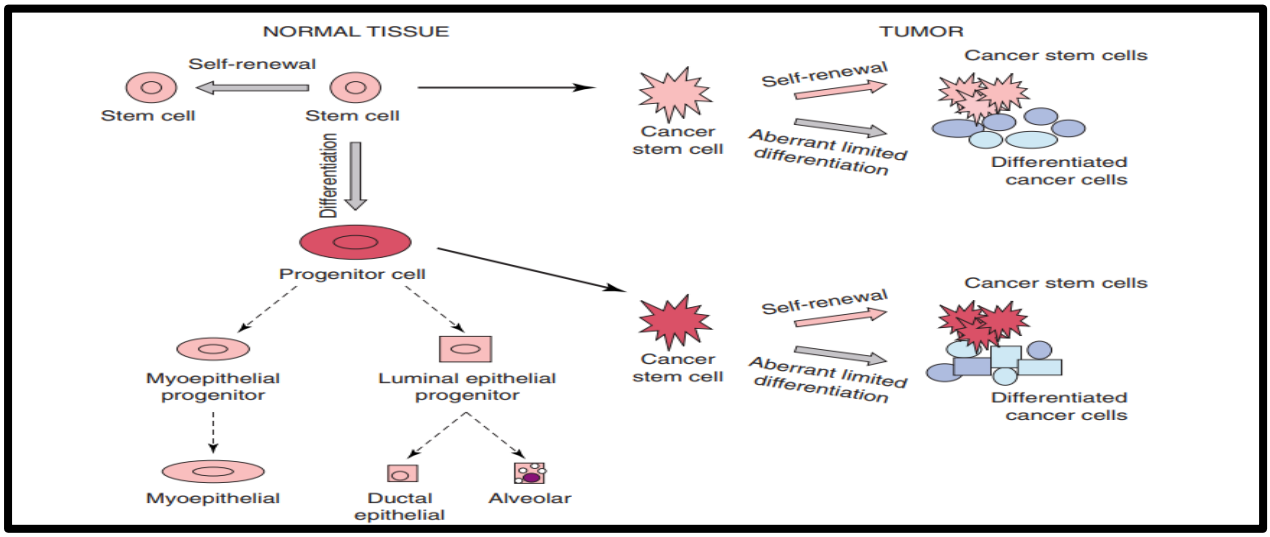
ETIOPATHOGENESIS: [40,41]

Breast cancer risk increases in patients whose families have the disease, especially those with certain genetic abnormalities including BRCA1, BRCA2, and p53. Symptoms of estrogen excess in women include early menarche, delayed menopause, extended reproductive life, nulliparity, and a low estrogen-secreting tumor prevalence in the ovaries. In industrialized nations, the prevalence of BC is higher due to numerous risk factors, such as increased intake of alcohol, smoking, & by utilising breast augmentation procedures.

CLINICAL FEATURES: [40,41]

Individuals who have a family or personal record of BC have greater likelihood of disease contraction, especially if they possess BRCA1, BRCA2, or p53 mutated genes. Nulliparity, early menarche, a high incidence of low Estrogen-secreting tumors in the ovaries, an extended reproductive lifespan, delayed menopause, and early menarche are all indications of an excess of Estrogen in women.

FIGURE-9: THE FLOW CHART PRESENTED BELOW ILLUSTRATES SEQUENTIAL EVENTS RESPONSIBLE FOR TUMOR PROPAGATION: [42]



INVASIVE DUCTAL CARCINOMA, (NOS) DEFINITION:[43]

The diverse collection of cancers that cannot be histologically defined is known as invasive ductal carcinoma, not otherwise specified (NOS). Of all forms of invasive BC, its occurrence is frequently encountered. Mammary duct epithelial tumors typically manifest in women over the age of 40.

MACROSCOPY: [43]

Malignant tumors can range in size from soft to hard, have a stellate pattern of irregular shapes, and have moderately to poorly defined borders. The tumor feels rough when sliced. Typically, the sliced surface has a greyish-white color and could have a desmoplastic reaction around it. In TNM staging, tumor size is a critical factor. Areas of bleeding and necrosis are also visible.

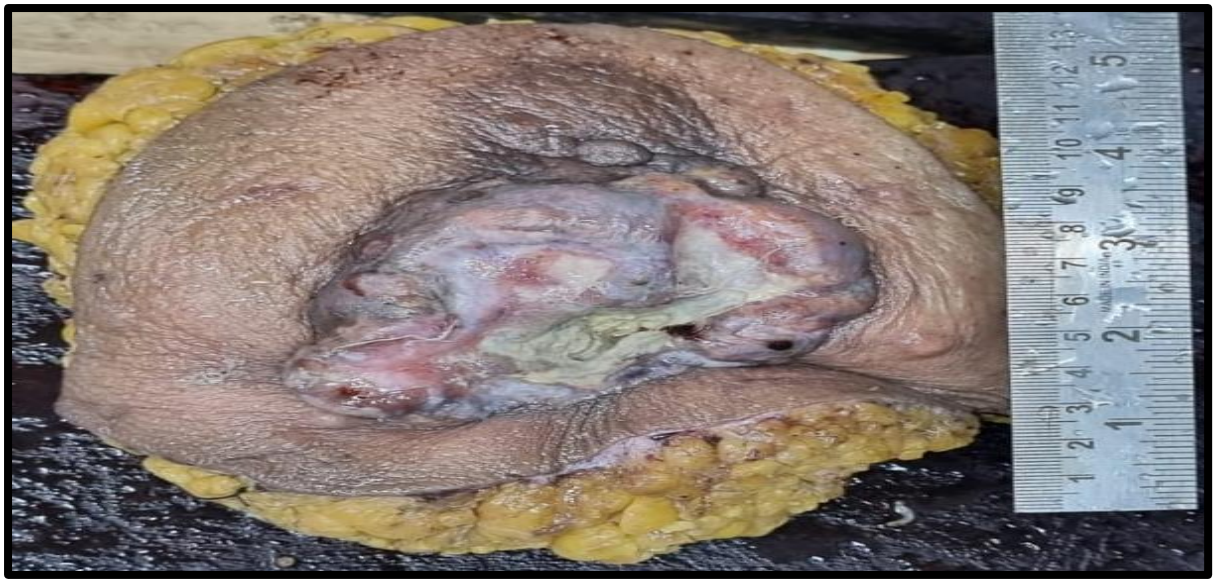
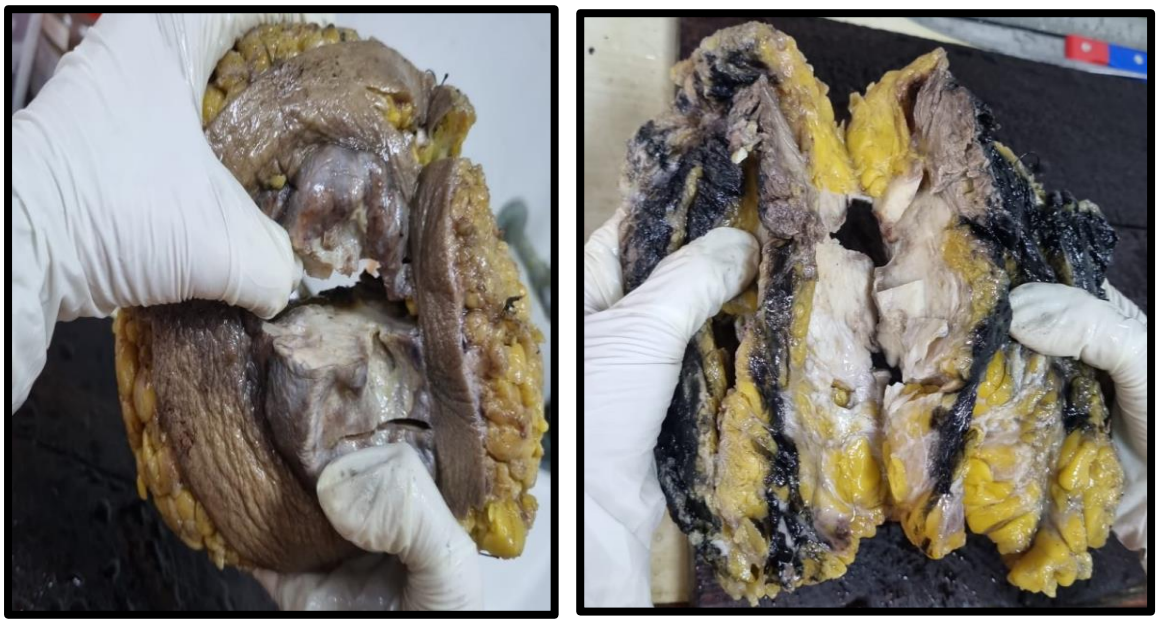


FIGURE-10: Gross image of mastectomy specimen showing skin ulceration and involvement of nipple areola complex by tumor (B/4213/23)



FIGURES-11&12: Cut surface showing homogenous grey-white lesion with surrounding desmoplasia and inked deep resected margin (B/4213/23)

HISTOPATHOLOGY: [43]

While most tumors display their cells in cords and trabeculae, some can have a solid or syncytial pattern with very little stroma surrounding them and even diffuse infiltration. The nuclear pleomorphism of individual cells can range from highly pronounced with numerous nucleoli to moderately pronounced with few nucleoli and an abundance of eosinophilic cytoplasm, and the sizes of these cells can vary widely. As many as 80% of tumors will have comedo type DCIS foci. Tumors are further categorized based on their differentiation into well, moderate and poor type of carcinomas based on nuclear atypia, mitotic activity, and tubular development. There was a marked decrease in tubule development, extreme nuclear atypia, and significant mitotic activity as the tumor grade increased. Grading will determine the prognosis.

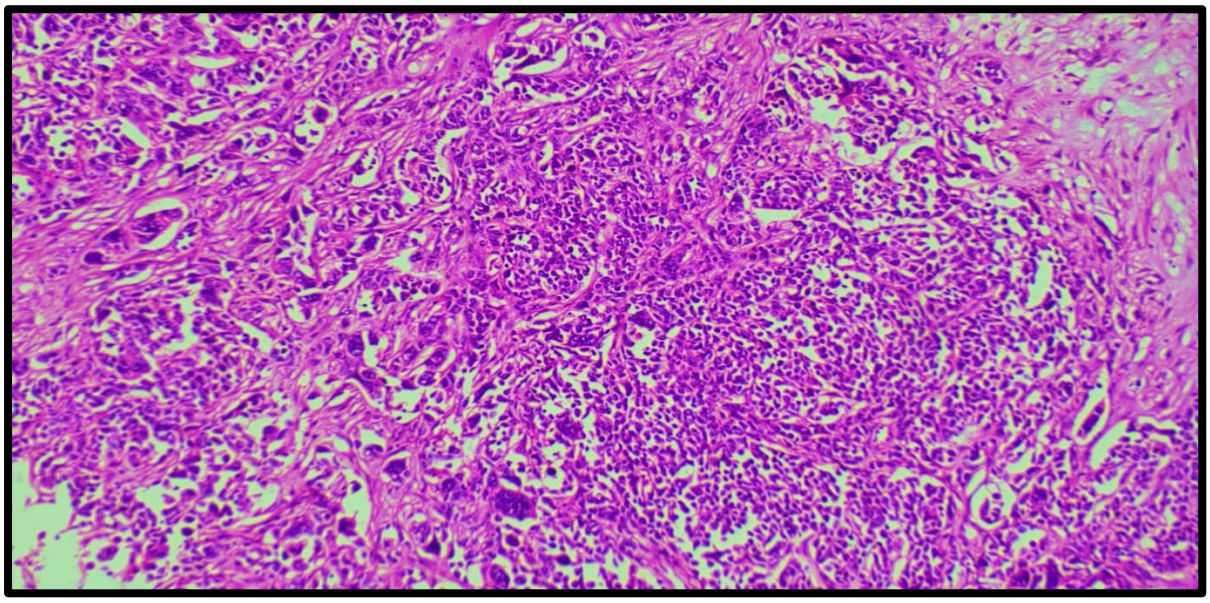


FIGURE-13: Microscopy (100X): showing tumor cells are organized in nests & sheets; individual cells are round to oval in shape having moderately pleomorphic nuclei with vesicular chromatin as well as inconspicuous nucleoli; Invasive Ductal Carcinoma, NST (B/297/24)

MICROGLANDULAR ADENOSIS: [42-45]

It consists of small glands that lack myoepithelial cells and are composed of solitary layer of epithelial cells. However, these glands do not compress the surrounding stroma.

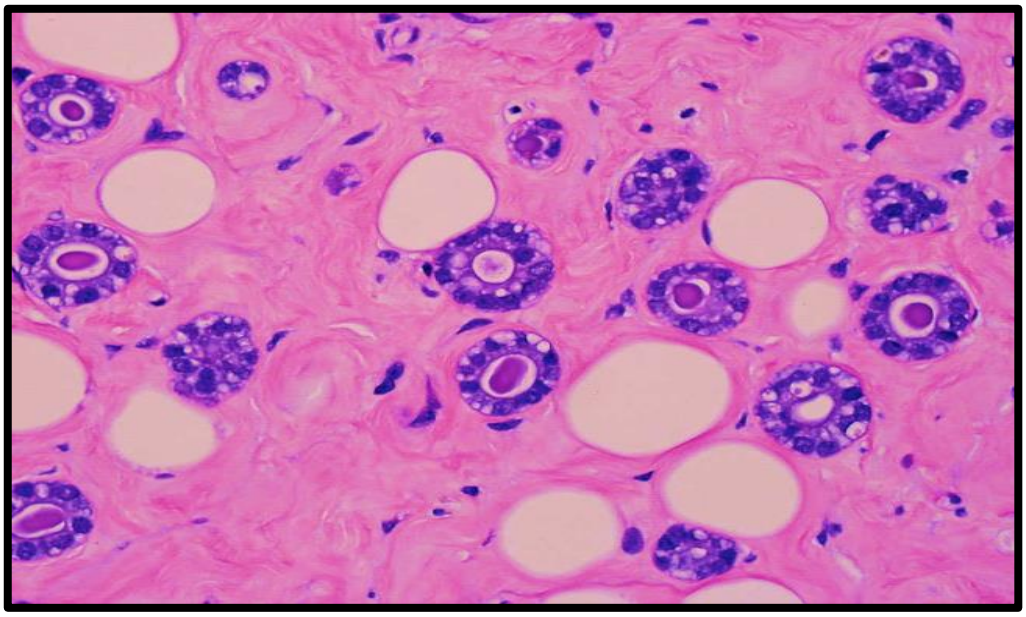


FIGURE-14: Microscopy (400X): Microglandular adenosis is defined by spherical glands devoid of myoepithelial cells and with a distinctive single-cell lining and central eosinophilic secretory substance. [44]

TUBULAR ADENOMA: [42-45]

A palpable, painless, solitary, freely mobile, and well-defined mass is the hallmark of tubular adenomas, which are slow-growing tumors. Hard tumors that are not encased and have a solid, uniform, or rubbery surface texture upon cut. Epithelial and myoepithelial cells form tightly packed benign ductules in tumor cells. These ductules have modest lumina and very little stroma and lymphocytes.

DUCTAL CARCINOMA IN SITU: [42-45]

Nuclear pleomorphism, size, nucleoli, mitotic figures, and comedo necrosis are used to further evaluate it as low, middle, or high. Typically found in conjunction with comedo necrosis, high-grade DCIS is defined by largely pleomorphic nucleus (almost double the RBC's size), vesicular chromatin, conspicuous nucleoli, and an abundance of mitotic figures.

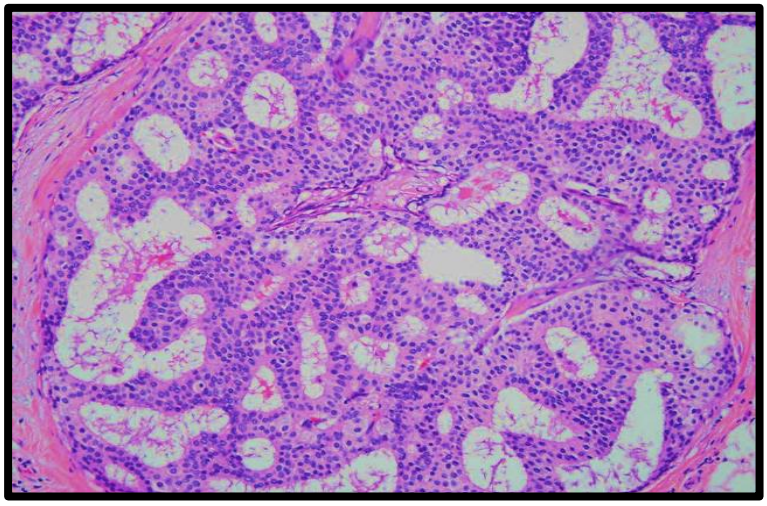


FIGURE-15: Microscopy (100X): Low grade DCIS: Contiguous spaces are involved by atypical cells exhibiting distinct cell border. [44]

INVASIVE CRIBRIFORM CARCINOMA: [42-45]

On the surface, tumors look like firm masses with a stellate shape. Under the microscope, a desmoplastic stroma with surrounding fat infiltration reveals irregularly formed epithelial nests with cribriform gaps. A cribriform DCIS is the most similar type; it has uniform cell proliferation and secondary spaces with a low to intermediate nuclear grade.

TUBULAR CARCINOMA: [42-45]

Typically, it is less than 2 centimeters in size and makes up 2% of breast malignancies. Because they are less aggressive, these tumors improve the prognosis and lead to more frequent mammograms. T1 is the most common stage for lesions, and ER positivity

is expressed by 90% of tumors. When observed under a microscope, the most prominent feature is the open Lumina, which is surrounded with just one layer of epithelial cells that stands out most.

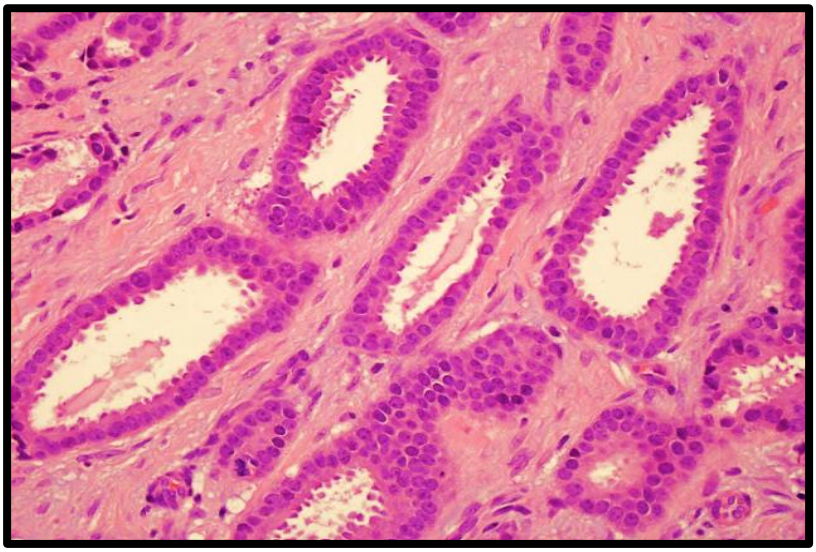


FIGURE-16: Microscopy (400X): Tubular carcinoma characterized by open glands and occasional bent “tear drop/apocrine snouts” structures with single cell layer showing mild pleomorphism. [44]

INVASIVE LOBULAR CARCINOMA: [42-45]

At least 90% of tumor cells do not form a cohesive mass and are organized in a single-file manner. It is possible to observe typical lobules and ducts surrounding the tumor. There are cytoplasmic inclusions seen and cell nucleus exhibit mild to moderate nuclear pleomorphism. Mitotic figures appear very often.

INVASIVE PAPILLARY CARCINOMA: [42-45]

Found mostly in women who have passed menopause. These are soft tumors with clear borders, around 1–3 cm across. Under the microscope, the papillary pattern reveals tumor cells encased in a fibrovascular core. Circumscribed papillary tumors without

myoepithelial cells are known as encapsulated or solid kinds, and they can be observed infiltrating the capsule. The nucleus and cytoplasm of individual tumor cells are of an intermediate grade, and they exhibit considerable pleomorphism. It has an abundant mitotic figure. An excellent prognosis is one of its defining features.

MUCINOUS CARCINOMA: [42-45]

Mucinous tumors can be classified histologically as: Tumors with mucinous A, B, or AB cellularity levels as hypocellular, hypercellular, or intermediate, respectively, and are associated with the worst prognosis. The tumor cells that originate from the epithelium appear morphologically as "floating" in the mucin pool and have a poor nuclear grade in nests and sheets.

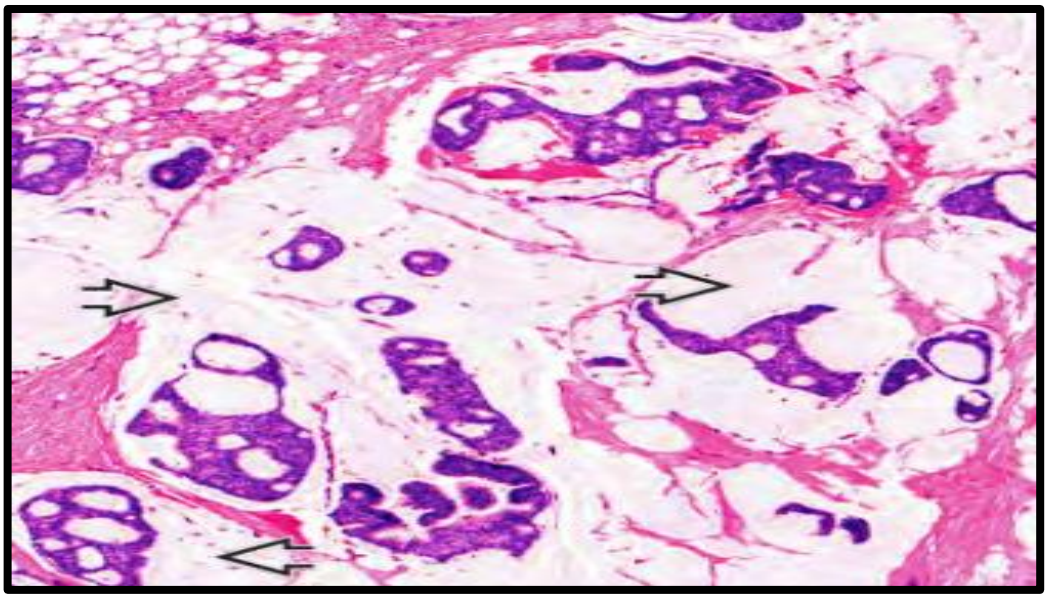


FIGURE-17: Microscopy (400X): Mucinous Carcinoma lacks myoepithelial cells and is marked by large extracellular stromal mucin pools and the tumor cells are seen floating within it. [43]

METAPLASTIC CARCINOMA: [42-45]

As a cystic lesion bordered by squamous cells exhibiting variable atypi, squamous cell carcinoma—the most prevalent form of metaplastic carcinoma—presents itself. Additionally, there are a number of uncommon subtypes of metaplastic carcinomas, including Adenosquamous, spindle cell, matrix generating, metaplastic, and low-grade mucoepidermoid carcinomas.

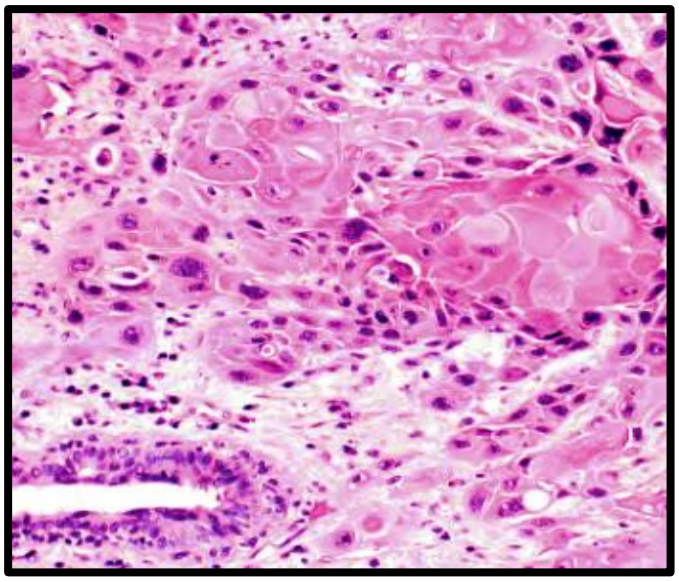


FIGURE-18: Microscopy (400X): Metaplastic Carcinoma (Squamous cell carcinoma) Cysts and squamous metaplasia are frequently related. A component of a spindle cell is almost always present in metaplastic carcinomas. They need to be differentiated from nipple skin carcinomas. [43]

METASTATIC CARCINOMA: [42-45]

We must first check for any peculiar morphological pattern. Metastases to the salivary glands and colon can be identified by the absence of ER staining. The following antibodies are part of an immunohistochemistry panel that can be used to determine the main

site of tumor origin: ER & GATA3 for breast; CK7, CK20, & CDX2 for GIT; TTF1 for lung; WT1, CA125, & PAX8 for ovary; CA19.9 for pancreatic & hepatobiliary origin; CD10 for renal; chromogranin and even synaptophysin for neuroendocrine tumors; Melan A/HMB 45 for melanoma; & CD45 to prevent lymphomas.

MIXED TYPE CARCINOMA: [42-45]

An extensive evaluation of sample sections determines if a tumor is ductal NOS based on the presence or absence of non-specialized pattern in more than 50% of its overall volume. So, let's pretend that, out of all the tumor types, 10% to 49% have the ductal NOS pattern. It will then be categorized depending on whether it is mixed ductal & particular type or mixed ductal and lobular carcinoma, two types of mixed cancers.

PLEOMORPHIC CARCINOMA: [42-45]

Pleomorphic carcinoma that occurs infrequently in subtypes of high-grade ductal carcinoma NOS include adenocarcinoma, adenocarcinoma with spindle or squamous differentiation, and presence of large pleomorphic cells comprising over 50% of the tumor cells. Average age of patients is 51 years, and their ages range from 28 to 96 years. A palpable mass is the first symptom experienced by the majority of patients; however, a metastatic tumor is the initial sign of the disease in 12% of instances. Tumors typically measure 5.4 cm in diameter. When tumors are large, they can cause cavities and necrosis. More than 75% of tumor cells are typically large tumor cells. There are more than 20 mitotic figures for every ten high power fields. Grade 3 carcinomas best describe all of these malignancies. There is usually high-grade necrosis and a ductal pattern in the intraepithelial component. In 19% of instances, Lymphovascular invasion is evident. BCL 2, ER, and PR do

not play an essential role. But TP53 is positive in 2/3rd of these pleomorphic carcinomas, and S-100 is positive in 1/3rd. CAM 5.2, EMA, & pan-cytokeratin (AE1/AE3, CK1) all exhibit positive results. Over 70% are aneuploid, and over half are triploid. Among them, 63% have a high S-phase content (> 10%). Fifty percent of patients show metastases to axillary lymph nodes; most of these cases involve three or more nodes. Unfortunately, many people come in with advanced illness.

GRADING OF INVASIVE CARCINOMAS: [46]

Common criteria for grading invasive tumors, including invasive ductal carcinomas, include the presence or absence of tubules or glands, nuclear pleomorphism, and mitotic numbers. The correlation among histological grade & prognosis in invasive BC has been well documented. It needs to be part of bare minimum of essential data required for histopathological BC reporting as it is currently validated as a significant prognostic factor. After Bloom & Richardson as well as Elston & Ellis revised the Pateley & Scarff approach, histological grade assessment became more objective.

METHOD OF GRADING: [47]

Nottingham–Bloom–Richardson (NBR) histologic grading system:

Three features of tumors are examined.

I. Formation of tubules as an indicator of glandular differentiation:

Score of One: Greater than 75% of tumor has tubular shape

Score of Two: 10-75% of the tumor forms tubules

Score of Three: Assuming the tumor's tubularity is less than 10%

II. Nuclear pleomorphism:

Score of One: Nuclei that exhibit slight size as well as shape variation

Score of Two: Nuclei that exhibit significant size as well as shape variation

Score of Three: Nuclei that exhibit major deviations in size and shape

III. Mitotic counts:

- Score of One: 0 to 5/10Hpf
- Score of Two: 6 to 10/ 10Hpf
- Score of Three: More than 11/10Hpf

To make sure each factor is evaluated separately, we use numerical scoring from 1 to 3. Only structures with visible central lumina are included in the evaluation of tubules and glandular acini; the score is assigned based on cutoff values of 75% and 10% of the epithelial/tumor area, respectively. In order to determine nuclear pleomorphism, one looks at the surrounding breast tissue for normal epithelial cells and how regular their size and shape are. Additional factors that aid in assigning pleomorphism scores include the growing nuclear contour variabilities as well as the quantity and nucleolar size. It is important to be cautious when evaluating mitotic figures; observers should only count well-defined ones; nuclei that are hyperchromatic or pyknotic are disregarded since they appear to be indicative of cell death as opposed to proliferation. Mitosis numbers are aggregated for every set of ten high-power fields. When doing mitotic screening, it is best to start at the tumor's periphery. Regions with a higher frequency should be chosen if heterogeneity is present. It is only appropriate to evaluate the field that has a typical tumor load. Scores between three and nine are generated by adding the three values. Below is the criteria for assigning each grade:

Grade One – Well differentiation is noted: (Score of Three to Five)

Grade Two – Moderate differentiation is noted (Score of Six to Seven)

Grade Three – Poor differentiation is noted (Score of Eight to Nine)

TNM CLASSIFICATION OF BREAST TUMORS: [39]

8th edition of the American Joint Committee on Cancer's staging system offers a way to classify individuals according to their prognosis. While tumor tissue ER and PR levels, lymph node status, menopause status, & overall patient health are important considerations for making therapeutic decisions, staging categories are not the only ones.

T – PRIMARY TUMOR ASSESSMENT:

T_X – Assessment of Primary tumor is not achievable

T_0 –If detection of Primary tumor is not possible

T_{is} - Carcinoma in Situ

T_{is} (DCIS) - Ductal Carcinoma in Situ

T_{is} (LCIS) - Lobular Carcinoma in Situ

T_{is} (Paget) - No invasive cancer evidence or carcinoma in situ (DCIS or LCIS) within breast parenchyma or nipple illness (Tis, Paget)

T_1 - The tumor's largest dimension is less than 2 cm

T_{1mi} - Microinvasion is limited to a maximum dimension of 0.1 cm

T_{1a} - Greatest size ranges around 0.1 and 0.5 cm

T_{1b} - Greatest size ranges around 0.5 and 1 cm

T_{1c} - 1-2 cms in the largest dimension

T_2 - 2-5 cms in largest dimension

T_3 – Maximum dimension more than 5 cms

T_4 - Any tumor, no matter how small, that has grown into skin (as ulcer/nodule) or the chest wall

T4a- Chest wall progression (without invading pectoralis muscles alone)

T4b- Some symptoms may include skin oedema, ipsilateral satellite nodules, or ulceration (also known as peau d'orange)

T4c-4a + 4b

T4d-Inflammatory type of carcinoma

N – REGIONAL LYMPH NODE SPREAD:

N_X - Nodes in the surrounding area cannot be evaluated since they have been removed

N_0 – Lack of metastasis to regional lymph nodes

N_1 - Metastases in internal mammary nodes that can be identified by sentinel lymph node biopsy but are not clinically detected; micro metastases; or metastases in 1 to 3 axillary ipsilateral lymph nodes

N1mi- Micro metastases (those exceeding 200 cells and/or 0.2 mm in diameter, but not exceeding 2.0 mm in length)

N1a- Metastasis in 1-3 axillary lymph nodes, with a minimum of one lymph node exceeding 2 mm in maximum size

N1b- Internal mammary lymph nodes that aren't detected on clinical examination

N1c- The absence of clinical detection of metastasis in 1-3 axillary lymph nodes & internal mammary lymph nodes

N2- 4–9 ipsilateral axillary lymph nodes exhibit metastasis. or in ipsilateral internal mammary lymph nodes detected clinically without metastasis to the axillary lymph nodes

N2a- 4-9 axillary lymph nodes have metastasized, with minimum one pN3 lymph node exceeding 2 mm in dimension

N2b- In the absence of axillary lymph node metastasis, but presence clinically detected internal mammary lymph node metastasis

N3a- Infraclavicular lymph nodes metastasis or level III lymph nodes, or at least 10 ipsilateral axillary lymph nodes with a minimum size of 2 mm in diameter

N3b- Metastasis in internal ipsilateral mammary lymph nodes that are clinically detected and have a positive axillary lymph node status; metastasis in internal mammary lymph nodes & beyond three axillary lymph nodes with macroscopic/microscopic metastasis identified through sentinel lymph node biopsy although clinically not detected; or both

N3c- Ipsilateral supraclavicular lymph node metastasis.

M – DISTANT METASTASIS:

M_0 - No distant metastasis

M_1 - Distant metastasis

PREFIXES:

y: preoperatively if the patient has taken radiotherapy or chemotherapy

r: recurrence of the tumor

TABLE-1 AJCC ANATOMIC STAGING: [39]

If TUMOR(T) is	If NODE(N) is	If METASTASIS (M) is	STAGING
Tis	NO	M0	0
TI	NO	M0	IA
T0	N1 mi	M0	IB
TI	N1mi	M0	IB
T0	N1	M0	IIA
TI	N1	M0	IIA
T2	N0	M0	IIA
T2	N1	M0	IIB

T3	N0	M0	IIB
T0	N2	M0	IIIA
TI	N2	M0	IIIA
T2	N2	M0	IIIA
T3	N1	M0	IIIA
T3	N2	M0	IIIA
T4	N0	M0	IIIB
T4	N1	M0	IIIB
T4	N2	M0	IIIB
Any T	N3	M0	IIIC
Any T	Any N	M1	IV

MOLECULAR CLASSIFICATION OF BREAST CARCINOMA: [48-54]

Perou et al. conducted the initial molecular classification at the start of current century. By utilizing complementary DNA microarrays that represented 8102 human genes, the researchers initially analyzed a collection of 65 surgical samples of breast tumors belonging to 42 women. Subtypes of these malignancies may be defined by notable variations in gene expression profiling (GEP). After conducting additional research and making improvements, the scientists put up a categorization system that categorized breast cancer into four distinct molecular subtypes: luminal A, luminal B, ERBB2)/HER2 gene-overexpressing, & basal-like. Luminal carcinomas are known for their distinctive expression of estrogen receptors (ER) along with varying levels of cell proliferation. The characteristic feature of ERBB2-overexpressing tumors is the excessive production of Her2 protein, while also showing the absence of ER and PR expression. Basal-like carcinoma does not exhibit the

ER, PR, or HER2, which classifies it as triple-negative carcinoma. Instead, it expresses markers associated with basal cells, such as CK 5/6 & EGFR. The above groups exhibited clear differences in histologic patterns, clinical characteristics, and prognosis. The progress appeared quite stimulating. Nevertheless, the implementation of the GEP test in routine labs posed challenges because of its intricate technical nature and lack of cost-effectiveness. Consequently, alternative methods were explored to replicate the GEP outcomes. Cheang et al. discovered a new immunohistochemistry (IHC) panel consisting of six IHC markers. They determined that this panel is capable of replicating the biological subgroups of breast cancer that are derived from complete gene expression profiling (GEP). Afterwards, Schnitt summed up the diagnostic criteria for intrinsic IHC classification in a nutshell as demonstrated in Table-2. 2013 European St Gallen Consensus made slight revisions to the criteria, including raising the Ki-67 threshold to 20% or higher and lowering the PR threshold to 20% or lower in order to improve distinctions. The implementation of intrinsic molecular subtyping has significantly advanced the field of breast cancer categorization. Nevertheless, it is not flawless. Initially, the classification of breast cancer is limited to only four sustainable types, which fails to adequately represent the intricate biochemical complexity of the underlying tumor. Each category remains diverse, with varying outlooks and different reactions to treatment. Secondly, it could be largely replaced by immunohistochemistry (IHC). As a result, its use has not become widely adopted in everyday life. However, it lays the groundwork for future research into molecular tests that predict patient's prognosis. Advancements in luminal malignancies have led to the development of assays that can guide a patient's clinical treatment, primarily because of the discovery of integral molecular categorizing.

TABLE-2 MOLECULAR CLASSIFICATION OF BREAST CARCINOMA: [48-54]

Molecular classification		Estrogen receptor	Progesterone Receptor	Her2neu	Ki67%
Luminal-A		+	+	_	Low
Luminal-B	**Her 2 Negative**	+	_	+	Low
Luminal-B	**Her 2 Positive**	+	+/-	+	High
Her2 Enriched		_	_	+	High
TNBC		_	_	_	High

Hormone therapy is effective for cases where estrogen and progesterone levels show positivity. Patients tested positive for Her-2Neu are only treated with Her-2Neu medications. Because TNBC patients do not react to standard hormonal treatment, chemotherapy was administered to these patients.

IMMUNOHISTOCHEMISTRY OF BREAST CANCERS: [45,55]

Both benign and malignant breast diseases rely heavily on immunohistochemistry in their pathology. In order to differentiate between benign and malignant tumors, myoepithelial markers are utilized. The most popular immunohistochemistry markers utilized in breast malignancies are ER, PR, HER-2 Neu, & Ki-67 which serve as both prognostic and therapeutic indicators.

TABLE-3 GIVES A BRIEF SUMMARY OF THE MULTIPLE MARKERS USED IN BREAST CANCER: [39,42-45&55]

MARKERS	STAIN TYPE	USE
p63	Nuclear	Myoepithelial differentiation
HMW CK (14 & 5/6)	Cytoplasmic	Lobular carcinoma from benign lesions can be differentiated
CK8	Peripheral cytoplasmic staining	Ductal Carcinoma
CK8	Perinuclear staining	Lobular Carcinoma
ER&PR	Nuclear	For Subtyping and defines tumor Origin from breast
HER-2/Neu	Membranous staining	For Subtyping
Mammaglobin A	Cytoplasmic	Metastatic carcinoma originating from breast
Carcino Embryonic Antigen (CEA)	Cytoplasmic	Metastatic mammary carcinoma

PROGNOSTIC AND PREDICTIVE FACTORS: [56]

Therapy of BC has seen tremendous change throughout the years due to novel therapeutic options. Predicting the potential prognosis of these individuals and picking appropriate treatment approaches are both greatly assisted by prognostic information. Their relative importance in determining prognosis and therapeutic results led to their previous categorization into three groups. These elements used to be mixed up; now, thanks to molecular pathology and improved imaging techniques, they're classified into two groups: those pertaining to the biology of cancer and those pertaining to the extent of carcinoma.

TUMOR SIZE: [45]

Lesion dimension is a crucial and critical indicator of how BC will affect the patient's behavior. Less tumor size means better prognosis; when lesions were smaller than 1 cm, only <20% cases experience nodal spread; and when tumors are lesser than 1 cm & without positive nodes, 90% of patients experience disease-free survival for 10 years. Microscopical measurements of an invasive component have the upper hand, but both gross and microscopic measurements of the tumor must be done and compared. Metastasis can be seen in HER-2+ and ER- malignancy regardless of size; hence, size is not a determining factor in these cases.

NODAL STATUS: [57-58]

Primary determinant for knowing the prognosis and overall survival rate of BC patients is condition of axillary region nodes. No nodal involvement has been associated with a ten-year disease-free survival rate of 70%-80%. Presence of at least three but not more than five positive lymph nodes, survival percentage fell as low as 35–40%, and after ten or more, it plummeted to 10–15%. An important factor considered in the Nottingham prognostic index is the absolute count of affected nodes, which also holds prognostic significance. By employing coloured dyes, the sentinel nodes—the initial one or two nodes from which lymphatic outflow of breast carcinomas reaches—can be readily identified. Differentiating reactive nodes from metastases requires biopsies taken from the clinically palpable nodes. When grossing lymph nodes, it is important to remember to submit all uninvolved nodes for histologic investigation. While small nodes can be implanted entirely, larger nodes require more thorough evaluation and numerous sections to prevent erroneous negative outcomes. With its excellent specificity and sensitivity in predicting nodal status, biopsy of sentinel node has been emerged as one of the important substitutes to axillary node removal.

CARCINOMA WITH INVASION VERSUS CARCINOMA IN SITU: [45]

When comparing carcinoma in situ to invasive cancer, prognosis is consistently worse for women with the latter.

LOCALLY ADVANCED LESION: [45]

Complete surgical excision becomes challenging when tumors infiltrate skin or underlying muscle, leading to a high recurrence rate. But these incidences have dropped significantly in recent years because of all the screenings and awareness campaigns.

HISTOLOGIC GRADE: [45&59]

Using these three criteria, all invasive ductal carcinomas must be classified: There is a strong correlation between the three categories of invasive carcinomas (grading of nucleus, tubule development, & mitotic figures) and likelihood to have survival both disease-free as well as overall. For cancer staging, AJCC Manual suggests using the Nottingham grading, which is an adaptation of Scarff Bloom Richardson grading by Elston Ellis.

HISTOLOGIC TYPE: [45&59]

Invasive carcinomas without any particular form, mucinous, tubular, papillary, lobular as well as adenoid cystic invasive carcinomas have a well-established favorable prognosis. Metaplastic carcinoma and micropapillary carcinoma, on the other hand, have been linked to more unfavourable prognosis in female patients. When it comes to certain subtypes of malignancies, such as low-grade Adenosquamous carcinoma as well as adenoid cystic carcinoma, which disproportionately affect younger females, histology triumphs over molecular status.

MOLECULAR PROGNOSTIC PARAMETERS IN BREAST CARCINOMA:[60]

The development of breast cancer and its course can be better understood with the use of molecular markers that help direct treatment. Pathologists increasingly use immunohistochemical examination of ER, PR, Ki-67, HER-2, as well as p53, which are prognostic as well as predictive indicators.

HORMONE RECEPTORS: [45&61]

One important step forward in the fight against breast cancer is the discovery that tumor tissue hormone receptors (estrogen and progesterone) strongly correlate with the efficacy of hormone therapy and chemotherapy. Both progesterone and estrogen have receptors in a normal breast epithelium. Cell proliferation and differentiation are induced by the interaction of these receptors with hormones. Approximately sixty to seventy percent of breast cancers display these receptors. Therefore, it is possible that circulating endogenous hormones interact with ER/PR-positive tumors to drive their growth. New medications have been created that bind to hormone receptors in ER/PR-positive cancers; this stops the growth of tumor cells, increases the patient's survival rate, and sometimes shrinks the tumors that are already there. Accordingly, survival rates are higher for patients whose tumors are estrogen receptor-positive, as confirmed either biochemically or immunohistochemically. Hormonal treatment is effective in eliminating breast cancers in 80% of cases where both ER and PR are positive, but in only 40% of cases when one of these markers is positive. Chemotherapy has a lower success rate with ER-positive malignancies. In contrast, tumors lacking ER nor PR expression with response rate of <10% to hormonal treatment, while chemotherapy achieves greater efficacy in these instances.

HER-2 Neu: [45&62]

HER-2/neu (c-erbB-2) oncogene encodes for the transmembrane glycoprotein p185, belonging to class of epidermal growth factor receptors, which has tyrosine kinase activity. Not only does Her-2/neu overexpression correlate with worse survival, but it also serves in forecasting outcome in the effect of drugs that aimed at transmembrane protein, which is of paramount importance.

Ki67: [62]

Histologic grading, S-phase fraction of flow cytometry, immunohistochemistry (for cellular proteins including cyclins and Ki-67), and mitotic counts are all ways to quantify proliferation. Despite a potential improvement in treatment response, the prognosis is worse for cancers with high proliferation rates.

VARIABLES INFLUENCING PROGNOSIS IN THE PATIENT MANAGEMENT-NPI: [63-65]

Three criteria were utilized in the initial NPI that is Grading of the lesion, nodal status, as well as dimensions of the lesion were considered. As a prognosis indicator, these three criteria were evaluated collectively. When added together, the worse the prognosis, the greater the number. Three groups of patients were formed based on a cut-off value between 3.4 and 5.4. There are three subsets: Group-1, which has an excellent prognosis (with a score of uptil 3.4) and an expected 5-year survival rate of 80%; Group-2, which has a moderate prognosis (scoring 3.4 to 5.4) and an estimated four-and-a-half percent 5-year survival; and even Group-3, which has a bad prognosis (score more than 5.4) and an estimated thirteen percent five-year survival.

It is determined using following formula: NPI is computed by multiplying pathological tumor size in centimeters by 0.2, adding the nodal staging (1, 2 or 3), and finally adding the histologic grading (1, 2 or 3).

TABLE 4: THE NPI IS DIVIDED INTO DIFFERENT CATEGORIES, EACH ASSOCIATED WITH A SPECIFIC ESTIMATE OF BC-SPECIFIC SURVIVAL FOR 10 YEARS:

NPI	SCORE	TEN-YEAR OVERALL SURVIVAL
I (Excellent)	≤2.4	96%
II (Good)	>2.4 -≤3.4	93%
III (Moderate I)	>3.4 - ≤4.4	81%
IV (Moderate II)	>4.4 - ≤5.4	74%
IV (Poor)	>5.4	38%-50%

MATERIALS

AND

METHODS

MATERIAL AND METHODS:

1. STUDY AREA:

The pathology department of R.L. Jalappa Hospital & Research Hospital, which is affiliated with Sri Devaraj Urs Medical College in Tamaka, Kolar, was the site of the current investigation.

2. STUDY POPULATION:

All cases with invasive ductal carcinoma of the breast who present to surgical oncology as well as surgery departments.

3. STUDY DESIGN:

An observational study (Cross-sectional analytical study).

4. SAMPLE SIZE:

In invasive breast cancer, MDM2 expression in tumor cell nuclei was found in 66.7% of cases, according to a study by Opoku.F et al. [20]

- Equation sample size = $\frac{Z_{1-\alpha}^2 p(1-p)}{d^2}$
- Here $Z_{1-\alpha}^2$ = Standard normal variant
- p = Expected proportion of population based on previous studies
- d = Absolute error of 10 %

The necessary sample size for the cross-sectional study on breast cancer, with a 95% confidence interval, was 93.

5. TIME FRAME TO ADDRESS THE STUDY:

Two years (July 2022 to March 2024).

6. INCLUSION CRITERIA:

Female patients diagnosed with infiltrating ductal carcinoma (NOS) who have undergone Modified radical mastectomy surgery.

7. EXCLUSION CRITERIA:

Women who have undergone neoadjuvant radiotherapy or chemotherapy mastectomy; women with recurring tumors; women who have received chemotherapy for a different type of cancer within the last five (5) years; and male patients with a diagnosis of breast cancer.

8. STUDY COURSE:

The paraffin blocks as well as slides had been obtained from Pathology department. Medical records & pathology reports had been used to gather clinical information. The histological type of all hematoxylin & eosin slides was examined, and suitable blocks were selected for Immunohistochemistry.

9. COLLECTION OF DATA:

A total of ninety-three (93) cases of Infiltrating Ductal Carcinoma were diagnosed and treated at R.L.Jalappa Hospital and Research Centre between March 2020 and March 2024, and these cases were included in this study. The study included all BC cases that were verified through histological testing. Following the removal of identifying patient information, data on patient's clinical information, tumor dimensions, and nodal status of axillary region were gathered. Paraffin blocks & even slides of these patients were obtained from the archives of Department of Pathology. The hematoxylin and eosin-stained slides were examined to determine histological type, tumor grade, and presence of nodal metastases. The slides for MDM2 immunohistochemistry were carefully chosen, with a suitable positive control (Liposarcoma) and negative control (Lipoma) being used.

METHODOLOGY:

IMMUNO HISTOCHEMICAL EXAMINATION:

Immunohistochemistry (IHC) was conducted on tissue slices that were 4 micrometers thick and derived from tissue blocks that were 10% formalin fixed and paraffin embedded. The peroxidase-antiperoxidase technique has been employed. A process of standardization involved conducting immunohistochemistry (IHC) on both positive and negative controls simultaneously.

TABLE:5 DISPLAYS THE SPECIFIC IHC DETAILS UTILIZED IN THE CURRENT STUDY:

Antigen	Clone	Species	Producer	Control	Stain
Synthetic peptide derived from N-terminal region of human MDM2	SMP 14	Mouse	Diagnostic Biosystems	Liposarcoma	Nucleus

THE IHC PROCEDURE INCLUDES THE FOLLOWING STEPS:

1. Tissue blocks fixed in 10% formalin were used to create sections that were 3-5μm thick.
2. These sections were then placed on slides that were coated with organosilane and had a positive charge.
3. Glass slides had been incubated over a hot plate for a temperature of 58°C overnight.
4. Deparaffinization was performed by immersing the sample in Xylene I and Xylene II for 15 minutes each.
5. The process of dexylinisation was carried out using pure alcohols I and II, with each step lasting for 1 minute.

6. The slides were dealcoholized using 90% and 70% alcohol for 1 minute each.
7. The slides were subsequently rinsed with distilled water, ensuring that the sections were not dried at any point during staining process.
8. The Antigen Retrieval approach involves subjecting the sample to enzymatic treatment using a microwave set at power 10 for a duration of 6 minutes. This is done in TRIS EDTA buffer with a pH of 6.0, and the process is repeated for two cycles.
9. Rinse with distilled water for a duration of 5 minutes.
10. Perform two consecutive 5-minute washes by transferring to Tris Buffer Solution (TBS) at a pH of 7.6.
11. Apply a peroxidase block for 10-15 minutes to inhibit the activity of the endogenous peroxidase enzyme.
12. Perform TBS buffer washes for three separate 5-minutes.
13. Power block over 10-15 minutes to prevent the non-specific reactivity with other tissue antigens.
14. Incubate sections with a specific primary antibody for 45 minutes to detect tissue markers by antigen-antibody reaction.
15. Before processing, give the sample a 5-minute rinsing in Tris buffer (pH 7.6). Repeat this process three times with gentle agitation to remove any antibodies that are not bound.
16. A super enhancer was introduced for a duration of 20 minutes to intensify the interaction between the primary and secondary antibodies.
17. Perform three 5-minute washes with TBS wash buffer to remove any antibodies that are not bound.
18. A highly responsive polymer horseradish peroxidase (poly HRP) was introduced for a duration of 30 minutes to extend the chain and additionally mark the enzyme.

19. Subsequently, the inclusion of DAB led to the production of a chromogen for a period of 5-8 minutes, resulting in the coloration of antigens.

20. Perform three washes with TBS wash buffer for 5 minutes each.

21. Rinse the sample with tap water for 5 minutes, then apply hematoxylin counterstain for 1 minute.

22. The specimen was dehydrated using 90% alcohol and absolute alcohol for a duration of 2 minutes.

23. It was then cleared using a mixture of alcohol and xylene in a 1:1 ratio for another 2 minutes.

24. Finally, the specimen was mounted with DPX.

25. The identification of antigens in cells as well as tissues is accomplished through an intricate procedure utilizing HRP technique.

DOCUMENTATION AND INTERPRETATION OF DATA:

93 cases of Infiltrating Ductal Carcinoma that were detected as well as treated at RLJH & Research Centre between March 2022 to March 2024. This study included all cases of breast cancer that were proven through histopathological testing.

Upon anonymizing the patient's personal information, clinical data, tumor dimensions, and axillary lymph node condition. The H&E-stained slides had been examined for determining the histological type, grading of tumor, and presence of nodal metastases. We chose suitable slides for MDM2 immunohistochemistry. The immunohistochemical (IHC) analysis assessed the expression of MDM2 by quantifying the proportion of tumor cells that exhibited nuclear staining, which was considered as a positive result.

Scoring system for tumor cells is as follows:

0 = 0%

1+ = 1-25%

2+ = 26-50%

3+ = >50%

A tumor is classified as MDM2 positive when it receives a score of 1+ or above. Positive interpretation is not given for cytoplasmic staining alone. [66]

Slides labelled with ER, PR, HER-2, and Ki67 were obtained from the immunohistochemical lab's archive. Scoring had been performed according to the guidelines set by the CAP-ASCO.

TABLE-6 PRESENTS THE ALLRED SCORING FOR ER & PR IHC IN BC: [67]

Score for staining cell proportion (PS)		**Score for staining intensity (IS)**	
Score	**Interpretation**	**Score**	**Interpretation**
0	No staining	**0**	None
1	<1%	**1**	Weak
2	1-10%	**2**	Average
3	11-33%	**3**	Strong
4	34-66%		
5	67-100%		

Allred Score = PS+IS.

The Allred score for ER as well as PR is determined using a scale of ≥3 to indicate positivity and ≤3 to indicate negativity.

The HER-2 neu staining had been evaluated and assigned a score ranging from 0 to 3, as per the 2018 ASCO guidelines.

TABLE-7 SCORING THE EXPRESSION OF HER2NEU IN BC USING IHC: [68]

Scoring	Over Expression Assessment	Protein Scoring Pattern
Score 0	Negative	There is no visible staining or percentage of tumor cells showing membrane staining is below 10%
Score 1+	Negative	>10% of tumor cells show very faint or undetectable membrane staining
Score 2+	Equivocal	>10% of the tumor cells exhibit a mild to moderate staining throughout entire membrane
Score 3+	Positive	Intense and widespread staining over the whole membrane circumference, detected in >10% of tumor cells

The Ki 67 was evaluated based on the methodology outlined by Kan Yilmaz G et al., utilizing the below specified criteria mentioned in Table-8. [69]

TABLE-8: SCORING THE EXPRESSION OF KI67 IN BREAST CANCER USING IMMUNOHISTOCHEMISTRY (IHC):

Ki67 Scoring	Result
<14%	Negative
>14%	Positive

STATISTICAL ANALYSIS:

Acquired information was inputted into a Microsoft Excel spreadsheet & evaluated with SPSS 22, a software package for statistical analysis. The representation of quantitative data will be done through the incorporation of the mean as well as standard deviation. Independent t-test had been utilized for statistical testing to determine the significance of the variation in means. The qualitative data will be expressed as frequencies as well as proportions. Chi-square test will be employed to detect disparities among the groups. Chi-square or Fischer's exact test (2x2 tables only) has been employed as test of significance for qualitative information. The results have been displayed in a tabular format and also visually represented using either a bar diagram or a pie diagram, depending on the nature of the data. A p-value below 0.05, when considering a 95% confidence level, signifies statistical significance.

RESULTS & OBSERVATIONS

RESULTS & OBSERVATIONS

MICROSCOPIC IMAGES:

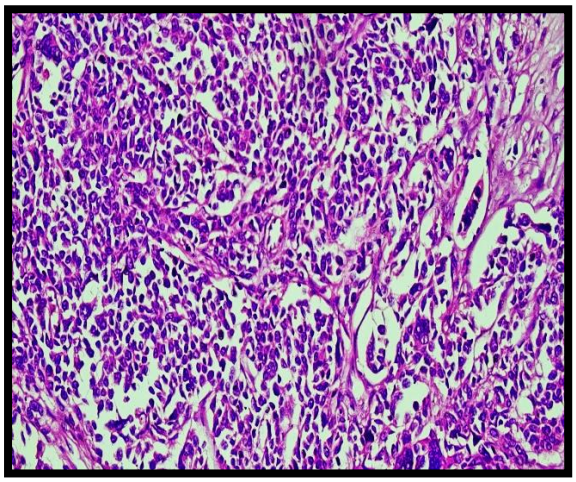


FIGURE-19: IDC Breast (NOS)

(H & E)-100x. (B/634/24)

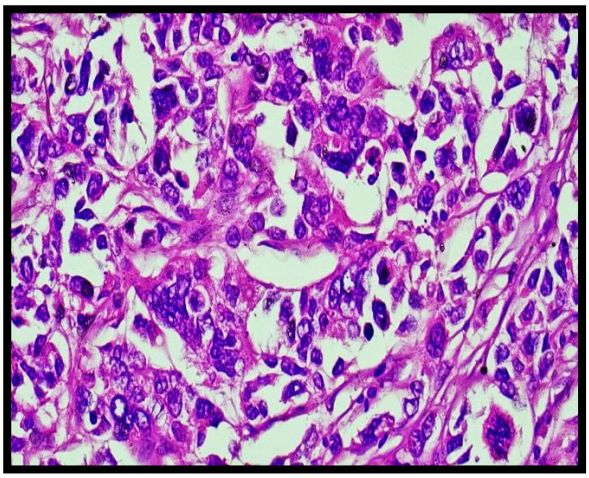


FIGURE-20: IDC Breast (NOS)

(H & E)-400x. (B/634/24)

MICROSCOPY OF ABOVE IMAGES: Tumor cells arranged in tubules and few in sheets. Individual tumor cells are moderately pleomorphic with high nuclear: cytoplasmic ratio, 1-2 notable nucleoli and moderate cytoplasm. 2-3 mitotic figures/Hpf are noted. These cells are seen compressing the surrounding stroma.

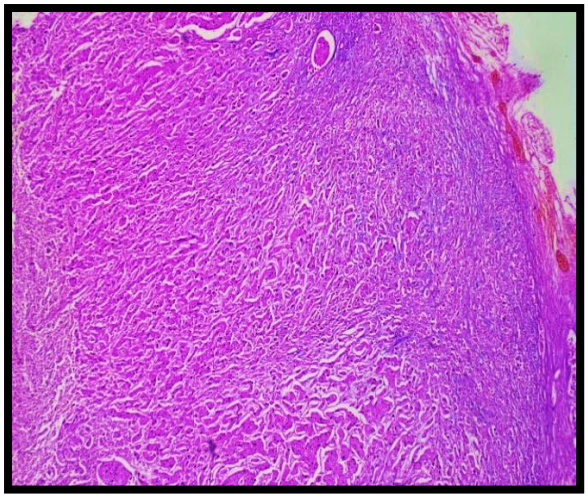


FIGURE-21: Invasive ductal carcinoma deposits in lymph node (H & E) – 40x (B/1112/24)

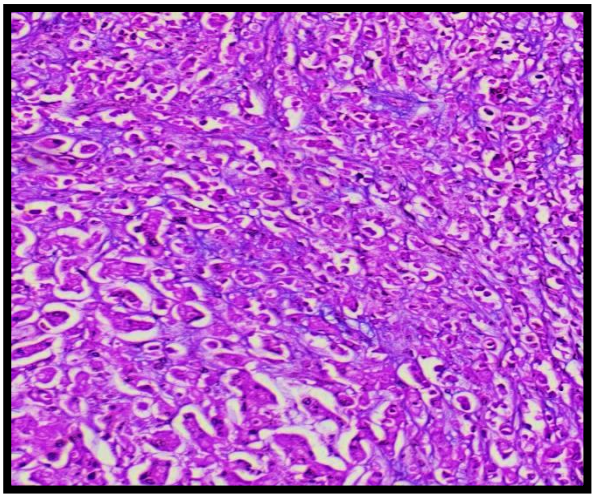


FIGURE-22: Invasive ductal carcinoma deposits in lymph node (H & E) – 400x (B/1112/24)

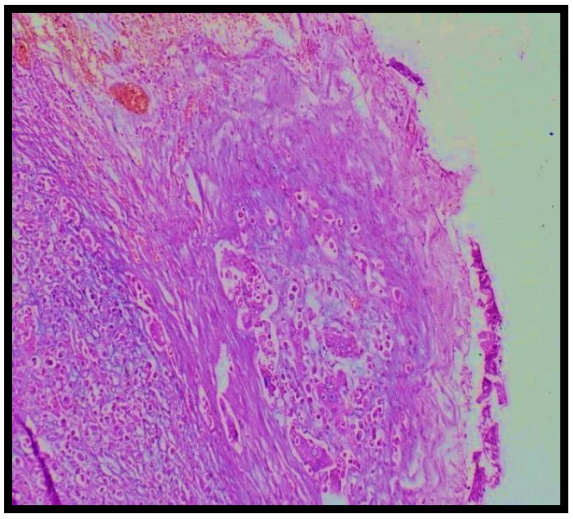


FIGURE-23: Extra capsular extension of IDC in lymph node (H & E) – 40x (B/1934/23)

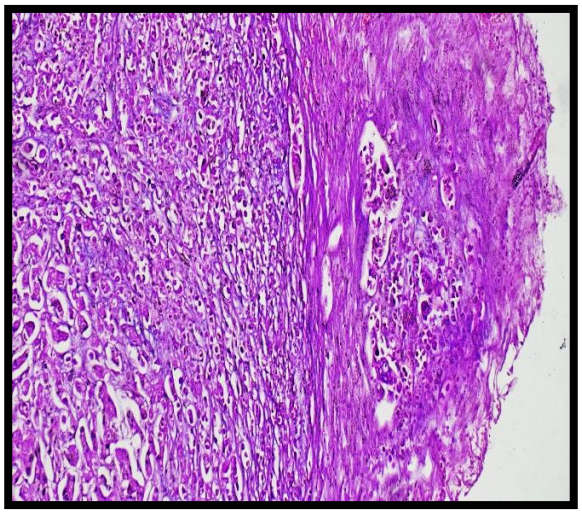


FIGURE-24: Extra capsular extension of IDC in lymph node (H & E) – 100x (B/1934/23)

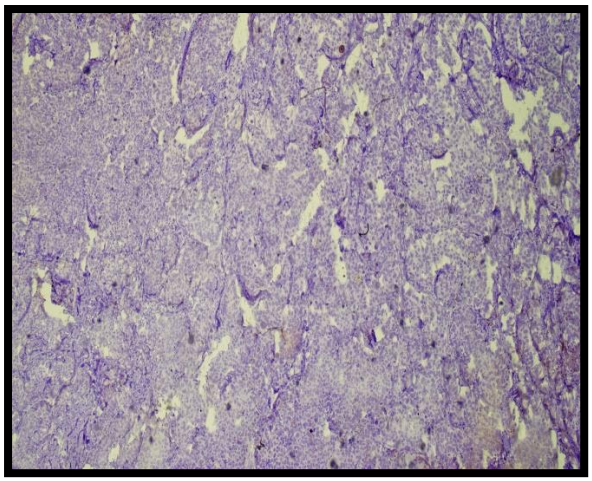


FIGURE-25: ER Negative 100x

(B/297/24)

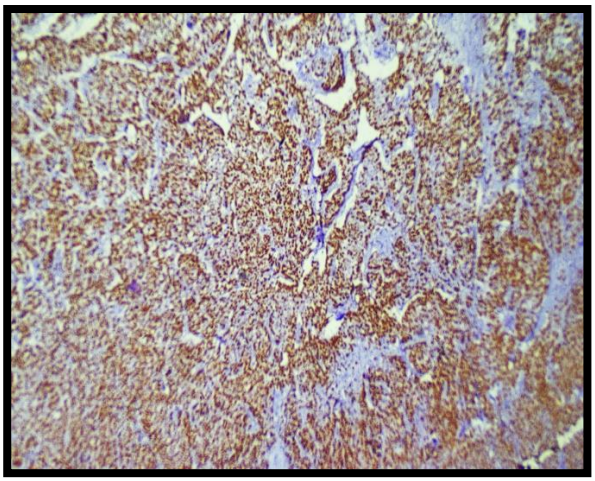


FIGURE-26: ER Positive 100x

(B/953/24)

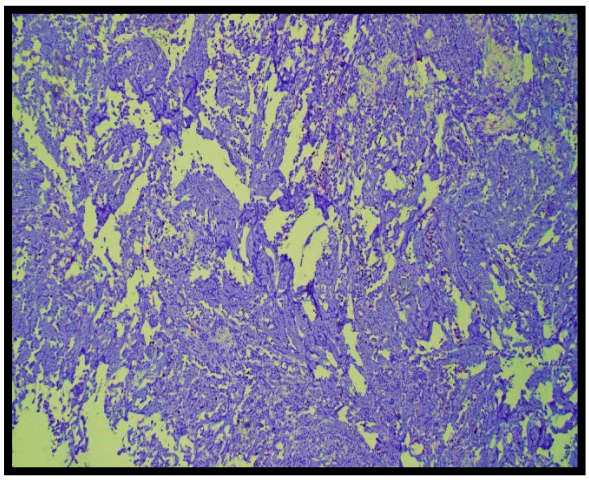


FIGURE-27: PR Negative 100x

(B/4335/23)

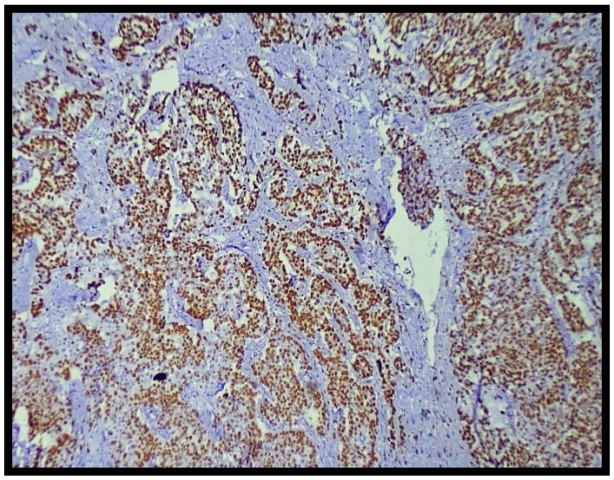


FIGURE-28: PR Positive 100x

(B/4686/23)

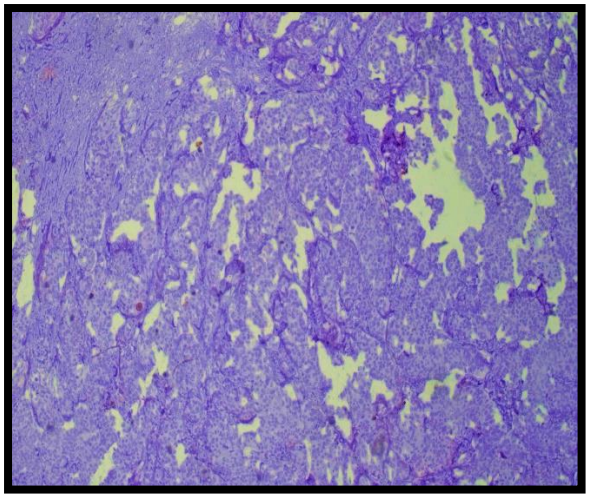


FIGURE-29: HER-2neu Negative

100x (B/4241/23)

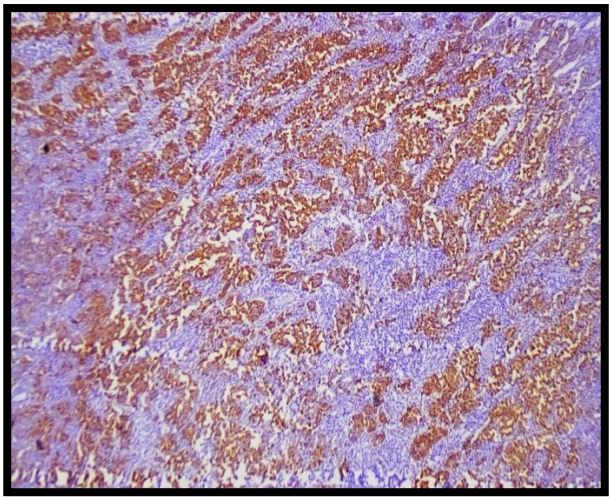


FIGURE-30: HER-2neu Positive

100x (B/1167/22)

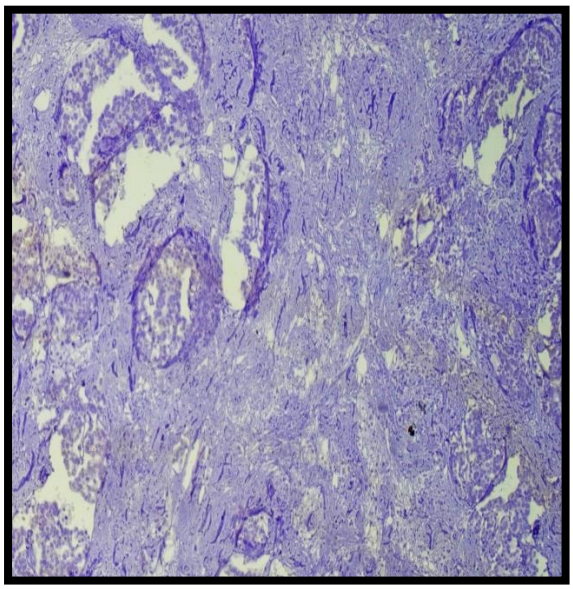


FIGURE-31: Ki67<14% 100x

(B/3089/23)

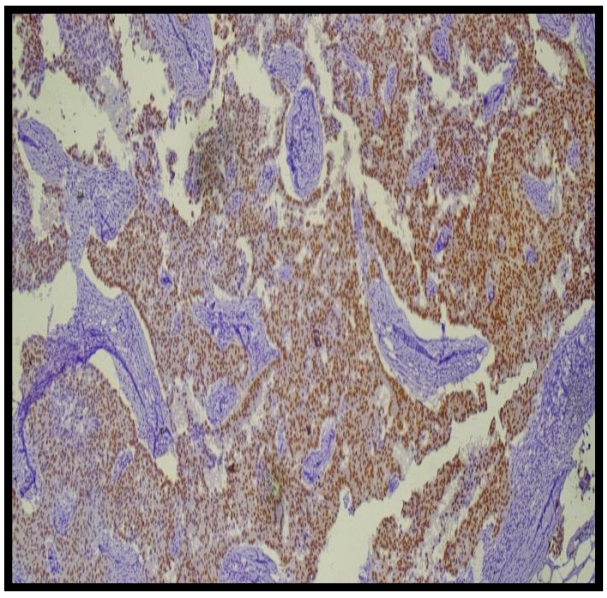


FIGURE-32: Ki67>14% 100x

(B/3103/23)

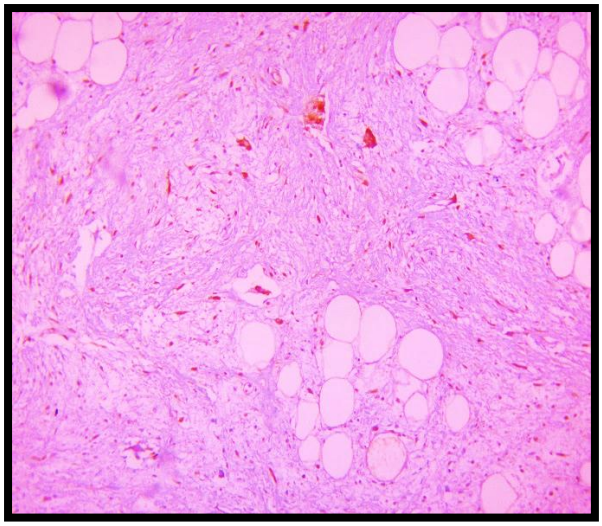


FIGURE-33: Positive Control-Liposarcoma-Showing strong nuclear positivity-100x

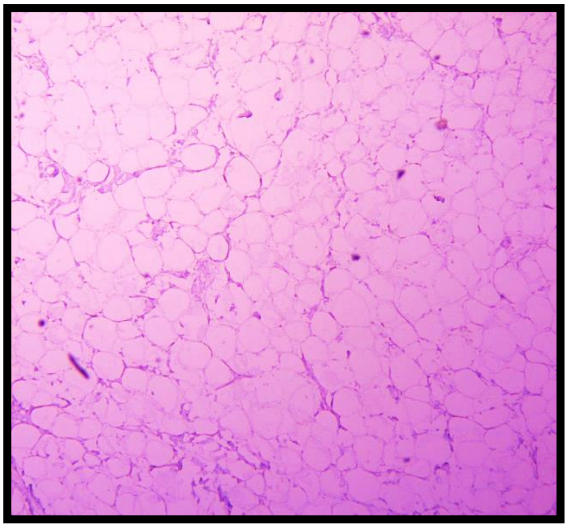


FIGURE-34: Negative Control-Lipoma-Negative staining-100x

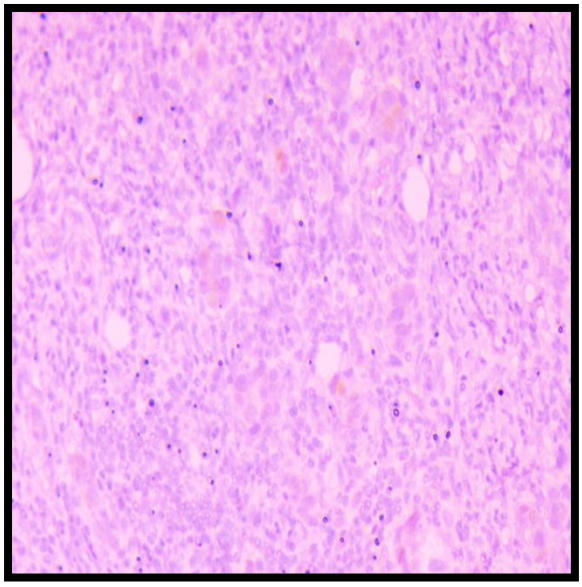


FIGURE-35: MDM2 IHC ON IDC – 100x 0% Nuclear staining (0%= Score 0)

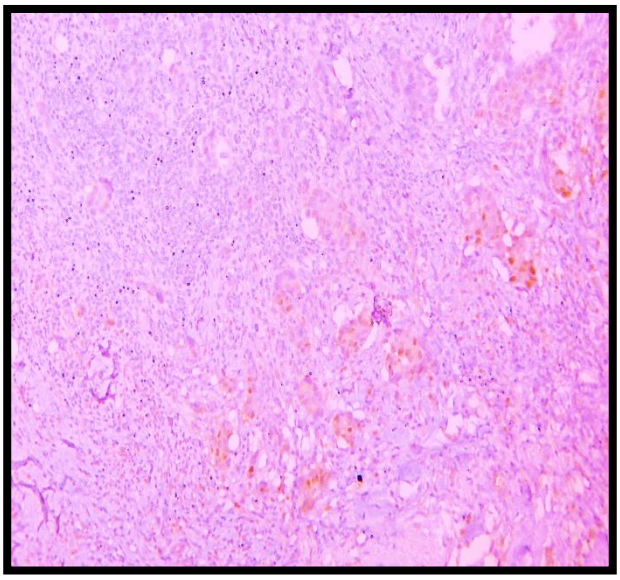


FIGURE-36: MDM2 IHC ON IDC – 100x 1%-25% Nuclear staining (1-25%= Score 1+)

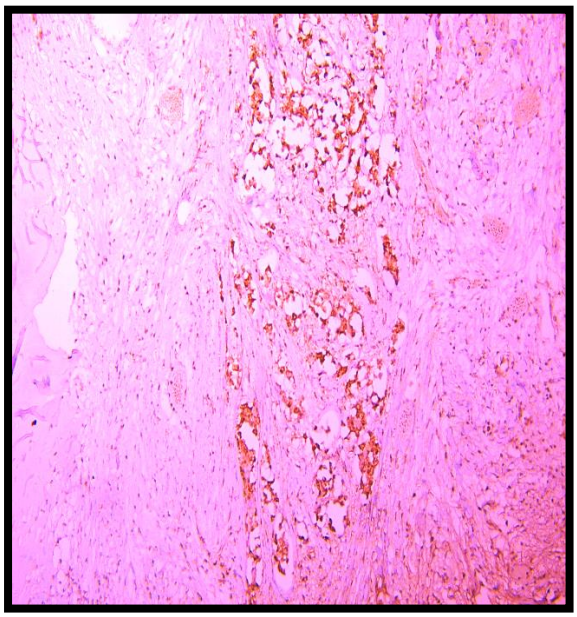


FIGURE-37: MDM2 IHC ON IDC – 100x

26%-50% Nuclear staining (0%= Score 2+)

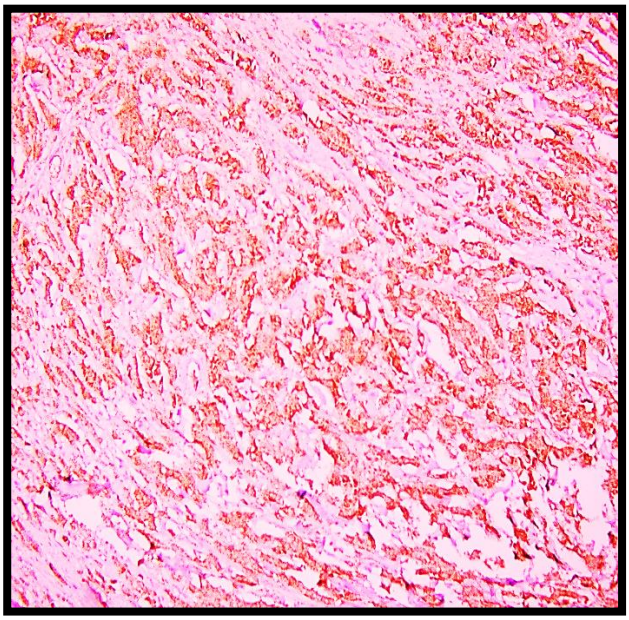


FIGURE-38: MDM2 IHC ON IDC – 100x

>50% Nuclear staining (>50%= Score 3+)

DEMOGRAPHIC DATA:

TABLE-9 AGE-SPECIFIC PATIENT DISTRIBUTION:

AGE	FREQUENCY	PERCENT
30-39yrs	18	19%
40-49yrs	15	16%
50-59yrs	32	35%
60-69yrs	17	18%
70-79yrs	11	12%
Total	93	100%

Average age of the patient in this study was around 53.7years. A large percentage of individuals fell within an age range of 50-59 years [35%], followed by 30-39 years [19%], 60-69 years [18%], 40-49 years [16%], & 70-79 years [12%].

GRAPH-1 AGE GROUP DISTRIBUTION OF PATIENTS:

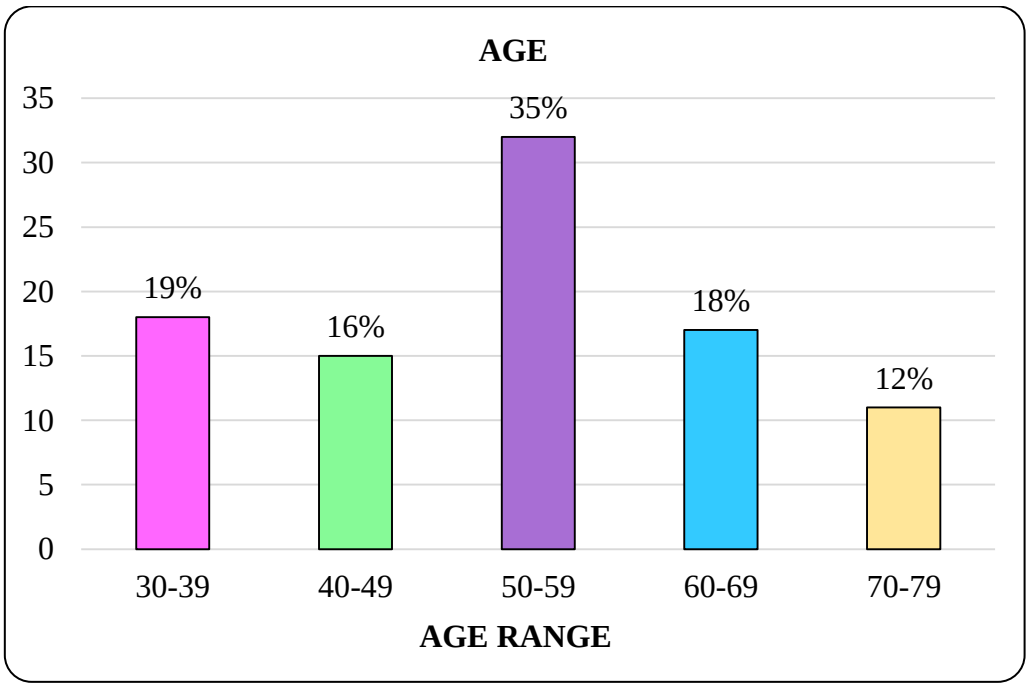


TABLE-10 CASE DISTRIBUTION IN ACCORDANCE WITH THE SIZE OF THE TUMOR:

TUMOR SIZE	FREQUENCY	PERCENT
<2cms	4	4%
2-5cms	50	54%
>5cms	39	42%
Total	93	100%

Among the patients, 4% had tumors smaller than 2 centimeters, 54% had tumors ranging from 2 to 5 centimeters, and 42% had tumors larger than 5 centimeters.

GRAPH-2 DISPLAYS THE DISTRIBUTION OF SUBJECTS GROUPED ACCORDING TO TUMOR SIZE:

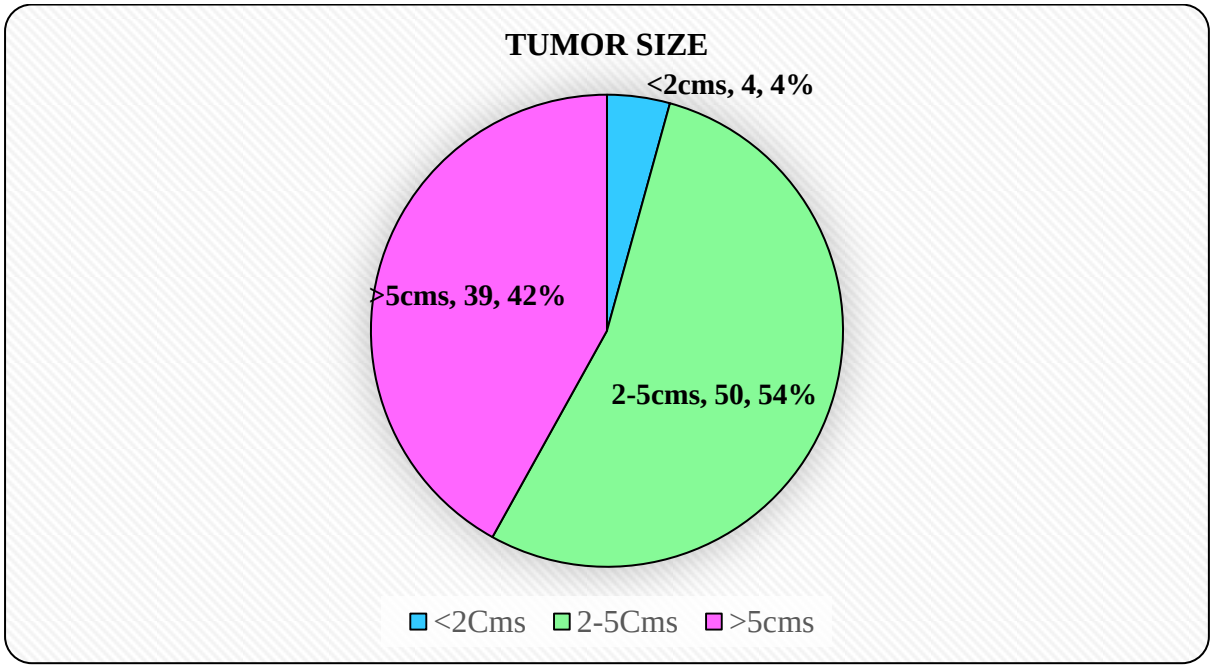


TABLE-11 PRESENTS THE DISTRIBUTION OF PATIENTS CATEGORIZED BASED ON MODIFIED SCARFF-BLOOM-RICHARDSON'S HISTOLOGICAL TUMOR GRADING (MBR):

GRADING	FREQUENCY	PERCENT
I	47	51%
II	32	34%
III	14	15%
Total	93	100%

51% of patients exhibited a Grade I tumor, whereas 34% had a Grade II tumor, and 15% showed a Grade III tumor.

GRAPH-3 SHOWS THE DISTRIBUTION OF PATIENTS GROUPED BY THE MODIFIED SCARFF-BLOOM-RICHARDSON'S HISTOLOGICAL TUMOR GRADE (MBR):

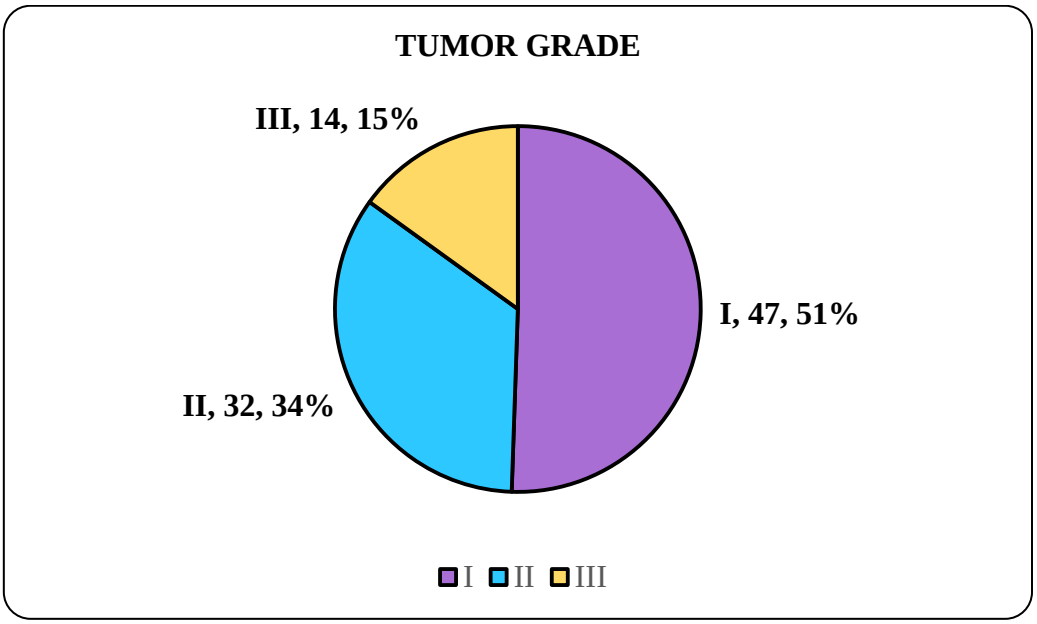


TABLE-12 DISPLAYS THE DISTRIBUTION OF PATIENTS CLASSIFIED ACCORDING TO THE TUMOR STAGING:

T STAGING	FREQUENCY	PERCENT
T1	2	2%
T2	41	44%
T3	40	43%
T4	10	11%
Total	93	100%

The overall distribution of tumor stages among patients was as follows: 2% had Stage I tumor, 44% had Stage II tumor, 43% had Stage III tumor, and 11% had Stage IV tumor.

GRAPH-4 ILLUSTRATING DISTRIBUTION OF PATIENTS CLASSIFIED ACCORDING TO THE TUMOR STAGING:

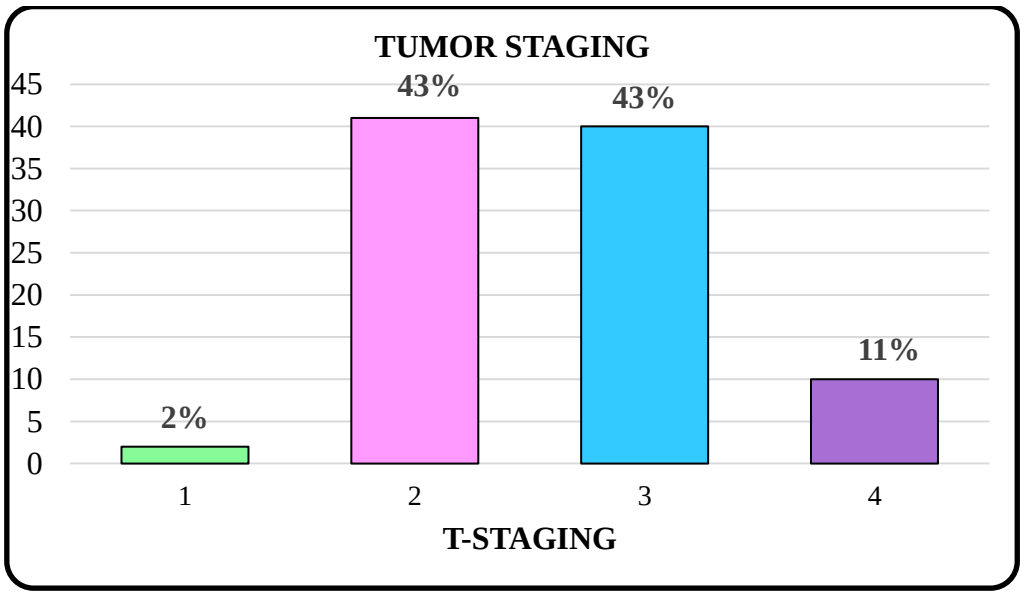


TABLE-13 UNVEILS DISTRIBUTION OF PATIENTS ACCORDING TO LYMPH NODE INVOLVEMENT:

NODAL STAGING	FREQUENCY	PERCENT
N0	41	44%
N1	21	23%
N2	20	21%
N3	11	12%
Total	93	100%

The nodal staging was classified as 0 in 44%, N1 in 23%, N2 in 21%, and N3 in 12% of patients.

GRAPH-5 CATEGORISING PATIENTS ACCORDING TO THE NODAL STAGING:

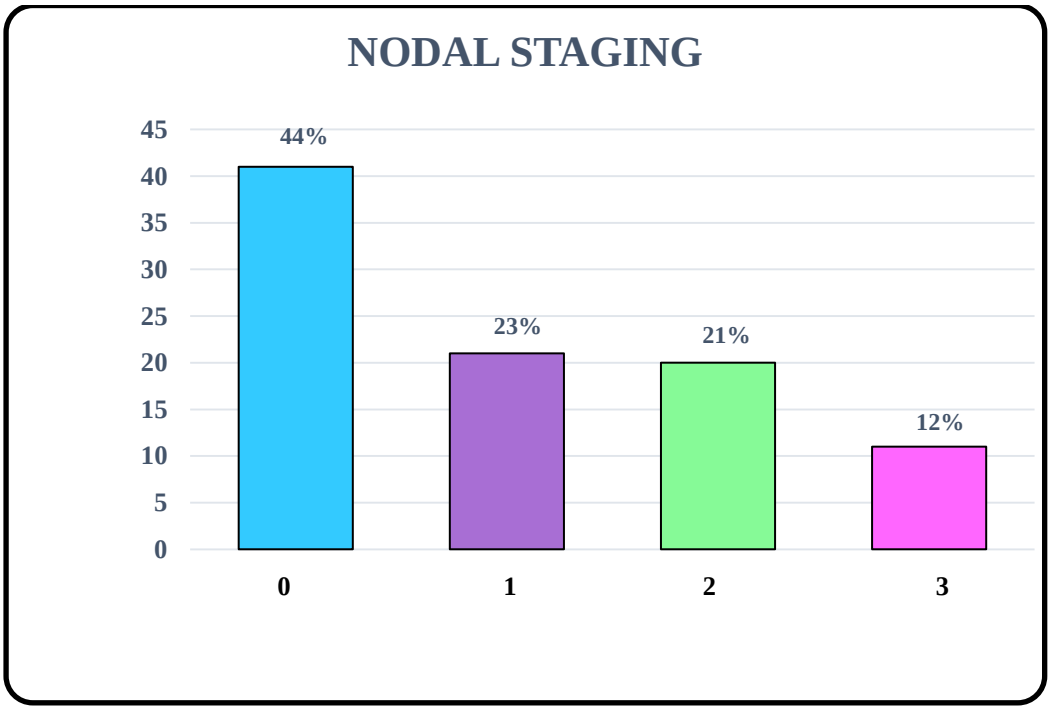


TABLE-14 DISTRIBUTION OF PATIENTS BASED ON THE EXTRANODAL EXTENSION:

EXTRA NODAL EXTENSION	FREQUENCY	PERCENT
Absent	80	86%
Present	13	14%
Total	93	100%

Extranodal extension was observed in 14%, while it was not present in 86% of patients.

GRAPH-6 DISTRIBUTION OF PATIENTS BASED ON THE EXTRANODAL EXTENSION:

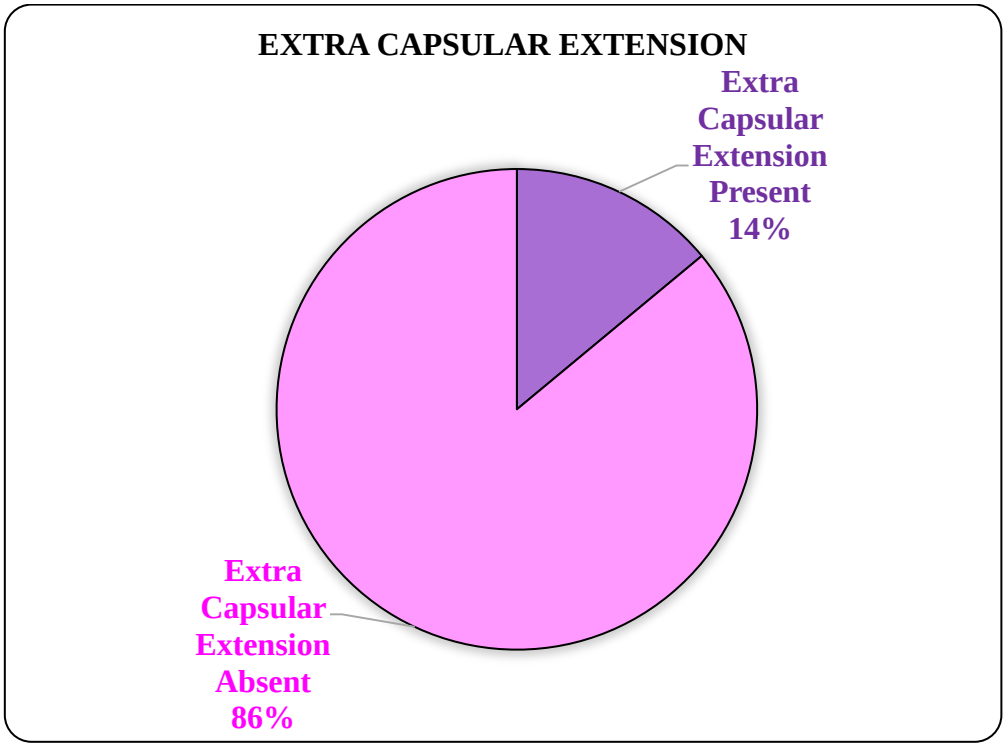


TABLE-15 CLASSIFICATION OF CASES BASED ON THE TNM STAGING:

TNM STAGING	FREQUENCY	PERCENT
I	3	3%
II	45	49%
III	44	47%
IV	1	1%
Total	93	100%

The distribution of tumors among patients was as follows: Stage I accounted for 3% of cases, Stage II for 48%, Stage III for 47%, and Stage IV for 1%.

GRAPH-7 REPRESENTATION OF PATIENTS ACCORDING TO THE TNM STAGING:

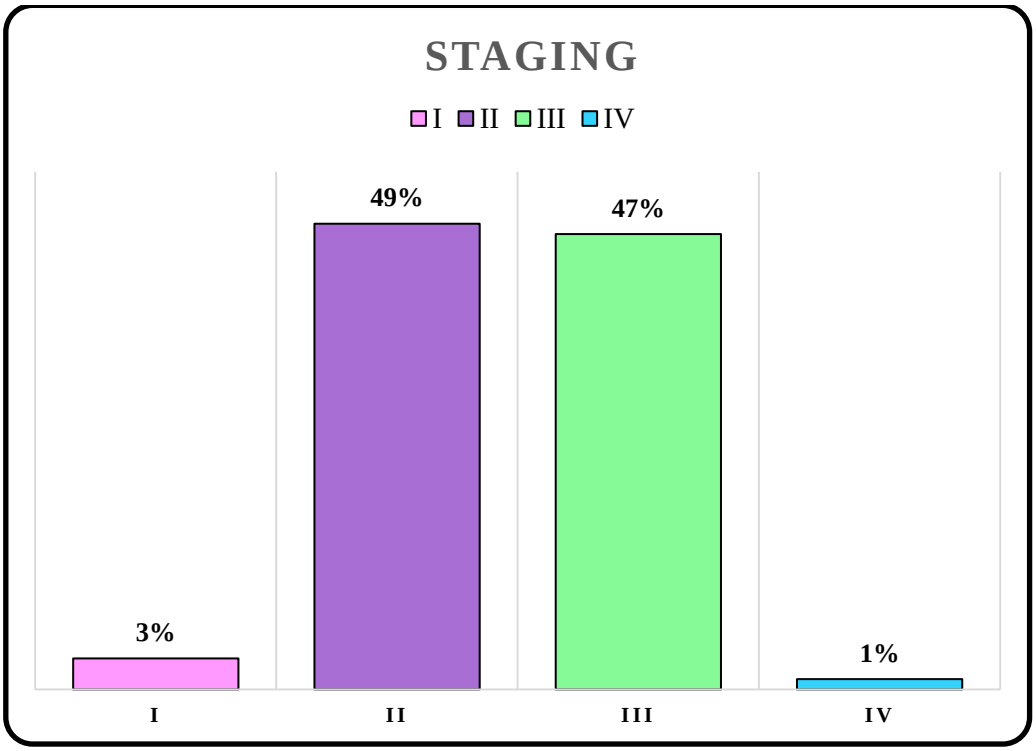


TABLE-16 PATIENT DISTRIBUTION IN BREAST CARCINOMA BASED ON THE NOTTINGHAM PROGNOSTIC INDEX(NPI):

NPI SCORE	FREQUENCY	PERCENT
≤2.4	16	17%
≤3.4	21	23%
≤4.4	23	25%
≤5.4	16	17%
≥5.4	17	18%

The probability of five-year survival rate was 96% in 17%, 93% in 23%, 81% in 25%, 74% in 17%, and ranged from 50% to 38% in 18% of subjects.

GRAPH-8 DEPICTION OF PATIENTS WITH BREAST CARCINOMA ARE CATEGORIZED USING NOTTINGHAM PROGNOSTIC INDEX(NPI):

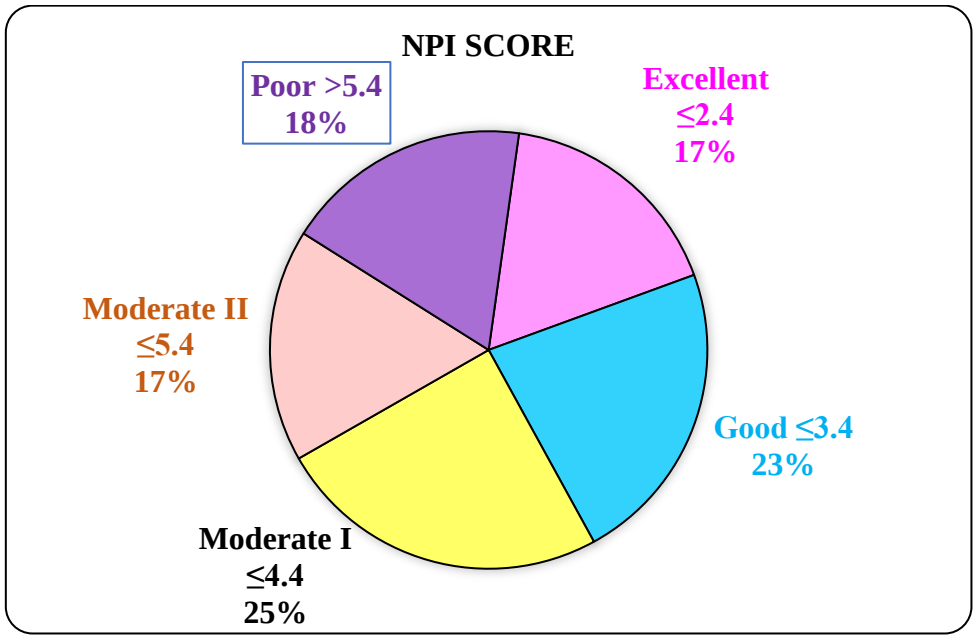


TABLE-17 DISTRIBUTION OF PATIENTS BASED ON THE ER STATUS:

ER STATUS	CASES	PERCENTAGE
Negative	40	43%
Positive	53	57%
Total	93	100%

Estrogen receptor (ER) tested on IHC showed positivity in 57% and negativity in 43% of individuals.

GRAPH-9 PRESENTATION OF PATIENTS DEPENDING ON THEIR ER STATUS:

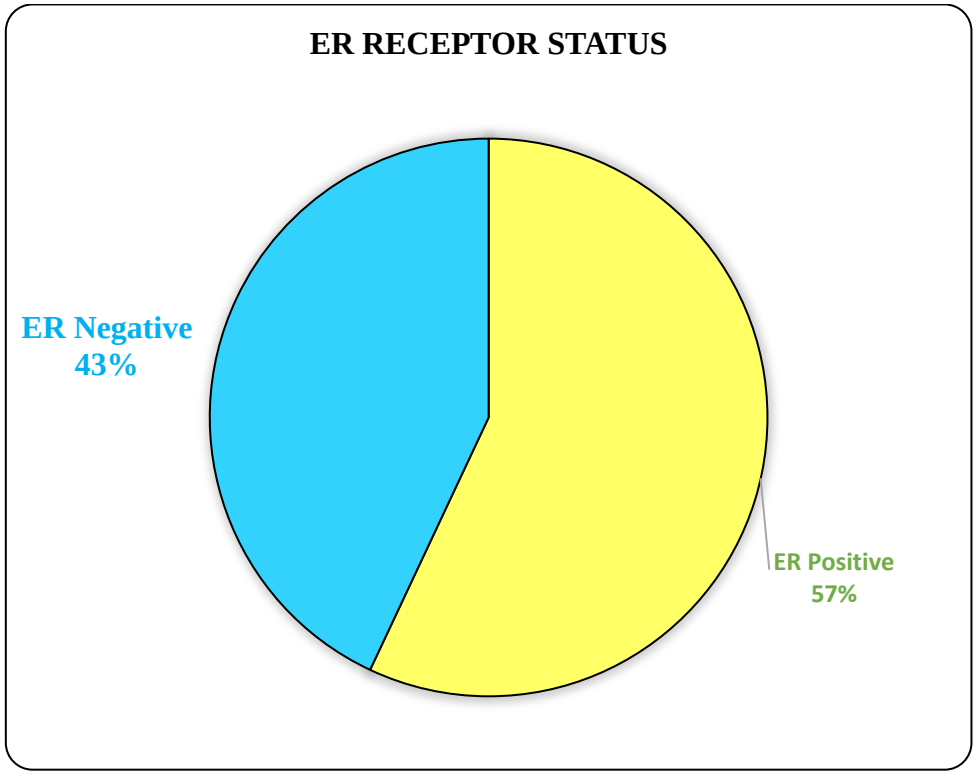


TABLE-18 PATIENTS ARE CATEGORIZED BASED ON THEIR PR STATUS:

PR STATUS	CASES	PERCENTAGE
Negative	38	41%
Positive	55	59%
Total	93	100%

IHC of PR yielded negative results in 41% and positive results in 59% of patients.

GRAPH-10 DEPICTION OF PATIENTS ACCORDING TO THEIR PROGESTERONE RECEPTOR STATUS:

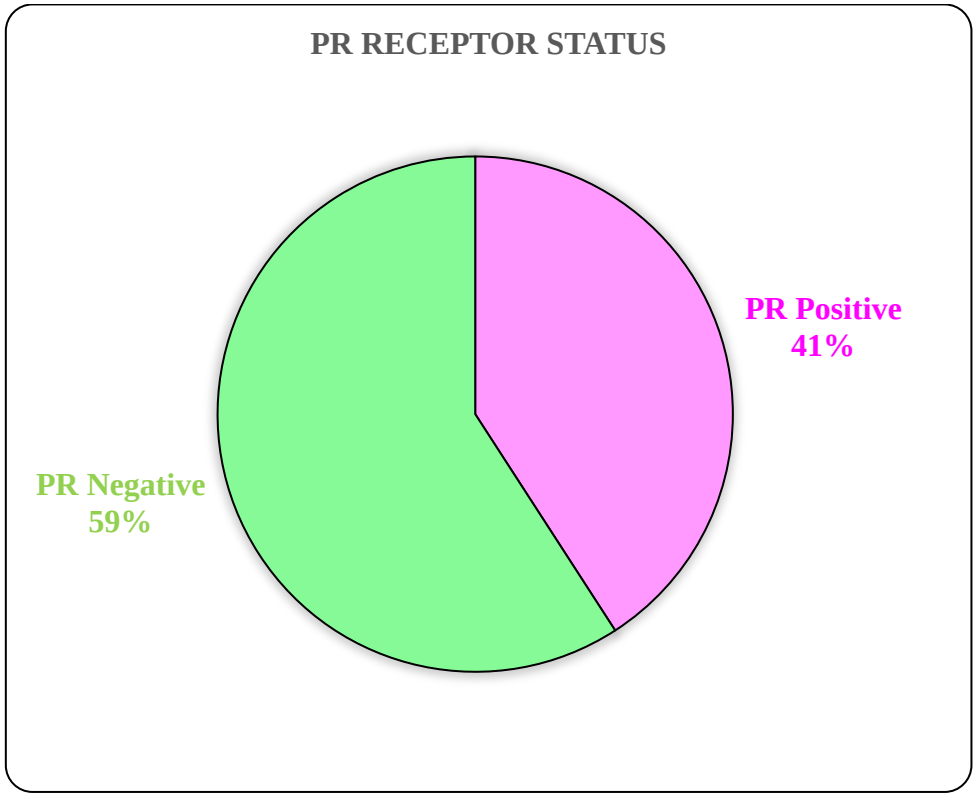


TABLE-19 PATIENT DISTRIBUTION CATEGORIZED ACCORDING TO HER2 NEU STATUS:

HER 2 NEU STATUS	CASES	PERCENTAGE
Negative	69	74%
Positive	24	26%
Total	93	100%

26% of patients had a positive IHC result for Her 2 neu, while 74% of patients had a negative result.

GRAPH-11 DIVISION OF PATIENTS INTO TWO GROUPS BASED ON THEIR HER 2 NEU STATUS:

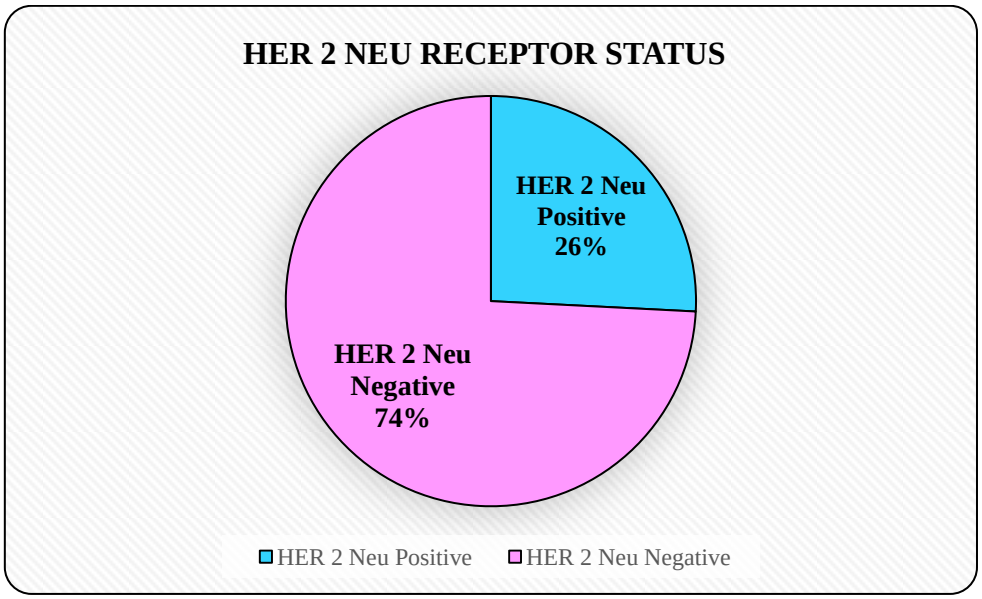


TABLE-20 DISTRIBUTION OF PATIENTS BASED ON THEIR KI67 STATUS:

Ki67 STATUS	FREQUENCY	PERCENT
>14%	58	62%
<14%	35	38%
Total	93	100%

Among the patients, 38% had a Ki67 value of less than 14%, while 62% had a Ki67 value greater than 14%.

GRAPH-12 PATIENT DISTRIBUTION DEPENDING ON THEIR KI67 STATUS:

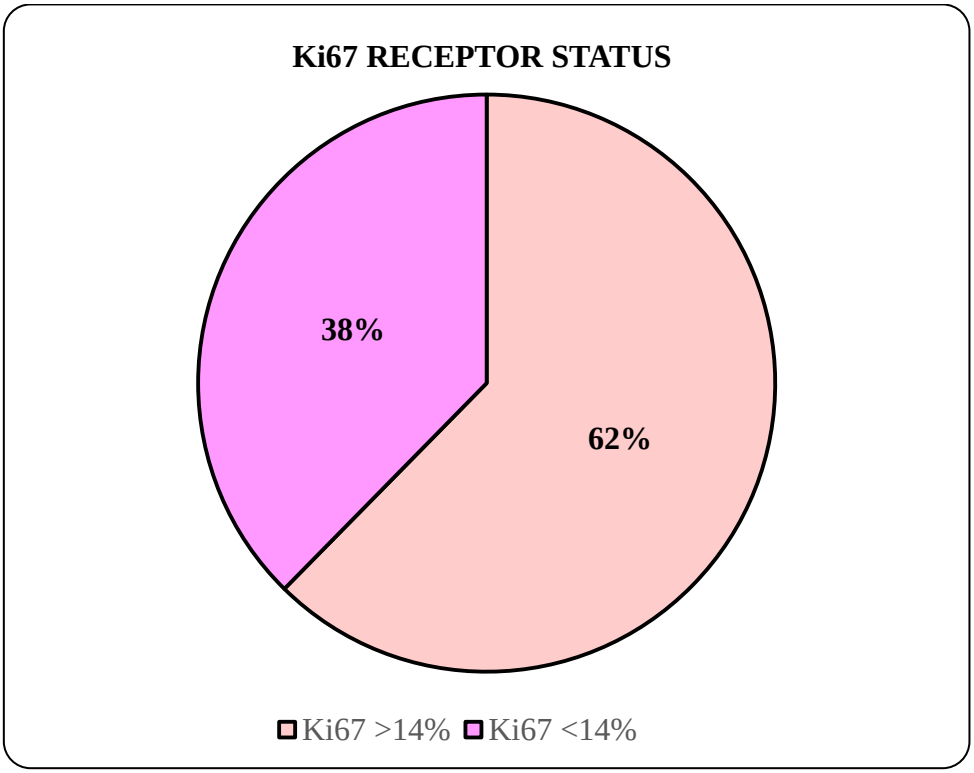


TABLE-21 CATEGORIZATION OF PATIENTS ACCORDING TO MOLECULAR TYPING:

MOLECULAR TYPING	FREQUENCY	PERCENT
Luminal A	32	34%
Luminal B	28	30%
Her2 Neu Enriched	13	14%
TNBC	20	22%
Total	93	100%

Molecular typing showed Luminal A in 34%, Luminal B in 30% of patients, Her2 Neu in 14% of patients and TNBC in 22% of patients.

GRAPH-13 DISTRIBUTION OF PATIENTS BASED ON THE MOLECULAR TYPING:

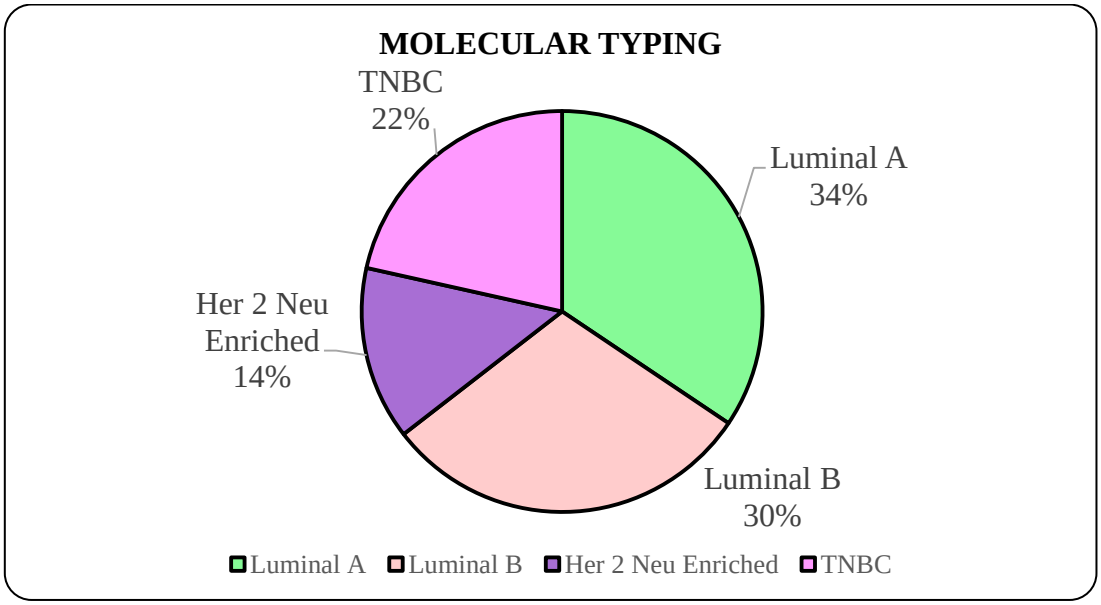


TABLE-22 EVALUATION OF PATIENTS ACCORDING TO THE MDM2 SCORE:

MDM2 SCORE	FREQUENCY	PERCENT
0	15	16%
1	26	28%
2	31	33%
3	21	23%
Total	93	100%

The MDM2 score demonstrated that 16% of patients had no staining, 28% had mild staining, 33% had moderate staining, and 23% had strong staining.

GRAPH-14 SORTING PATIENTS ACCORDING TO THEIR MDM2 SCORE:

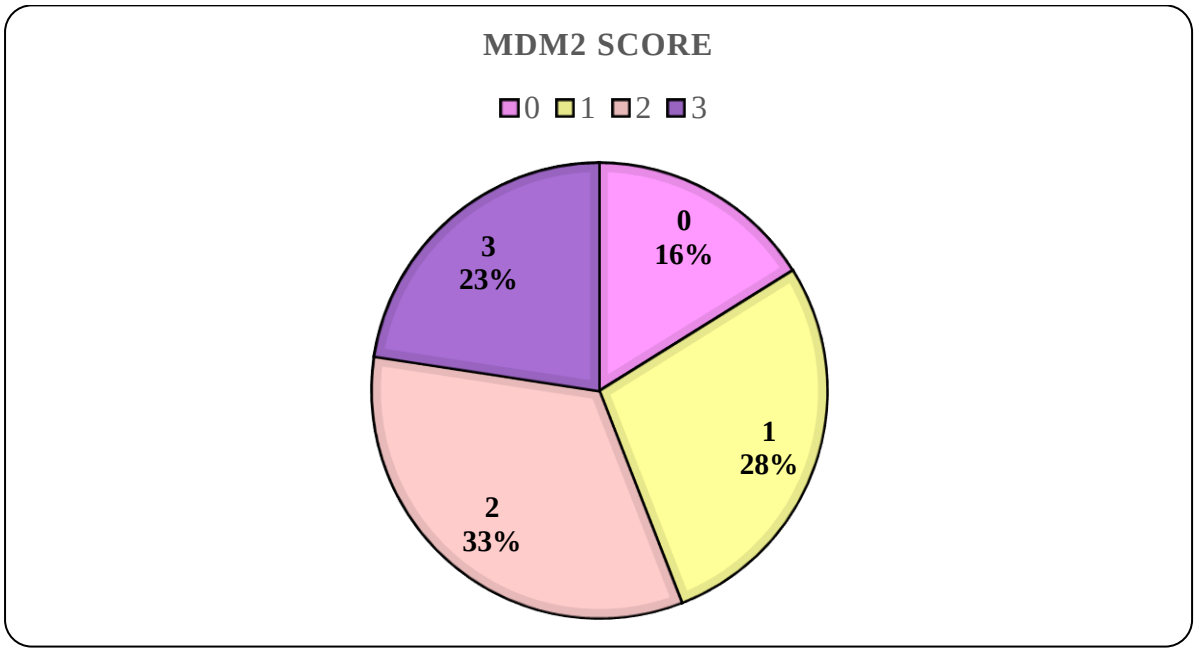


TABLE-23 SUBJECTS ARE ELUCIDATED BASED ON THEIR MDM2 SCORE AND AGE GROUP:

MDM2 SCORING	0		1		2		3	
AGE GROUP	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
30-39yrs	4	22.2%	5	27.8%	4	22.2%	5	27.8%
40-49yrs	3	20.0%	4	26.7%	6	40.0%	2	13.3%
50-59yrs	3	9.4%	10	31.3%	13	40.6%	6	18.8%
60-69yrs	2	11.8%	4	23.5%	5	29.4%	6	35.3%
70-79yrs	3	27.3%	3	27.3%	3	27.3%	2	18.2%

The majority of subjects with a score of 3 were observed in 6 of 17 patients that is 35.3% aged 60-69, while 3 of 11 cases that is 27.3% of patients aged 70-79 had an equal distribution of score of 0,1 and 2. The p -value of 0.880 indicates that there is no noteworthy correlation between the MDM2 score and age group.

GRAPH-15 SUBJECT DISTRIBUTION STRATIFIED BY MDM2 SCORE AND AGE GROUP:

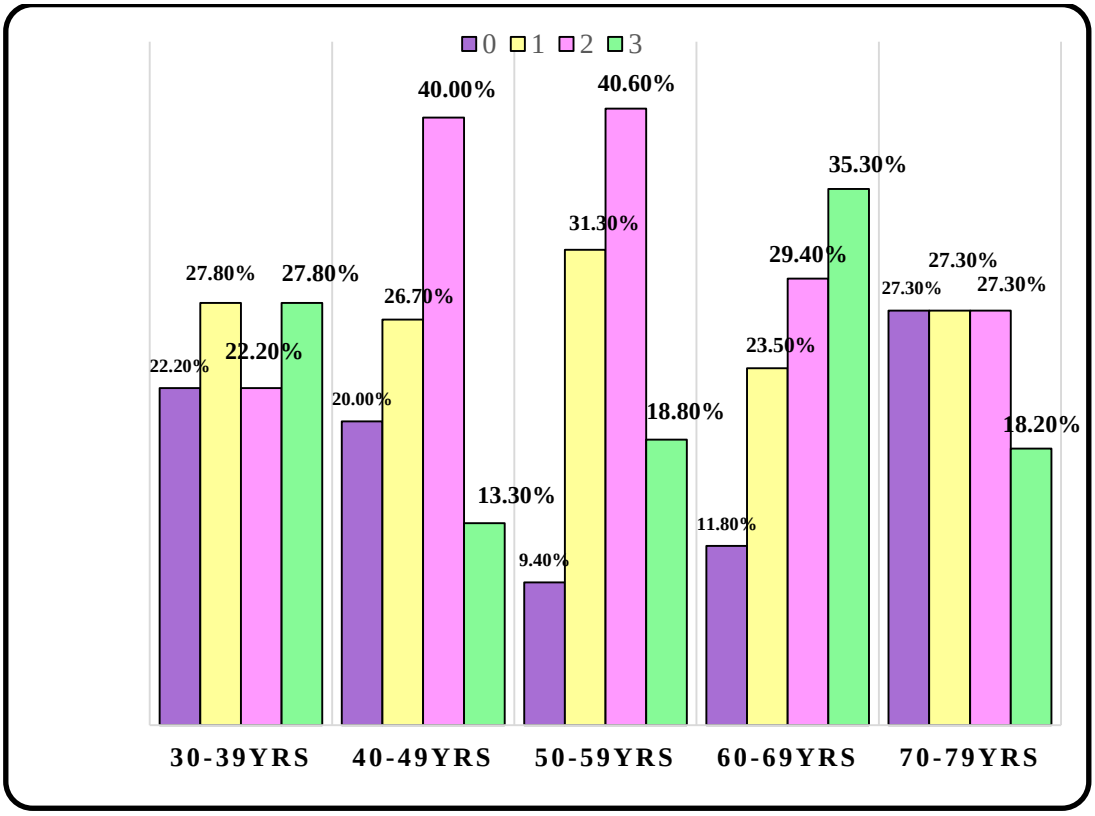


TABLE-24 SUBJECTS WERE REPRESENTED BASED ON THEIR MDM2 SCORE AND TUMOR SIZE:

MDM2 SCORING	0		1		2		3	
TUMOR SIZE	n	%	n	%	n	%	n	%
<2cms	0	0%	1	25%	0	0%	3	75%
2-5cms	8	16%	15	30%	16	32.0%	11	22%
>5cms	7	17.9%	10	25.6%	15	38.5%	7	17.9%

In the group of patients with an MDM2 score of 3, 75% that is 3 of 4 subjects had a tumor dimension <2 cms, 22.0% (11 of 50) with a dimension of between 2 and 5 cm, and 7 (17.9%) of 39 cases had a lesion measurement larger than 5 cm. There were no patients with lesions lesser than 2 centimetres who had a score of 0. However, 16.0% (8 of 50) of participants with tumors measuring 2-5 centimetres and 7 cases (17.9%) with tumors larger than 5 centimetres had a score of zero. The obtained p-value of 0.253 indicates that there was no noteworthy association seen between MDM2 score and lesion dimensions.

GRAPH-16 SUBJECT ILLUSTRATION BASED ON MDM2 SCORE AND TUMOR SIZE:

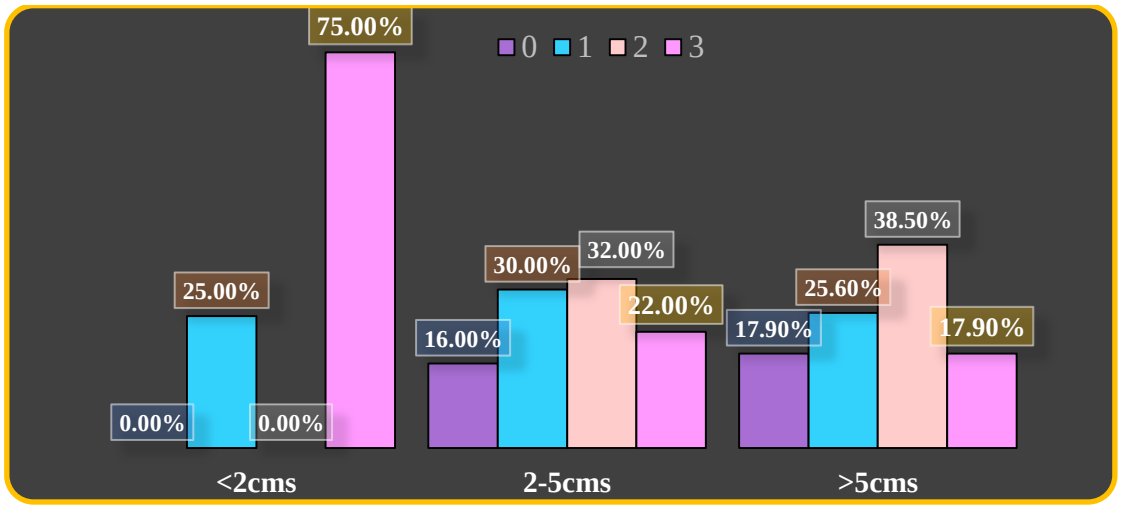


TABLE-25 SUBJECTS ARE CATEGORISED BASED ON MDM2 SCORE AND MODIFIED SCARFF-BLOOM-RICHARDSON'S HISTOLOGICAL TUMOR GRADE (MBR):

MDM2 SCORING	0		1		2		3	
TUMOR GRADE	n	%	n	%	n	%	n	%
I	12	25.5%	18	38.3%	17	36.2%	0	0%
II	3	9.4%	8	25%	11	34.4%	10	31.3%
III	0	0%	0	0%	3	21.4%	11	78.6%

The histological Grade I participants had a majority of zero scores 12 of 47 cases with 25.5%, while Grade III patients had a score of three in about 78.6% (11 of 14) of cases. A substantive association was seen among the MDM2 score & histological grading of tumor, with a p-value of less than 0.001.

GRAPH-17 DISTRIBUTION OF PATIENTS BY MDM2 SCORE AND MODIFIED SCARFF-BLOOM-RICHARDSON'S HISTOLOGICAL TUMOR GRADE (MBR):

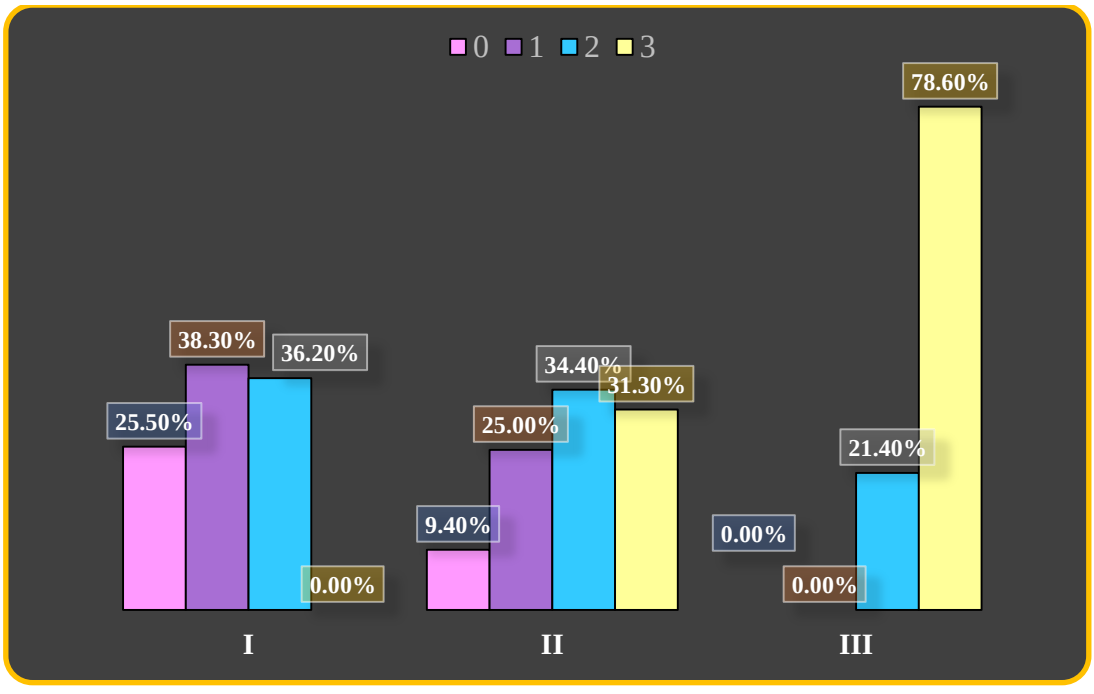


TABLE-26 SUBJECT DISTRIBUTION DIVIDED BY MDM2 SCORE AND TUMOR STAGING:

MDM2 SCORE	0		1		2		3	
TUMOR STAGING	n	%	n	%	n	%	n	%
T1	0	0%	2	100%	0	0%	0	0%
T2	14	34.1%	13	31.7%	12	29.3%	2	4.9%
T3	1	2.5%	8	20.0%	15	37.5%	16	40.0%
T4	0	0%	3	30.0%	4	40.0%	3	30.0%

All T1 cases exhibited a score of 1, whereas 14 out of 41 (34.1%) T2 cases demonstrated a score of zero. Among the 40 cases in T3, 16 instances, or 40.0%, exhibited a score of 3. Similarly, among the 10 T4 patients, 3 cases, or 30.0%, showed a score of 3. The statistical analysis revealed a noteworthy agreement between the MDM2 score and Tumor staging, with a p-value less than 0.001.

GRAPH-18 SUBJECTS ARE DISTRIBUTED BASED ON THEIR MDM2 SCORE AND TUMOR STAGING:

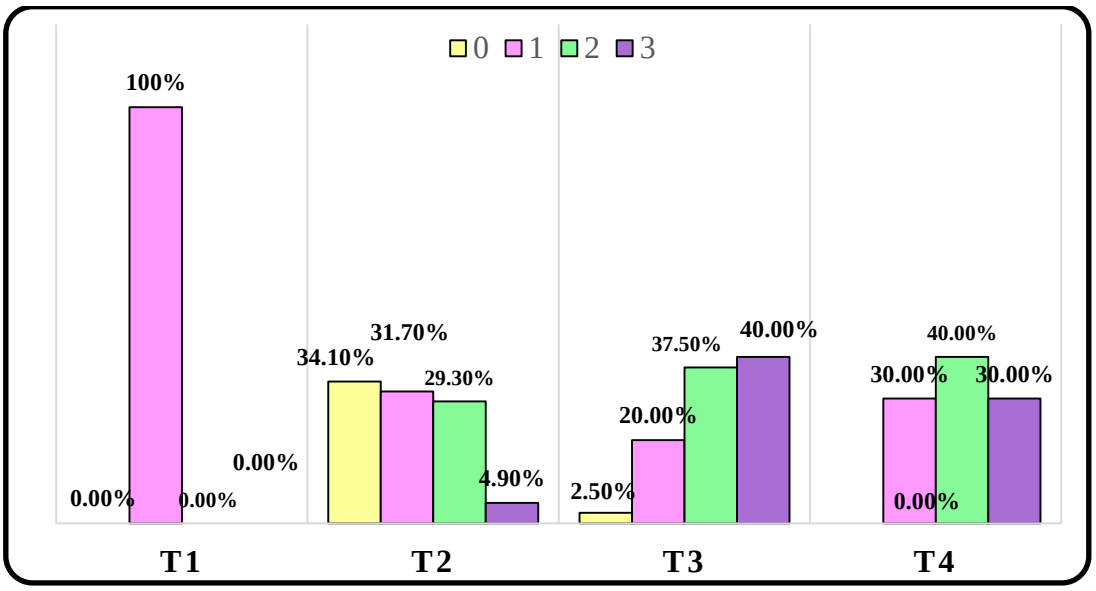


TABLE-27 CLASSIFICATION OF PARTICIPANTS BY MDM2 SCORE AND NODAL STAGING:

MDM2 SCORE	0		1		2		3	
NODAL STAGING	n	%	n	%	n	%	n	%
N0	13	31.7%	14	34.1%	14	34.1%	0	0%
N1	2	9.5%	5	23.8%	8	38.1%	6	28.6%
N2	0	0%	5	25.0%	4	20.0%	11	55.0%
N3	0	0%	2	18.2%	5	45.5%	4	36.4%

Out of the 41 patients without lymph node involvement (N0), around 31.7% had a score of zero, whereas among the 20 patients with lymph node involvement (N2), 55.0% had an MDM2 score of 3. 4 out of 11 patients that is 36.4% with lymph node involvement N3 showed a score of 3. With a probability value less than 0.001 which was correlating.

Out of the patients without nodal metastasis (N0), around 31.7% had a score of 3.

GRAPH-19 SUBJECT ILLUSTRATION ON THE BASIS OF MDM2 SCORE AND NODAL STAGING:

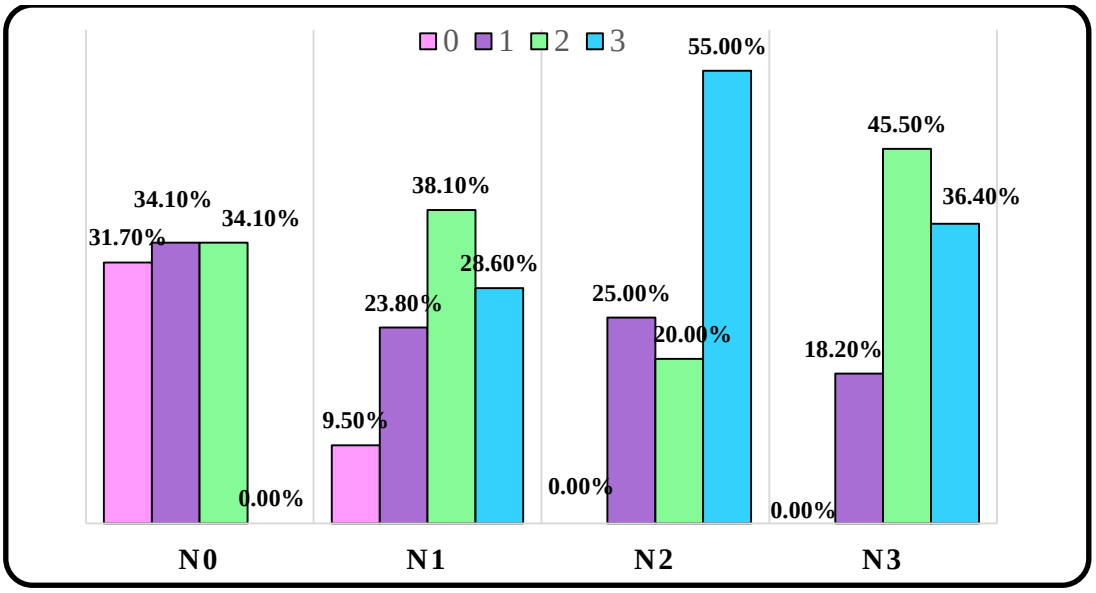


TABLE-28 SUBJECT DISTRIBUTION BASED ON MDM2 SCORE AND EXTRA NODAL EXTENSION:

MDM2 SCORING	0		1		2		3	
EXTRA NODAL EXTENSION	n	%	n	%	n	%	n	%
ABSENT	13	16.3%	23	28.7%	26	32.5%	18	22.5%
PRESENT	2	15.4%	3	23.1%	5	38.5%	3	23.1%

Of the 80 patients who did not have an extra nodal extension, 16.3% got a score of 0. On the other hand, out of 13 subjects 23.1% of patients with extra nodal extension had a score of 3. No relation was observed between the MDM2 score and extra nodal extension (probability Value>0.968).

GRAPH-20 DISTRIBUTION OF SUBJECTS ACCORDING TO MDM2 SCORE AND EXTRA NODAL EXTENSION:

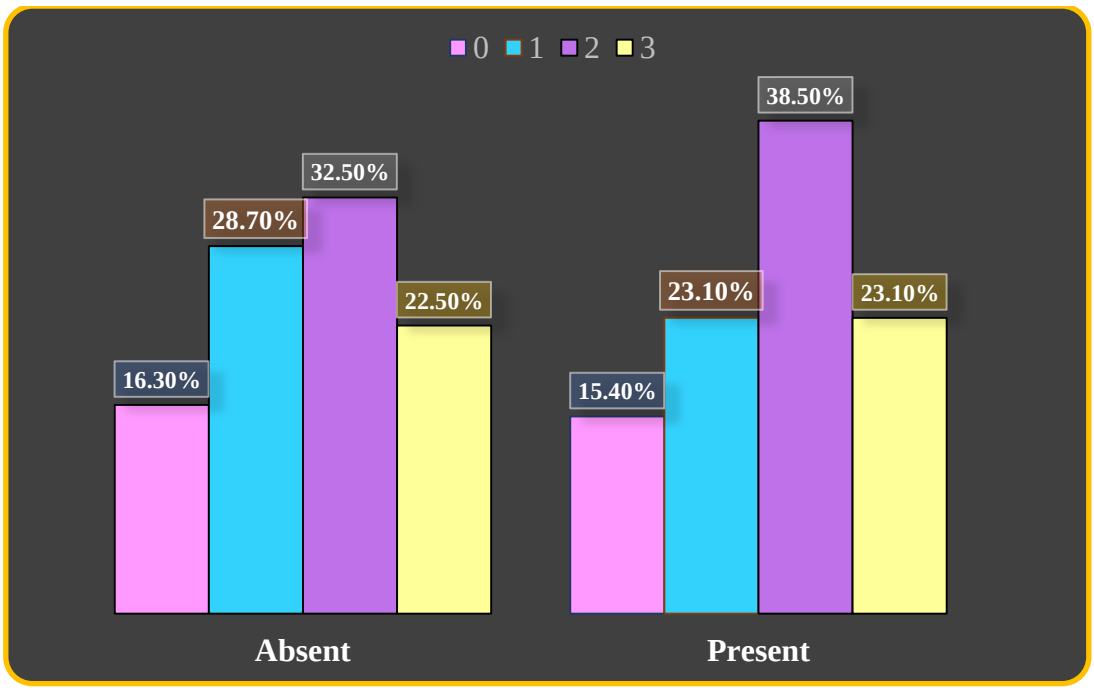


TABLE-29 SUBJECT DIVISION BASED ON MDM2 SCORE AND TNM STAGING:

MDM2 SCORING	0		1		2		3	
TNM STAGING	n	%	n	%	n	%	n	%
I	0	0%	2	66.7%	0	0%	1	33.3%
II	13	28.9%	12	26.7%	15	33.3%	5	11.1%
III	2	4.5%	12	27.3%	15	34.1%	15	34.1%
IV	0	0%	0	0%	1	100.0%	0	0%

Out of the three cases associated with TNM stage 1, two cases (66.7%) had an MDM2 score of 1 positive. On the other hand, from the 45 cases in TNM stage II, 15 cases (33.3%) had a score of two. Among of 44 cases with TNM stage III, 15 instances (34.1%) had a score of III. Only one case was noted with TNM stage IV which showed a score of 3. Almost 28.9% of the 45 cases, or 13 of them, had an MDM2 score of zero. Statistically significant difference was observed between MDM2 score & TNM staging, as indicated by the p-value of 0.028.

GRAPH-21 DEPICTS SUBJECTS IN ACCORDANCE WITH MDM2 SCORE AND TNM STAGING:

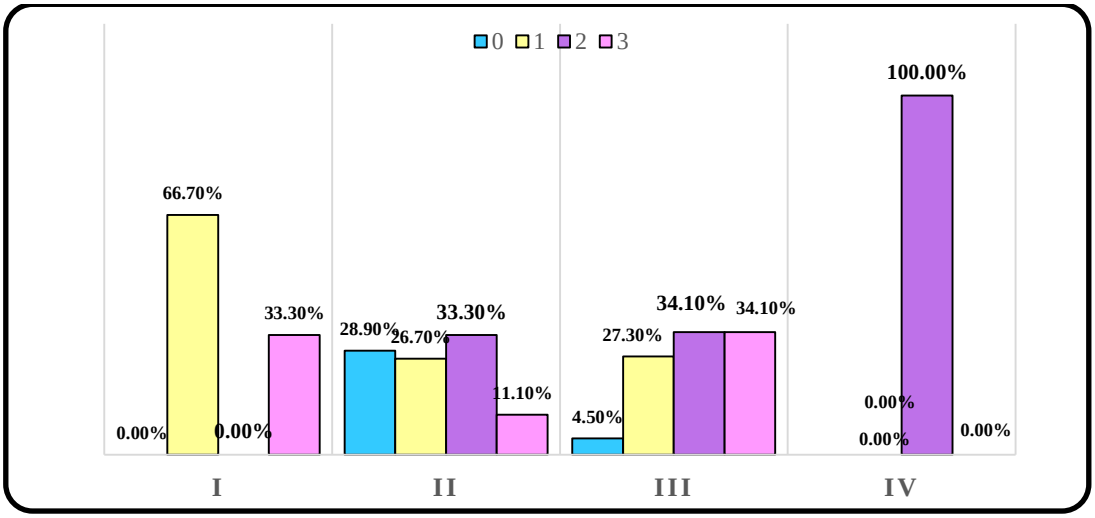


TABLE-30 DESCRIPTION OF DISTRIBUTION OF SUBJECTS ACCORDING TO MDM2 SCORE AND NOTTINGHAM PROGNOSTIC INDEX (NPI):

MDM2 SCORING	0		1		2		3	
NPI	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
≤2.4	2	12.5%	5	31.3%	3	18.8%	6	37.5%
≤3.4	3	14.3%	9	42.9%	6	28.6%	3	14.3%
≤4.4	4	17.4%	5	21.7%	10	43.5%	4	17.4%
≤5.4	3	18.8%	3	18.8%	6	37.5%	4	25.0%
≥5.4	3	17.6%	4	23.5%	6	35.3%	4	23.5%

Out of the 16 subjects with an NPI score of less than 2.4, 6 of them (37.5%) had a score of 3. Similarly, out of the 21 patients with an NPI score of less than 3.4, 9 of them (42.1%) had a score of 1. On the other hand, a score of 2 was observed in 10 out of the 23 patients (43.5%) with an NPI score of less than 4.4. 18.8% of participants with an NPI of less than 5.4 had a score of zero. Most of the cases with an NPI score more than 5.4 had scores of 1, 2, and 3 in 23.5%, 35.3%, and 23.5% of cases respectively. A probability value of 0.826, without any positive correlation.

GRAPH-22 CATEGORISES SUBJECTS BASED ON THEIR MDM2 SCORE AND NOTTINGHAM PROGNOSTIC INDEX (NPI):

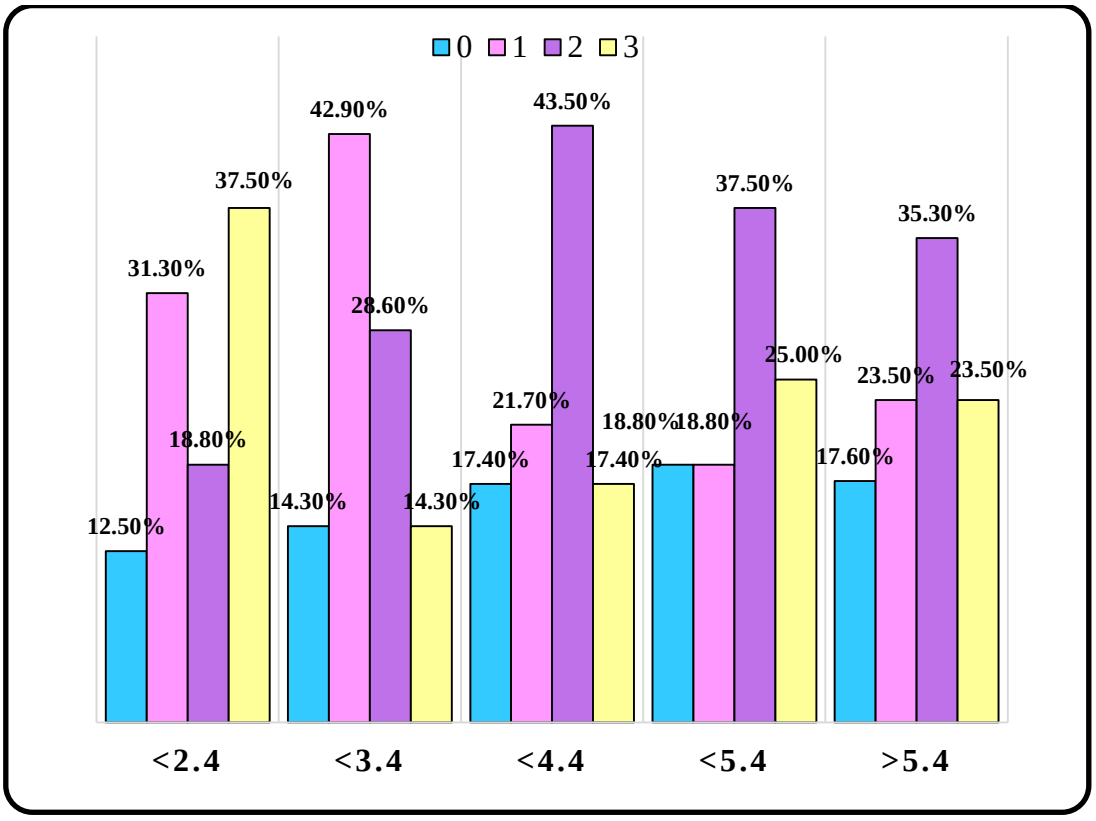


TABLE-31 ILLUSTRATION OF SUBJECTS ACCORDING TO MDM2 SCORE AND MOLECULAR TYPING:

MDM2 SCORE	0		1		2		3		p-value
MOLECULAR TYPING	n	%	n	%	n	%	n	%	
Luminal A	7	21.9%	15	46.9%	7	21.9%	3	9.4%	<0.004
Luminal B	5	17.9%	8	28.6%	12	42.9%	3	10.7%	0.299
Her 2 Neu Enriched	3	23.1%	2	15.4%	5	38.5%	3	23.1%	0.689
TNBC	0	0%	1	5.0%	7	35.0%	12	60.0%	<0.001

The majority of cases with HER2+ exhibited a score of 2, accounting for 38.5% (5 of13) among the overall. Of all the 32 Luminal A cases, approximately 21.9% (7 of 32) had a score of 0, while 46.9% (15 of 32) had a score of 1. A significant proportion of the luminal B patients exhibited a score of 1 in 8 out of 28 cases (28.6%), whereas a score of 2 was observed in 12 out of 28 instances (42.9%). 60.0% (12 out of 20) of TNBCs exhibited a score of three. Among the four molecular types luminal A and TNBC has shown noteworthy correlation with probability values of <0.004 and <0.001 correspondingly.

GRAPH-23 SUBJECTS WERE CATEGORIZED BASED ON THEIR MDM2 SCORE AND MOLECULAR TYPING FOR THE PURPOSE OF DISTRIBUTION

ANALYSIS:

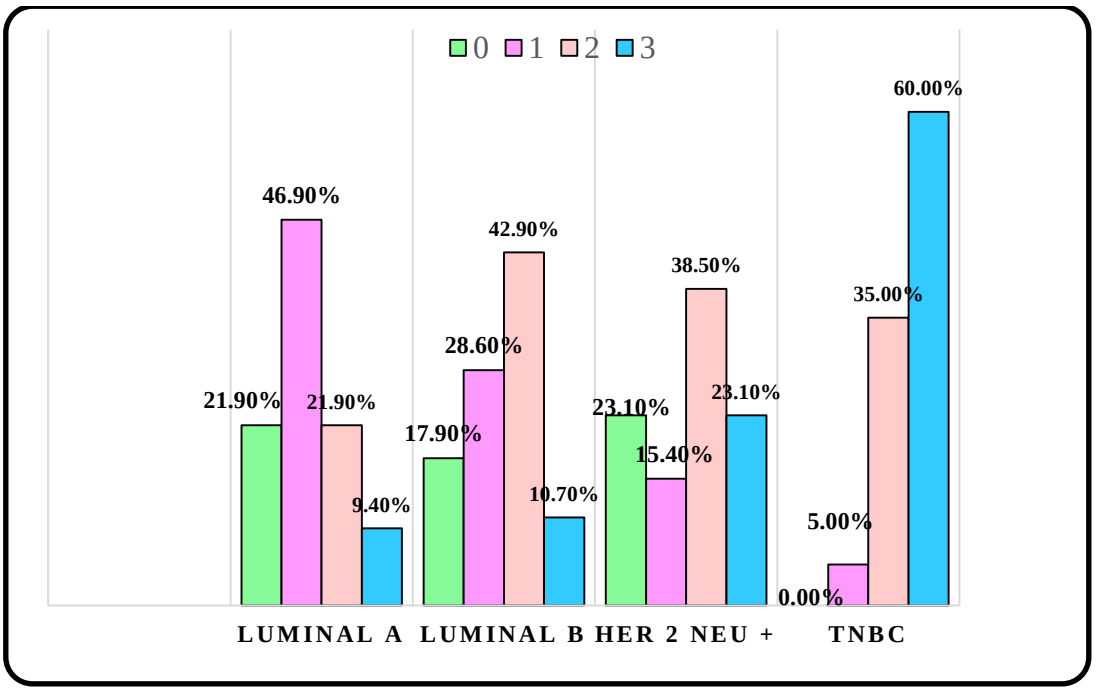
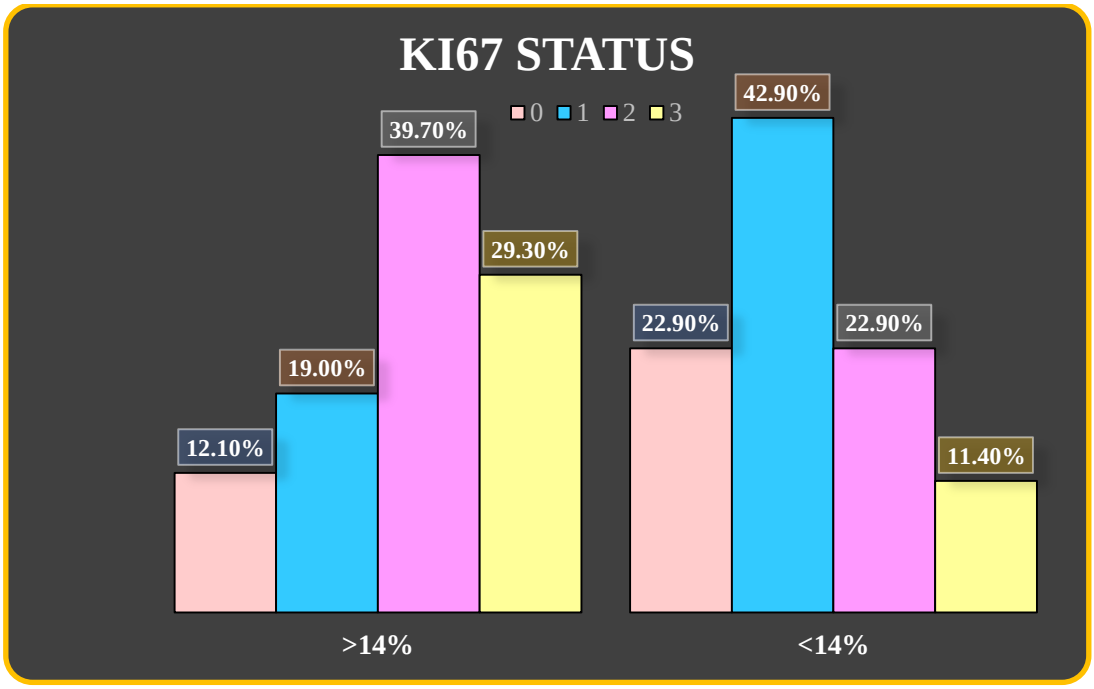


TABLE-32 DISTRIBUTION OF SUBJECTS ACCORDING TO THEIR MDM2 SCORE AND Ki67 STATUS:

MDM2 SCORE	0		1		2		3	
Ki67	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
>14%	7	12.1%	11	19.0%	23	39.7%	17	29.3%
<14%	8	22.9%	15	42.9%	8	22.9%	4	11.4%

Among 35 patients with a Ki67 level greater than 14%, an MDM2 score of 3 was found in 29.6% of them. On the other hand, among 58 patients with a Ki67 level less than 14%, an MDM2 score of 0 was recorded in 22.9% of them. A total of 42.9% of individuals with a Ki67 value below 14% exhibited a score of 1. The association between MDM2 score and Ki67 was found correlating with a probability value of 0.012.

GRAPH-24 SUBJECT DISTRIBUTION IN ACCORDANCE WITH MDM2 SCORE AND KI67 SCORES:



DISCUSSION

DISCUSSION:

Carcinoma of breast constitutes as a diverse illness characterized by different biology and clinical features. It is the predominant form of cancer in women globally, representing a quarter of all cancer instances. [70] The GWAS (Genome-Wide Association Study) summary statistics were acquired from the Breast Cancer Association Consortium, which included data from 122,977 cases and 105,974 controls. Excessive MDM2 production contributes to the growth and propagation of cancers via its involvement in various cellular signalling pathways, including P53 inhibition. The cell cycle, cell death, invasion of the cancer cells, repair of the DNA, migration of the tumor along with angiogenesis, and chemo resistance are all pathways that fall into this category. They have discovered six notable connections between MR (Mendelian randomization) and gene expression levels. These interactions involve the genes TUBB, MDM2, CSK, ULK3, MC1R, & KCNN4. Furthermore, there were two notable interactions observed between MR and the levels of gene methylation in 21 CpG islands. These associations involve the genes RPS23 and MAPT. [71]

Although just a couple of studies focussed on a link between immunohistochemistry (IHC) of MDM2 and histopathological characteristics, as well as hormonal status. Several literatures investigated the function of MDM2 within different types of cancer and its underlying mechanisms. There were gaps detected in the relationship between the immunohistochemistry (IHC) of MDM2 and the parameters stated above. MDM2 is mutated in 3.71% of BC cases, with over expression of MDM2 occurring in 2.55% of all BC cases. [27]

TABLE-33 SHOWS MEAN AGE OF VARIOUS STUDIES ALONG WITH PRESENT STUDY:

STUDY CONDUCTED BY	MEAN AGE (IN YEARS)
Opoku F et al. [20]	49.3
Floris M et al. [23]	51.68
Han M et al. [24]	TCGA (The Cancer Genome Atlas)-61; METABRIC (Molecular Taxonomy of Breast Cancer International Consortium)-63.02
Purshotham MK et al. [72]	52.10
Present study	**53.7**

Within this investigation, we analyzed a total of 93 instances of BC. The clinicopathological demography reveals an age range of 30-80 years, having an average age occurrence of 53.7 years.

Most of patients in the present study were within age ranging 50-59 years, accounting for 35% within the study population, which contrasts the findings of previous study conducted by Opoku F et al. which reported an age range of around 40-49 years, accounting for 31% of the total population with mean age of the participants as 49.3 years. He has also done a comparative analysis of age and MDM2 score and found no statistically significant correlation, as indicated by a p-value exceeding 0.597. This finding is consistent with the findings of the current investigation. [20]

Han M et al. published a research paper on copy number variations within the MDM2 gene in two groups, namely TCGA and METABRIC with a mean age more than the

present analysis. The study found no link between these changes and age. However, when comparing the age group with MDM2 IHC in the present study, the same result was observed. [24]

Present investigation is similar to the average age conducted in previous studies conducted by Floris et al. & Purshotham MK et al., which reported mean ages of approximately around 51.68 and 52.10 years, respectively. [23,72]

TABLE-34 DEPICTS TUMOR SIZE OF FEW STUDIES ALONG WITH PRESENT STUDY:

TUMOR SIZE	PURUSHOTHAM MK et al. [72]	KWON GY et al. [73]	OGAWA Y et al. [74]	PRESENT STUDY
<2cms	14%	42%	24.5%	4%
2-5cms	55%	47.7%	55.5%	54%
>5cms	31%	10.3%	20%	42%

No correlation was noted among size of the lesion & MDM2 in current study with 54% of patients showing a tumor size of 2-5cms. In this study, most of the cases had tumor sizes ranging from 2 to 5 centimeters, followed by tumor sizes greater than 5 centimeters without a significant p-value. There was limited literature available to compare MDM2 and tumor size.

Similar findings of the tumor size were reported by Purushotham MK et al., Kwon GY et al., and Ogawa Y et al., reported significant number of subjects with tumor sizes between 2 to 5 centimeters. [72-74]

TABLE-35 DISPLAYS THE HISTOLOGICAL GRADING (MBR) OF VARIOUS RESEARCHES ALONG WITH CURRENT RESEARCH:

HISTOLOGICAL GRADE	OPOKU et al. [20]		PRESENT STUDY	
	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
GRADE I	7.7%	92.3%	85.2%	14.8%
GRADE II	2.3%	97.7%	83.9%	16.1%
GRADE III	7.0%	93.0%	75%	25%

Opoku et al. reported observed no relationship (p-value 0.528) between the grade and MDM2 score, which contradicts the findings of the present investigation. [20]

Opoku et al, Tang Y et al, Hemalatha et al, as well as Purushotham et al observed that majority of cases had a tumor grade of II, which contradicts current research as most of the participants exhibited grade I tumors. [20,27,72&75]

TABLE-36 DEPICTS THE MDM2 SCORE OF VARIOUS STUDIES ALONG WITH PRESENT STUDY:

MDM2 SCORE	Opoku et al. [20]	Han M et al. [24]		Qi M et al. [26]	PRESENT STUDY
		TCGA	METABRIC		
0	NEGATIVE=93.1%	82.5%	88.9%	LOW=63.5%	16%
1	POSITIVE=6.9%	12.7%	8.2%		28%
2		4.8%	2.9%	HIGH=28.8%	33%
3					23%

Within this study, the MDM2 score was absent in 16% of patients, while a score of 1 was observed in 28% of patients, a score of 2 was observed in 33% of patients, and a value of 3 was observed in 23% of patients.

Opoku et al., observed that the majority of breast tumors had MDM2 negative results, contradicting the findings of the current investigation. [20] In their study, Han M et al., analyzed large cohorts from TCGA and METABRIC and found that the majority of patients having negative score, which contradicts the findings of the current study. [24]

Qi M et al., categorized the scoring of MDM2 as either low or high, with majority of patients exhibiting a low score. However, this finding does not align with the results of the current investigation. [26]

TABLE-37 ILLUSTRATES THE TUMOR STAGING OF VARIOUS STUDIES INCLUDING THE CURRENT STUDY:

T stage	Purushotham MK et al. [72]	Pistelli M et al. [76]	Wang M et al. [77]	Present study
T1	11%	53.6%	18.1%	4%
T2	48%	30.8%	43.4%	49%
T3	22%	12.7%	14.5%	35%
T4	19%	2.6%	23.9%	12%

Research by Pistelli M et al on early-stage breast cancers, noted that patients in stage T1 (53.6%) were higher, but this research found that most of them were in stage T2 which accounted for 49% of cases. [76]

In Purushotham MK et al. and Wang M et al., in their studied noticed predominance of T2 staging which is similar to present study. [72,77]

However, we have noticed correlation between tumor staging and MDM2 score due to paucity of the studies there were comparable literature.

N STAGING:

The majority of the patients (52.5%) for the research conducted by Tang Y et al were classified as being in N0 stage without any extra nodal extension. This finding corresponds to current investigation (53%). [27]

However, this research observed a statistically significant relationship between nodal staging and MDM2 scoring. There was no observed association between MDM2 and extra nodal extension. The literature did not include any research that compared MDM2 and N staging.

TNM STAGING:

In this particular research, specifically stage I and II accounts for 54%. The findings are consistent to those of Floris et al., who also found a similar proportion of 66.18%. [23]

The accessible papers did not include any research comparing the MDM2 score with TNM staging. However, this particular study found a significant p-value when comparing it with TNM staging.

NPI SCORE:

Analysis of statistical data revealed no relationship with NPI score & MDM2. However, none of the papers were available to verify the result. The probability of five-year survival rate was 96% in 17%, 93% in 23%, 81% in 25%, 74% in 17%, and ranged from 50% to 38% in 18% of subjects.

TABLE-38 ILLUSTRATES THE MOLECULAR TYPING OF VARIOUS STUDIES INCLUDING CURRENT STUDY:

MOLECULAR TYPE	Opoku et al. [20]		Qi M et al. [26]		Herok M et al. [78]		PRESENT STUDY	
MDM2 SCORE	_	+	LOW	HIGH	LOW	HIGH	_	+
LUMINAL A	96.4%	3.6%	63.2%	36.8%	49.7%	50.2%	22%	78%
LUMINAL B	87.5%	12.5%			50.2%	49.7%	19%	81%
HER 2 ENRICHED	88%	12%	65.2%	34.8%	50.7%	49.2%	33%	67%
TNBC	93.9%	6.1%	63%	37%	50.3%	49.6%	0%	100%

Opoku et al found a lack of association with regard to MDM2 & luminal A (p-value >0.691), luminal B (p-value >0.323), and triple negative (p-value>0.756) breast cancer subtypes. Despite their research's findings, authors did find an important association (p-value <0.027) among MDM2 & Her2 overexpression. [20]

By a p-value of 0.982, the researchers Qi M et al. discovered a lack of relationship with MDM2 & molecular typing. Nevertheless, the current investigation revealed a strong association between MDM2 and luminal A and TNBC subtypes, contradicting this finding. [26]

Herok M et al. in his research found no considerable link between gene expression and luminal-A, luminal-B, and TNBC subtypes, with probability values of 0.4583, 0.7848, & 0.4591, accordingly. However, the Her-2 enriched group exhibited a noteworthy probability value of 0.0001, which contradicts the current study findings where Luminal A and TNBC groups showed significant p-values with MDM2 IHC. [78]

TABLE-39 SHOWS VARYING HORMONE RECEPTOR STATUS AMONG DIFFERENT STUDIES INCLUDING CURRENT RESEARCH:

STUDY CONDUCTED BY	ER		PR		HER 2 NEU	
	+	–	+	–	+	–
Opoku et al. [20]	29%	71%	10.9%	89.1%	20.7%	79.3%
Han M et al. [24]	97.4%	2.6%	87.3%	12.7%	5.8%	94.2%
Bartnykaitė A et al. [79]	57%	43%	48%	52%	22%	78%
Present study	55%	45%	41%	59%	14%	86%

Opoku et al, Bartnykaitė A et al, & even Han et al demonstrated greater prevalence for ER positivity compared to ER negativity, which aligns with the findings of the present investigation. [20,24&79]

The PR status yielded predominantly unfavorable results in our investigation, which aligns in accordance with outcome of Opoku et al & Bartnykaitė A et al, but not with those of Han et al. Findings of the three researches mentioned previously thereby indicating that Her 2 Neu had a higher degree of negativity compared to positivity, which aligns with the findings of the present investigation. [20,24&79]

Ki67:

Opoku et al observed a substantial association between MDM2 and Ki67, with a p-value of <0.011, which aligns with the findings of the current investigation.[20]

High-grade tumors and higher TNM staging were associated with MDM2 overexpression, indicating a high-risk status in patients. Administering MDM2 inhibitors as a form of intensive therapy can be considered a top priority.

Various types of MDM2 inhibitors for many malignancies including solid tumors such as colorectal carcinoma and malignant mesothelioma, as well as haematological neoplasms and soft tissue sarcoma, are being studied in phase I clinical trials, which are promising developing therapies for these conditions. [80]

CONCLUSION

CONCLUSION:

As the histological grading & nodal staging with the lesion increases, MDM2 score was observed to be high. The MDM2 score reached its peak at 3 in TNM stage III and stage IV, indicating the enhanced aggressiveness of the tumor as the score increases. Prior treatment with MDM2 could potentially reduce the tumor burden and metastasis as most of the cases with MDM2 over expression was noted in subjects with extra nodal extension and higher TNM staging. As many MDM2 inhibitors are in clinical trials MDM2 can be considered as a adjunctive targeted therapy potential for TNBC's and patients with drug resistance.

SUMMARY

SUMMARY:

- A total of 16% had no MDM2 score, 28% had 1, 33% had 2, and 23% had 3.
- The age spectrum of patients was predominantly concentrated in the 50-59 age range, followed by the age groups of 30-39 and 60-69. There was no discernible correlation among age & MDM2 score.
- 54% patients had tumor size measuring between 2-5 centimeters; 42% had >5 centimeters; and 5% with tumor size <2 centimeters. It was determined that the correlation between tumor size and MDM2 score lacked statistical significance.
- 51% of cases had a tumor with histological grade I, 34% with histological grading II, and 15% had a histological grading of III. The correlation between tumor grade and MDM2 score was noteworthy.
- 13% of patients exhibited extranodal extension. The correlation between Extra nodal extension and MDM2 was determined to be statistically insignificant.
- In terms of tumor stage, 3% of individuals were in Stage I, 49% in Stage II, 47% in Stage III, and 1% in Stage IV. The correlation between tumor stage and MDM2 score was deemed considerable.
- Strong correlation had been discovered with regard to MDM2 and histological grading of tumor (MBR), tumor staging, nodal staging and TNM staging with a p-value of <0.001, <0.001, <0.001 and <0.028.
- On the other hand, no notable correlation reported was concerning MDM2 & age group, lesion size, extra nodal staging, and NPI score. The p-values for these associations were all more than 0.880, 0.253, 0.968, and 0.086 respectively.

- Majority of the tumors with Ki67 >14% showed an MDM2 score of 2 (39.7%) and 3 (29.3%) which implies that proliferative index was more in MDM2 positive cases with a remarkable probability value of less than 0.012.
- NPI score was categorized as excellent, good, moderate I, moderate II, and poor in 17%, 23%, 25%, 17%, and 18% correspondingly. The likely overall survival rates corresponding to these categories were 96%, 93%, 81%, 74%, and 38-50%.
- The molecular typing analysis revealed that 34% cases had Luminal-A, 30% had Luminal-B, 14% had Her2 Enriched, & 22% had TNBC. Among the four molecular types Luminal A and TNBC was remarkably noteworthy with probability values of less than 0.004 & 0.001 correspondingly, whereas no significant association was seen between Luminal B and Her 2 Neu Enriched, with probability values of greater than 0.299 and 0.689, accordingly.
- Almost all Luminal A cases showed score of zero and 1 that is 21.9% and 46.9% respectively. Almost 60% of patients with TNBC showed a score of 3.

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ANNEXURES

ANNEXURE - I

INFORMED CONSENT FORM

STUDY TITLE: IMMUNOHISTOCHEMICAL EXPRESSION OF MDM2 IN INVASIVE DUCTAL CARCINOMA OF BREAST AND ITS ASSOCIATION WITH HISTOPATHOLOGICAL PARAMETERS AND HORMONAL STATUS

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

Name and signature / thumb impression Date:

(Subject) Place:

Name and signature / thumb impression Date:

Place:

(Witness/Parent/ Guardian/ Husband)

ANNEXURE –II

PATIENT INFORMATION SHEET

STUDY TITLE: IMMUNOHISTOCHEMICAL EXPRESSION OF MDM2 IN INVASIVE DUCTAL CARCINOMA OF BREAST AND ITS ASSOCIATION WITH HISTOPATHOLOGICAL PARAMETERS AND HORMONAL STATUS

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

The main aim of the study is to find the expression of MDM2 in invasive ductal carcinoma. The specimens from post-surgery will be collected from the department of pathology, SDUMC, Kolar and will be subjected to immunohistochemistry. 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.

All the cost incurred for collection of data, performing the immunohistochemistry tests, analysis; printing publication will be borne by the post graduate student (Dr Priyanka DVS)

For any clarification you are free to contact the investigator.

PRINCIPAL INVESTIGATOR: Dr. Priyanka DVS, Mobile No: +918121319835.

ANNEXURE III

PATIENT PROFORMA:

IMMUNOHISTOCHEMICAL EXPRESSION OF MDM2 IN INVASIVE DUCTAL CARCINOMA OF BREAST AND ITS ASSOCIATION WITH HISTOPATHOLOGICAL PARAMETERS AND HORMONAL STATUS

Name: Age: Hospital Number:

Anonymized Sample No:

Chief complaint:

History of presenting illness:

Past history:

Personal history:

Local examination:

Biopsy Number:

Gross:

Microscopy:

Histopathological diagnosis:

Staging of the disease:

IHC scoring for MDM2 (Score 0 = 0%, 1+ = 1-25%, 2+ = 26-50%, 3+ = >50%)

A tumor was considered MDM2 positive when a score of ≥ 1+ was assigned. Non-Nuclear cytoplasmic staining was not interpreted as positive.

All the cost incurred for collection of data, performing the immunohistochemistry tests, analysis; printing publication will be borne by the post graduate student (Dr Priyanka DVS).

KEY TO MASTER CHART:

T – Tumor staging according to 8th edition of AJCC

N – Nodal staging according to 8th edition of AJCC

M – Metastasis staging according to 8th edition of AJCC

NPI – Nottingham Prognostic Index

IDC – Infiltrating Ductal Carcinoma of breast

ER – Estrogen Receptor

PR -Progesterone Receptor

Her2 neu – Human Epidermal Growth Factor Receptor 2 neu protein

MDM 2 score- Mouse Double Minute 2 score

Neg –Negative

TNC – Triple Negative Breast carcinoma

Her2+ - Her 2 enriched

MBR – Modified Bloom Richardson Score (Histological Grading)

UHID – Hospital Number

MASTER CHART:

Sl No	YEAR	Biopsy no	Age	UHID	Tumor size(cm)	Tumor Grade	Lymph node	Extra nodal extension	T	N	M	Stage	NPI	Proforma	Tumor proper	ER	PR	Her 2 neu	Ki67	Molecular typing	MDM2 SCORE
1	2020	1354	59	857344	3.5	I	0 OF 11	0	2	0	X	II	2.7	IDC	A,B	6	7	Neg	<14 %	Luminal A	0
2	2020	1407	72	863435	3.5	II	0 OF 13	0	2	0	X	II	2.7	IDC	L,M,Q,	Neg	Neg	Neg	<14 %	TNBC	3
3	2020	1445	31	852475	3	II	1 OF 9	0	2	1	X	II	3.6	IDC	F,G	Neg	7	3	>14%	Luminal B	2
4	2020	1462	73	865603	3	I	0 OF 15	0	2	0	X	II	1.6	IDC	C,D	6	5	Neg	<14 %	Luminal A	1
5	2020	1476	60	865757	3.3	I	7 OF 9	0	2	1	X	III	4.6	IDC	B,C	6	5	Neg	<14 %	Luminal A	1
6	2020	1648	45	873038	2.5	II	0 OF 1	0	2	0	X	II	1.5	IDC	F	6	7	Neg	<14 %	Luminal A	2
7	2020	1878	36	878950	1.5	I	0 OF 1	0	2	0	X	I	1.4	IDC	A,B,C	6	7	Neg	<14 %	Luminal A	3
8	2020	1913	67	879823	6.5	II	0 OF 1	0	3	1	X	III	5.2	IDC	P,Q	Neg	Neg	2	<14 %	HER2+	0
9	2020	1920	45	882814	8	III	6 OF 22	0	3	1	X	III	3.9	IDC	B,C	6	7	2	>14%	Luminal B	2
10	2020	2017	50	876387	4.5	II	0 OF 11	0	3	0	X	II	2.89	IDC	A,B,C	6	7	5	>14%	Luminal B	1
11	2021	33	76	885577	6	II	0 OF 11	0	3	0	X	II	4.2	IDC	C,H,U	Neg	Neg	Neg	>14%	TNBC	3
12	2021	256	48	892991	7	III	2 OF 33	2	4	1	X	III	6.6	IDC	F,G	6	7	Neg	<14 %	Luminal A	0
13	2021	410	35	897214	4	I	5 OF 12	0	3	2	X	III	4.8	IDC	J,K	6	7	5	>14%	Luminal B	0
14	2021	544	37	905154	9	I	0 OF 8	0	2	0	X	IIB	3.8	IDC	F	Neg	Neg	3	>14%	HER2+	2
15	2021	582	57	903629	4	II	0 OF 10	0	2	0	X	IIA	3.8	IDC	G	Neg	Neg	Neg	>14%	TNBC	2
16	2021	893	50	923478	1.7	I	0 OF 4	0	1	0	X	II	2.24	IDC	F	Neg	Neg	Neg	>14%	TNBC	3
17	2021	910	52	923327	3	I	0 OF 16	0	2	0	X	IIA	2.6	IDC	B	Neg	Neg	Neg	>14%	TNBC	2
18	2021	1408	71	938765	6.2	I	0 OF 25	0	3	0	X	IIB	2.24	IDC	F	7	6	Neg	<14%	Luminal A	2
19	2021	1452	53	933465	3.5	I	0 OF 7	0	2	0	X	IIA	2.62	IDC	S	5	Neg	Neg	>14%	Luminal B	1
20	2021	1477	54	936249	10.5	I	2 OF 10	0	2	1	X	IIIA	3.8	IDC	F	5	5	Neg	>14%	Luminal B	2
21	2021	1540	70	940132	2.5	I	1 OF 10	0	2	1	X	IIIA	3.5	IDC	C	7	3	Neg	<14%	Luminal A	1
22	2021	1570	54	936249	5	I	13 OF 21	0	3	3a	X	IIIC	6	IDC	J	Neg	Neg	2	>14%	HER2+	2
23	2021	1586	51	941346	1.5	III	0 OF 10	0	1c	0	X	IA	2.3	IDC	C	Neg	Neg	Neg	>14%	TNBC	3
24	2021	1678	65	946403	3.5	I	0 OF 4	0	2	0	X	IIA	2.7	IDC	G	Neg	Neg	3	>14%	HER2+	3
25	2021	1705	65	945963	6	II	1 OF 19	1	4b	1a	X	IIIB	3.3	IDC	L	Neg	Neg	3	>14%	HER2+	2
26	2021	1852	42	39485	3.5	I	5 OF 21	0	3	1	X	IIIA	4.7	IDC	G	6	6	3	<14%	Luminal A	2
27	2021	1970	65	39318	14	I	18 OF 21	3	4c	3c	X	IV	11	IDC	E	6	2	Neg	>14%	Luminal B	2
28	2021	2155	55	39217	8	I	0 OF 10	0	2	0	X	IIB	3.6	IDC	N	5	5	Neg	<14%	Luminal A	0
29	2022	165	67	58769	4	II	1 OF 17	0	2	1a	X	IIB	4	IDC	F	6	7	Neg	>14%	Luminal B	2
30	2022	319	58	62864	2.3	I	0 OF 14	0	2	0	X	IIIA	2.6	IDC	F	Neg	6	3	>14%	Luminal B	2
31	2022	493	41	65320	3	I	0 OF 11	0	2	0	X	IIIA	2.6	IDC	F,P,Q	7	7	Neg	<14%	Luminal A	1
32	2022	551	56	68097	3	I	9 OF 14	0	4a	2a	X	IIIB	3.6	IDC	G	7	7	Neg	<14%	Luminal A	2
33	2022	618	48	67214	3	I	0 OF 5	0	2	0	X	IIA	2.6	IDC	A14	Neg	Neg	Neg	>14%	TNBC	3
34	2022	645	37	69623	3.5	II	5 OF 8	3	4a	3	X	IIIC	6.2	IDC	F,H	Neg	4	Neg	<14%	Luminal A	1
35	2022	690	61	71018	5	I	8 OF 16	1	2	2a	X	IIIA	4.1	IDC	F,G	7	7	Neg	<14%	Luminal A	1
36	2022	717	37	71915	4.5	I	0 OF 5	0	2	0	X	IIA	1.9	IDC	F	Neg	4	3	>14%	Luminal B	3
37	2022	857	39	75439	9	I	0 OF 6	0	1	0	X	IA	2.8	IDC	F	7	6	Neg	<14%	Luminal A	1
38	2022	876	75	77136	5	I	1 OF 13	0	2	1a	X	IIIA	3	IDC	E,G	7	6	Neg	<14%	Luminal A	0
39	2022	877	75	77136	8.5	II	11 OF 24	2	3	2	X	IIIA	5.7	IDC	G,H	7	6	Neg	<14%	Luminal A	0
40	2022	955	38	34745	3.5	II	0 OF 24	0	2	0	X	IIA	4.7	IDC	F	6	6	3	<14%	Luminal A	3
41	2022	960	51	74798	10	II	0 OF 21	0	2	0	X	IIA	4	IDC	G	7	6	3	<14%	Luminal A	1
42	2022	993	52	75084	2.8	II	0 OF 10	0	2	0	X	IIA	2.36	IDC	N,P	Neg	Neg	Neg	>14%	TNBC	3
43	2022	1084	54	55208	6.5	III	5 OF 22	1	3	3	X	IIIC	6.3	IDC	H,J	Neg	Neg	Neg	>14%	TNBC	3
44	2022	1088	56	88209	8	III	3 OF19	0	3	2a	X	IIIA	6.6	IDC	P,Q	Neg	Neg	Neg	>14%	TNBC	2

MASTER CHART:

45	2022	1097	68	81315	7	I	7 OF 19	0	3	2a	X	IIIA	4.4	IDC	F,G	6	7	Neg	<14%	Luminal A	1
46	2022	1167	30	83399	6	I	0 OF 6	0	3	0	X	IIB	2.1	IDC	J	6	7	5	>14%	Luminal B	1
47	2022	1472	60	73975	6	II	0 OF 14	0	3	0	X	IIB	3.2	IDC	F,G	Neg	4	3	>14%	Luminal B	1
48	2022	1817	40	114769	2.8	II	3 OF 41	0	3	2a	X	IIIA	4.56	IDC	G	Neg	Neg	3	>14%	HER2+	0
49	2022	2204	74	130798	6	II	17 OF 21	0	4b	2a	X	IIIB	6.2	IDC	F,G	6	6	3	<14%	Luminal A	2
50	2022	2717	48	105872	5.6	II	7 OF 14	3	3	2a	X	IIIA	6.1	IDC	F,G	Neg	Neg	Neg	>14%	TNBC	2
51	2022	2820	55	143267	4	I	3 OF 19	0	2	1a	X	IIB	2.8	IDC	F	4	3	Neg	<14%	Luminal A	1
52	2022	2935	53	155702	4	II	0 OF 10	0	2	0	X	IIB	3.8	IDC	G,H,J	7	Neg	3	>14%	Luminal B	2
53	2022	2975	55	144626	5.3	II	0 OF 9	0	3	0	X	IIB	4.1	IDC	G,H,J	7	Neg	3	>14%	Luminal B	0
54	2022	3061	55	155400	3	II	0 OF 12	0	2	0	X	IIA	7.1	IDC	H,J	Neg	Neg	3	>14%	HER2+	3
55	2022	3399	49	137723	7.5	II	2 OF 31	0	3	2a	X	IIIA	6.5	IDC	D,E	Neg	4	Neg	<14%	Luminal A	1
56	2022	3490	32	177274	9	I	4 OF 18	0	3	2a	X	IIIA	5.8	IDC	G,H,J	Neg	Neg	2	>14%	HER2+	3
57	2023	17	31	176388	6.5	I	4 OF 15	0	3	2a	X	IIIA	4.3	IDC	W,X,Y	6	7	3	>14%	Luminal B	0
58	2023	103	33	177470	2.4	I	0 OF 19	0	2	0	X	IIA	1.5	IDC	G,H	Neg	Neg	2	>14%	HER2+	0
59	2023	114	46	185791	4	I	0 OF 12	0	2	0	X	IIA	1.8	IDC	H,J	Neg	Neg	2	>14%	HER2+	2
60	2023	252	48	185625	4.2	I	10 OF 12	3	2	3a	X	IIIC	4.84	IDC	K,L	8	Neg	3	>14%	Luminal B	2
61	2023	272	61	92481	7.5	I	2 OF 27	0	4a	2a	X	IIIB	4.5	IDC	K,L,M	Neg	Neg	Neg	>14%	TNBC	3
62	2023	513	36	134501	5	I	0 OF 20	0	2	0	X	IIA	3.1	IDC	F,G	5	3	2	>14%	Luminal B	2
63	2023	625	39	197794	6	I	2 OF 18	0	3	1a	X	IIIA	3.2	IDC	N,P	8	6	Neg	>14%	Luminal B	1
64	2023	1074	38	167783	2.2	II	3 OF 32	0	3	2a	X	IIIA	4.6	IDC	K,L	8	6	Neg	>14%	Luminal B	3
65	2023	1082	55	209821	8	I	1 OF17	0	3	1b	X	IIB	5	IDC	G,H,J,K	8	Neg	3	>14%	Luminal B	2
66	2023	1150	52	211098	9.5	II	1 OF 22	0	4b	1	X	IIIB	4.9	IDC	F,G,H,J	5	8	3	>14%	Luminal B	3
67	2023	1232	38	215963	5.2	I	14 OF 25	3	3	3a	X	IIIC	4.6	IDC	G,H,J	6	3	Neg	<14%	Luminal A	2
68	2023	1281	57	56961	4.8	I	0 OF 14	0	3	0	X	IIB	3.2	IDC	E,F	Neg	Neg	2	>14%	HER2+	1
69	2023	1287	63	218496	4	I	3 OF 16	1	2	1	X	IIB	3.8	IDC	M,N,R	Neg	Neg	Neg	>14%	TNBC	3
70	2023	1686	28	234597	2	I	0 OF13	0	1c	0	X	IA	1.2	IDC	G,H,J,K	Neg	Neg	Neg	>14%	TNBC	1
71	2023	1719	61	163720	8	I	10 OF 11	1	4b	3	X	IIIC	5.6	IDC	K,L	4	3	Neg	<14%	Luminal A	3
72	2023	1721	52	229831	4.3	I	0 OF 21	0	3	0	X	IIB	2.6	IDC	K,L	7	3	Neg	>14%	Luminal B	2
73	2023	1934	40	241407	4.5	I	23 OF 29	1	2	3a	X	IIIC	6.25	IDC	D,A9	6	6	3	>14%	Luminal B	1
74	2023	1971	28	240102	2.4	II	0 OF14	0	2	0	X	IIA	2.8	IDC	A1	7	3	Neg	>14%	Luminal B	0
75	2023	2384	55	250519	8	I	0 OF 21	0	3	0	X	IIB	2.6	IDC	F,H	7	3	2	>14%	Luminal B	1
76	2023	2605	57	224942	7	I	0 OF 27	0	4a	0	X	IIIB	2.4	IDC	E,F	7	5	3	<14%	Luminal A	1
77	2023	2640	52	257482	5.4	I	1 OF 21	0	3	1a	X	IIIA	4.08	IDC	G,H,J	5	Neg	2	<14%	Luminal B	2
78	2023	2748	65	264889	3.5	I	0 OF 12	0	2	0	X	IIA	1.7	IDC	E,F	Neg	Neg	Neg	>14%	TNBC	3
79	2023	2800	59	247042	2.2	II	0 OF 14	0	2	0	X	IIA	2.4	IDC	F,G	6	3	Neg	<14%	Luminal A	1
80	2023	2825	62	267367	2.5	II	0 OF 13	0	2	0	X	IIA	3.5	IDC	G,H	6	3	Neg	<14%	Luminal A	2
81	2023	3042	61	274309	3	I	0 OF15	0	2	0	X	IIA	2.6	IDC	E,F	Neg	Neg	Neg	>14%	TNBC	2
82	2023	3089	45	280007	5	II	0 OF19	0	2	0	X	IIA	4	IDC	G,H	6	6	0	<14%	Luminal A	1
83	2023	3103	50	279012	3	I	1 OF 14	0	2	1	X	IIB	6.4	IDC	F	8	7	2	>14%	Luminal B	1
84	2023	3172	58	271590	8	II	4 OF 23	0	3	3	X	IIIA	7.6	IDC	G	Neg	Neg	2	>14%	HER2+	2
85	2023	3947	72	203764	9	III	0 OF 10	0	3	0	X	IIIA	4.8	IDC	F	Neg	Neg	Neg	>14%	TNBC	2
86	2023	4213	53	272010	9	I	6 OF 26	0	4b	2a	X	IIIB	4.8	IDC	G	Neg	Neg	2	>14%	HER2+	1
87	2023	4241	48	310904	5	III	3 OF 9	0	2	2a	X	IIIA	6	IDC	E	Neg	4	Neg	<14%	Luminal A	0
88	2023	4335	47	310910	4.5	I	0 OF 12	0	2	0	X	IIA	3.9	IDC	F	Neg	Neg	Neg	>14%	TNBC	3
89	2023	4686	78	317553	5.2	I	0 OF 12	0	3	0	X	IIB	4.04	IDC	F	7	8	Neg	>14%	Luminal B	0
90	2024	297	69	236402	5.5	III	0 OF 2	0	3	0	X	IIIA	4.1	IDC	G	Neg	Neg	Neg	>14%	TNBC	3
91	2024	634	56	350383	6	II	0 OF 6	0	3	0	X	IIB	5.2	IDC	G,H	Neg	Neg	Neg	>14%	TNBC	2
92	2024	953	69	358389	2.2	I	0 OF 17	0	2	0	X	IIA	2.44	IDC	G,H	8	7	Neg	<14%	Luminal A	0
93	2024	1112	76	362712	3.2	II	5 OF 11	0	2	2a	X	IIIA	5.4	IDC	F,G	8	7	Neg	<14%	Luminal A	1