"IMMUNOHISTOCHEMICAL EXPRESSION OF ALPHA SMOOTH MUSCLE ACTIN IN STROMA OF INFILTRATING DUCTAL CARCINOMA OF BREAST AND ITS ASSOCIATION WITH HISTOPATHOLOGICAL AND HORMONAL FACTORS-A LABORATORY OBSERVATIONAL STUDY"

BY

Dr. VAJJA NAGARAJU, MBBS

DISSERTATION SUBMITTED TO

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH

TAMAKA, KOLAR, KARNATAKA

IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF MEDICINE

IN

PATHOLOGY

UNDER THE GUIDANCE OF

Dr. HEMALATHA.A MBBS, MD

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DEPARTMENT OF PATHOLOGY

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Date: *Signature of the Candidate*

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LIST OF ABBREVATIONS

IHC – Immunohistochemistry

Alpha-SMA – Alpha smooth muscle actin

ER – Estrogen Receptor

PR – Progesterone Receptor

Her 2 – Human epidermal growth factor receptor 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

PARP – Poly (ADP –ribose) polymerase

ABSTRACT

BACKGROUND:

Breast carcinoma is the second most common cause of cancer worldwide and death in women. Ductal carcinoma is the most common type of breast cancer.

Among various theories regarding the pathogenesis of cancer, the tumor microenvironment is known to play an essential role in cancer development and progression. Cancer-associated fibroblasts show abundant myofibroblasts in the stoma. These are directly associated with the induction of angiogenesis release of growth factors contributing to tumor progression. Immunohistochemical markers Alpha smooth muscle actin(α-SMA) is used to detect myofibroblasts. This study has been taken up to see the expression of cancer-associated fibroblasts in the stroma of infiltrating ductal carcinoma and its relationship with other factors.

AIMS & OBJECTIVES:

To determine the proportion of alpha SMA in the stroma of Infiltrating ductal carcinoma of the breast and to correlate with histopathological and hormonal expression.

MATERIALS AND METHODS:

One hundred cases of infiltrating ductal carcinoma who underwent surgical resection were studied. H & E Slides of all patients were reviewed, and performed immunohistochemistry against Alpha smooth muscle actin. Alpha smooth muscle actin stomal expression was evaluated and correlated with clinicopathological data of cases such as age, histological grading, lymph node status, Extranodal extension, tumor staging, and hormonal expression.

RESULTS: Maximum number of cases were seen in 51-60 age group (33 %). In most cases, tumor size was 2-5 cm (62%), and Grade I tumors (58%). Most cases are ER and PR positive, showing 54 % and 52 %, respectively. Most of the cases are falling in Luminal A and TNBC molecular typing having 38 % and 30 %, respectively. The high stromal expression of the alpha-smooth muscle actin was statistically significant in tumor grade, Lymph node status, ER, PR, and Molecular typing.

CONCLUSION: SMA positivity can be an important prognostic factor for a poor clinical prognosis as its stromal expression correlated with Grade and triple-negative breast cancer (TNBC).

KEYWORDS: Alpha smooth muscle actin, Infiltrating ductal carcinoma, Hormonal expression, and Cancer associated fibroblast.

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INTRODUCTION

INTRODUCTION

Breast cancer (BC) is the commonest malignancy among women globally. It has now surpassed lung cancer as the leading cause of global cancer incidence in 2020, with an estimated 2.3 million new cases, representing 11.7% of all cancer cases.[1] Epidemiological studies have shown that the global burden of BC is expected to cross almost 2 million by the year 2030.[2] In India, the incidence increased significantly, almost 50%, between 1965 and 1985.[3] The estimated number of incident cases in India in 2016 was 118000 (95% uncertainty interval, 107000 to 130000), 98.1% of which were females, and the prevalent cases were 526000 (474000 to 574000). Over the last 26 years, the age-standardized incidence rate of BC in females increased by 39.1% (95% uncertainty interval, 5.1 to 85.5) from 1990 to 2016, with the increase observed in every state of the country.[4] As per the Globocan data 2020, in India, BC accounted for 13.5% (178361) of all cancer cases and 10.6% (90408) of all deaths (Figure 1 and Figure 2), with a cumulative risk of 2.81.[5] Current trends point out that a higher proportion of the disease is occurring at a younger age in Indian women, as compared to the West. The National Cancer Registry Program analyzed data from cancer registries from 1988 to 2013 for changes in cancer incidence. All population-based cancer registries have shown a significant increase in the trend of BC.[6] In India in 1990, the cervix was the leading site of cancer, followed by BC in the registries of 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%), while in Mumbai, the breast was the leading site of cancer (24.1% vs. 16.0%). By 2000-2003, the scenario had changed, and breast had overtaken as the leading site of cancer in all the registries except in the rural registry of Barshi (16.9% vs. 36.8%). In the case of BC, a significant increasing trend was observed in Bhopal, Chennai, and Delhi registries.[7] Regarding the 5-year overall survival, a study reported it to be 95% for stage I patients, 92% for stage II, 70% for stage III, and only 21% for stage IV patients.[8] The survival rate of patients with breast cancer is poor in

India compared to Western countries due to earlier age at onset, late stage of disease at presentation, delayed initiation of definitive management, and inadequate/fragmented treatment.[9] According to the World Cancer Report 2020, the most efficient intervention for BC control is early detection and rapid treatment.[10] A 2018 systematic review of 20 studies reported that BC treatment costs increased with a higher cancer stage at diagnosis. Consequently, earlier diagnosis of BC can lower treatment costs.[11]

The incidence of breast carcinoma in Kolar was 10.8%. Ductal carcinoma is the most common type of breast cancer and is subdivided into ductal carcinoma in situ and infiltrating ductal carcinoma, which constitutes 70-80 % of breast carcinomas cases.[12]

Need For Study:

Among various cancer pathogenesis theories, the tumor microenvironment plays an essential role in cancer development and progression. Cancer-associated fibroblasts (CAFs) like myofibroblasts, smooth muscle cells, endothelial cells, mesenchymal cells, and immune cells form this microenvironment.[13] Carcinoma of the breast, colon, and other solid tumors shows abundant myofibroblasts in the stroma.[14] These are directly associated with the induction of angiogenesis and the release of growth factors contributing to tumor progression.[14] Vimentin, Desmin, Paladin 41g, Alpha smooth muscle actin(α-SMA), and Cadherin 11 are commonly used immunohistochemical markers for detecting myofibroblasts, among which α-SMA is a well-established marker of myofibroblasts.[13] However, the role of myofibroblasts in pathogenesis and treatment resistance has not been well established.[13,14]

Despite tailored treatments protocol for carcinoma breast, recurrences and mortality are still high. Hence this study was taken up to see the expression of cancer-associated fibroblasts in the stroma of infiltrating ductal carcinoma and its relationship with other factors.

AIMS & OBJECTIVES

OBJECTIVES OF THE STUDY

1. To determine the proportion of alpha SMA in the stroma of Infiltrating ductal carcinoma of breast carcinoma.
2. To correlate the expression of alpha SMA in the stroma of Infiltrating ductal breast carcinoma with histopathological and hormonal expression.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

MACROSCOPIC ANATOMY OF BREAST[15]:

The breast is found in both males and females but is rudimentary in males. It is well-developed in females after puberty. The breast is a modified sweat gland. It forms a vital accessory organ of the female reproductive system.

DEVELOPMENT:

The breast will develop from ectodermal thickening called the mammary ridge, milk line, or line of Schultz. This line extends from the axilla to the groin. It appears during the 4th week of intrauterine life, but in humans, it disappears over most of its extent persisting only in the pectoral region. The gland is ectodermal, and the stroma is mesodermal in origin. The persisting part of the mammary ridge is converted into a mammary pit. Secondary buds (15 – 20) grow down from the pit's floor. These buds divide and subdivide to form the lobes of the glands. The entire system is first solid but is later canalized. At birth or later, the nipple is everted at the site of the original pit. The lactogenic hormone of the hypophysis cerebri causes the growth of the mammary glands at puberty.

SITUATION:

The breast lies in the superficial fascia of the pectoral region. A small extension called the axillary tail (of Spence) pierces the deep fascia and lies in the axilla.

EXTENT:

Vertically extends from the second to the sixth rib. Horizontally extends from the lateral border of the sternum to the mid-axillary line.

DEEP RELATIONS:

The breast lies on the deep fascia (pectoral fascia), covering the pectoralis major. Still deeper, there are the parts of 3 muscles, pectoralis major, serratus anterior, and the external oblique muscle of the abdomen. The breast is separated from the pectoralis fascia by loose areolar tissue (retro mammary space). Because of this loose tissue, the average breast can be moved freely over the pectoralis major.

STRUCTURE OF THE BREAST:

[A] THE SKIN:

It covers the gland and presents the following features:

1. A conical projection called the nipple is present just below the centre of the breast at the level of the 4th intercostal space. 15 – 20 lactiferous ducts pierce the nipple. It contains circular and longitudinal smooth muscles.

2. The skin surrounding the base of the nipple is pigmented and forms a circular area called the areola. The nipple is devoid of hair, and there is no fat subjacent to it.

[B] THE PARENCHYMA:

It is made up of glandular tissue which secretes milk. The gland consists of 15 – 20 lobes. Each lobe is an alveoli cluster drained by a lactiferous duct. The lactiferous ducts converge towards the nipple and open on it. Near its termination, each duct has a dilatation called a lactiferous sinus. Within each lobe of the breast, the main duct branches repeatedly to form several terminal ducts, each leading to a lobule consisting of multiple acini. Each terminal duct and its associated lobule is called a terminal duct–lobular unit.

[C] THE STROMA:

It forms the supporting framework of the gland. It is partly fibrous and partly fatty. The fibrous stroma forms septa, known as the suspensory ligaments of cooper, which anchor the skin and gland to the pectoral fascia. The fatty stroma forms the main bulk of the gland. It is distributed all over the breast except beneath the areola and nipple.

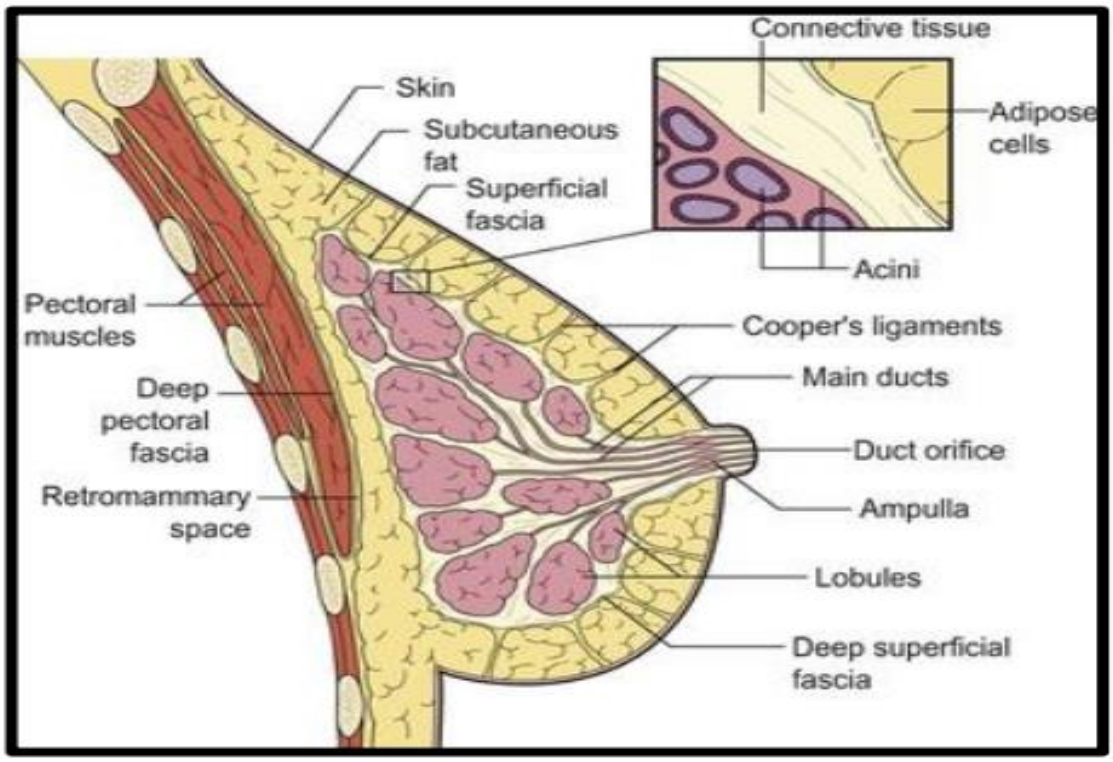


Figure 1: Normal Anatomy of The Breast

BLOOD SUPPLY:

The mammary gland is highly vascular. It is supplied by the branches of the following arteries:

1. Internal thoracic artery – branch of the subclavian artery through its perforating branches.
2. The axillary artery's lateral thoracic, superior thoracic, and Acromiothoracic branches.
3. Lateral branches of the posterior intercostal arteries.

The arteries converge on the breast and are distributed from the anterior surface. The posterior surface is relatively avascular. The veins follow the arteries. They first converge towards the base of the nipple, forming an anastomotic venous circle from where veins run in superficial and deep sets.

The superficial veins drain into the internal thoracic vein and the superficial veins of the lower part of the neck. The deep veins drain into the internal thoracic, axillary, and posterior intercostal veins.

NERVE SUPPLY:

The breast is supplied by the anterior and lateral cutaneous branches of the 4th to 6th intercostal nerves. The nerves convey sensory fibres to the skin and autonomic fibres to smooth muscle and blood vessels. The nerves do not control the secretion of milk. Secretion is controlled by the hormone prolactin, secreted by the pars anterior of the hypophysis cerebri.

LYMPHATIC DRAINAGE OF THE BREAST:

The lymphatic drainage of the breast assumes tremendous importance to the surgeon because carcinoma of the breast spreads mostly along the lymphatics to the regional lymph nodes.

LYMPH NODES DRAINING THE BREAST:

Lymph from the breast drains into the following lymph nodes: The axillary lymph nodes, chiefly the anterior (or pectoral) group. The posterior, lateral, central, and apical nodes also receive lymph directly or indirectly from the breast. The internal mammary (parasternal) nodes lie along the internal thoracic vessels. Some lymph from the breast also reaches the

supraclavicular lymph nodes, the cephalic (deltopectoral) node, the posterior intercostal nodes, and the subdiaphragmatic and sub peritoneal lymphatic plexuses.

LYMPHATIC VESSELS OF THE BREAST:

The superficial lymphatics drain the skin over the breast except for the nipple and areola. The lymphatics pass radially to the surrounding lymph nodes (axillary, internal.

Mammary, supraclavicular and cephalic). The deep lymphatics drain the parenchyma of the breast. They also drain the nipple and areola. About 75% of the breast drains into the axillary nodes, 20% into the internal mammary nodes, and 5% into the posterior intercostal nodes. The internal mammary nodes drain the lymph not only from the inner half of the breast but also from the outer half. A plexus of lymph vessels is present deep in the areola. This is the subareolar plexus of Sappy. The subareolar plexus and most of the lymph from the breast drains into the anterior or pectoral group of lymph nodes. The lymphatics from the deep surface of the breast pass through the pectoralis major muscle and the clavipectoral fascia to reach the apical and internal mammary nodes. Lymphatics from the lower and inner quadrants of the breast may communicate with the subdiaphragmatic and sub-peritoneal lymph plexuses after crossing the costal margin and then piercing the anterior abdominal wall through the upper part of the Linea alba.

NORMAL HISTOLOGY OF THE BREAST[16]:

Understanding diseases of the breast requires a working knowledge of its normal histology. However, what constitutes normal varies based on gender, age, menstrual phase, pregnancy, lactation, and menopausal status. The breast represents a modified skin adnexal structure composed of central lactiferous ducts that originate from the nipple, progressively branching until eventually inducing grape-like clusters of secretory glands as lobules. Breast development starts during the 5th week of gestation when ectoderm thickenings appear on

the fetus ventral surface, extending from the axilla to the groin (mammary ridges or milk lines). Most of these thickening regresses except for an area in the pectoral region. The adult female breast consists of branching ducts and ductless and lobulated acinar units embedded within a fibro adipose stroma. Terminal duct lobular units (TDLUs) are composed of lobules, groups of alveolar glands embedded in loose intralobular connective tissue that connect to a single terminal ductule. These are the structural and functional units of the breast, and most pathologic processes arise within them. The lining throughout the duct/lobular system comprises two distinct layers: an inner (luminal) epithelial layer with a cuboidal to columnar appearance and an outer (basal) myoepithelial layer. The myoepithelial cells have variable morphologies ranging from flattened to epithelioid with clear cytoplasm to a myoid appearance. The intralobular stroma is usually sharply demarcated from a denser, collagenized, paracellular interlobular stroma. The proportion of dense stroma to adipose tissue is variable, with younger women having denser connective tissue.

Breast lobules can be classified based on their morphology into three major types. Type 1 lobules are the most primitive and rudimentary and are usually seen in prepubertal and nulliparous women. Type 3 lobules are the most developed and are usually present in parous and postmenopausal women. The progression from type 1 to type 3 is accompanied by additional branching and increased alveolar buds.

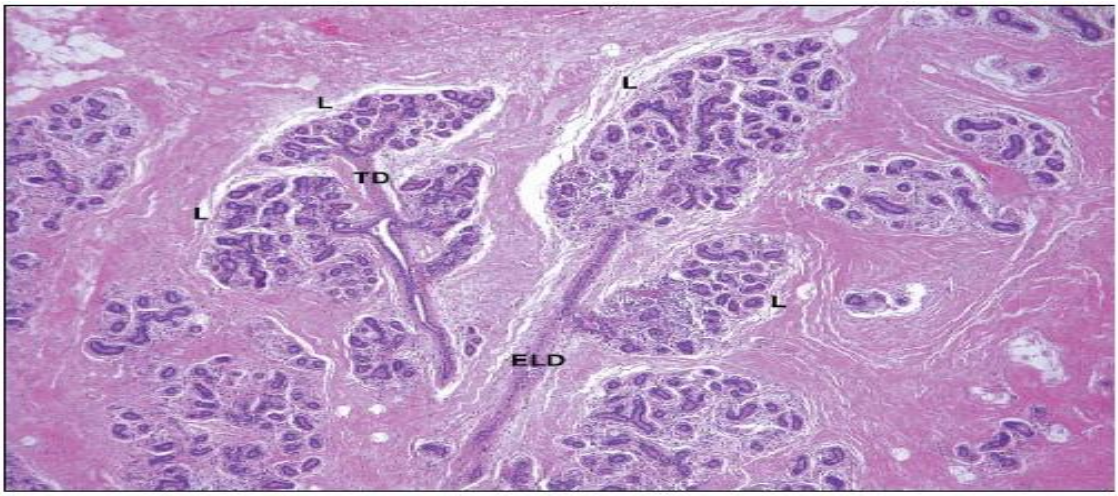


Figure 2 : **Normal Histology of Breast** tissue showing extralobular ducts (ELD), terminal ducts (TD), and lobules (L), the latter composed of groups of small glandular structures, the acini.

BREAST CARCINOMA:

WHO CLASSIFICATION OF TUMOURS OF THE BREAST 5th EDITION 2019[17]

I. Epithelial Tumors - Micro invasive carcinoma

A) Invasive Breast Carcinoma

- Invasive carcinoma of no special type (NST)
  - Pleomorphic carcinoma
  - Carcinoma with osteoclast-like stromal giant cells
  - Carcinoma with choriocarcinomas features
  - Carcinoma with melanotic features
- Invasive lobular carcinoma
  - Classic lobular carcinoma
  - Solid lobular carcinoma

- Alveolar lobular carcinoma
- Pleomorphic lobular carcinoma
- Tubulo lobular carcinoma
- Mixed lobular carcinoma

- Tubular carcinoma

- Cribriform carcinoma

- Mucinous carcinoma

- carcinoma with medullary features

  - Medullary carcinoma
  - Atypical medullary carcinoma
  - Invasive carcinoma NST with medullary features

- carcinoma with apocrine differentiation

- carcinoma with signet-ring cell differentiation

- Invasive micropapillary carcinoma

- Metaplastic carcinoma of no special type

  - Low-grade Adenosquamous carcinoma
  - Fibromatosis- like metaplastic carcinoma
  - Squamous cell carcinoma
  - Spindle cell carcinoma
  - Metaplastic carcinoma with mesenchymal differentiation

- Chondroid differentiation

- Osseous differentiation

- Other types of mesenchymal differentiation

  - Mixed metaplastic carcinoma
  - Myoepithelial carcinoma

•Rare types

- carcinoma with neuroendocrine features
    - Neuroendocrine tumor, well-differentiated
    - Neuroendocrine carcinoma, poorly differentiated (small cell carcinoma)
    - Carcinoma with neuroendocrine differentiation
- Secretory carcinoma
- Invasive papillary carcinoma
- Acinic cell carcinoma
- Mucoepidermoid carcinoma
- Polymorphous carcinoma
- Oncocytic carcinoma
- Lipid- rich carcinoma
- Glycogen-rich, clear-cell carcinoma
- Sebaceous carcinoma
- Salivary gland/skin adnexal type tumors
    - Cylindroma
    - Clear cell hidradenoma

B) Epithelial-myoepithelial tumors

- Pleomorphic adenoma
- Adenomyoepithelioma
- Adenomyoepithelioma with carcinoma
- Adenoid cystic carcinoma

C) Precursor lesions

- Ductal carcinoma in situ
- Lobular neoplasia
- Lobular carcinoma in situ
- Classic lobular carcinoma in situ
- Pleomorphic lobular carcinoma in situ
- Atypical lobular hyperplasia

D) Intraductal Proliferative Lesions

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

E) Papillary lesions

- Intraductal papilloma
  - Intraductal papilloma with atypical hyperplasia
  - Intraductal papilloma with ductal carcinoma in situ
  - Intraductal papilloma with lobular carcinoma in situ
- Intraductal papillary carcinoma
- Encapsulated papillary carcinoma
  - Encapsulated papillary carcinoma with invasion
- Solid papillary carcinoma
  - In situ
  - Invasive

F) Benign epithelial proliferation

- Sclerosing adenosis
- Apocrine adenosis

- Micro glandular adenosis
- Radial scar/ complex sclerosing lesion

G. Adenomas

- Tubular adenoma
- Lactating adenoma
- Apocrine adenoma
- Ductal adenoma

II. Mesenchymal Tumors

- Nodular fasciitis
- Myofibroblastoma
- Desmoid- type fibromatosis
- Inflammatory Myofibroblastic tumor
- Benign vascular lesions
  - Haemangioma
  - Angiomatosis
  - Atypical vascular lesions
- Pseudo angiomatous stromal hyperplasia
- Granular cell tumor
- Benign peripheral nerve-sheath tumor
  - Neurofibroma
  - Schwannoma
- Lipoma
  - Angiolipoma

- Liposarcoma
- Angiosarcoma
- Rhabdomyosarcoma
- Osteosarcoma
- Leiomyoma
- Leiomyosarcoma

III. Fibroepithelial Tumors

- Fibroadenoma
- Phyllodes tumor
    - Benign
    - Borderline
    - Malignant
    - Periductal stromal tumor, low grade
- Hematoma

IV. Tumors of the Nipple

- Nipple adenoma
- Syringomatous tumor
- Paget's disease of the nipple

V. Malignant Lymphoma

- Diffuse large B- cell lymphoma
- Burkitt lymphoma
- T-cell lymphoma

- Anaplastic large cell lymphoma
- ALK-negative

- Extranodal marginal zone B- cell lymphoma of MALT type

- Follicular lymphoma

VI. Metastases to breast

VII. Tumors of the Male Breast

- Gynecomastia

- carcinoma

  - Invasive carcinoma
  - In situ carcinoma

VIII. Clinical Patterns

- Inflammatory carcinoma,Bilateral breast carcinoma

INVASIVE DUCTAL CARCINOMA, NOT OTHERWISE SPECIFIED (NOS)[17]

DEFINITION:

Invasive ductal carcinoma, not otherwise specified (NOS), comprises the largest group of invasive breast cancers. It is a heterogeneous group of tumors that fail to exhibit sufficient characteristics to achieve classification as a specific histological type, such as lobular or tubular carcinoma. It is the most common invasive breast carcinoma comprising 40% and 75% of the population. These tumors are derived exclusively from mammary ductal epithelium in distinction from lobular carcinomas, which arise from within lobules. Ductal NOS tumors are rare below the age of 40 years.

SYNONYMS:

Invasive ductal carcinoma, no specific type (Ductal NST); infiltrating ductal carcinoma.

MACROSCOPY:

The tumors have no specific macroscopic features. There is a marked variation in size, from under 10 mm to over 100 mm. They can have an irregular, stellate outline or nodular configuration. The tumor edge is usually moderately or ill-defined. Classically ductal NOS carcinomas are firm or hard on palpation and have a gritty feel when cut with a knife. The cut surface is usually grey-white and may have a few yellow streaks.

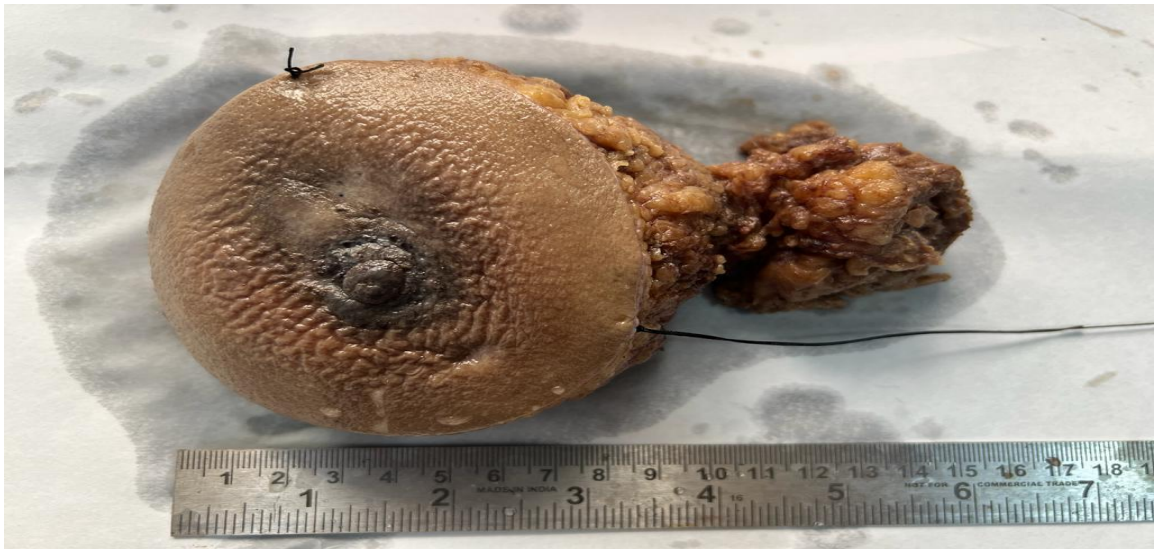


Figure 3: Gross image of mastectomy specimen with axillary clearance B/2204/22

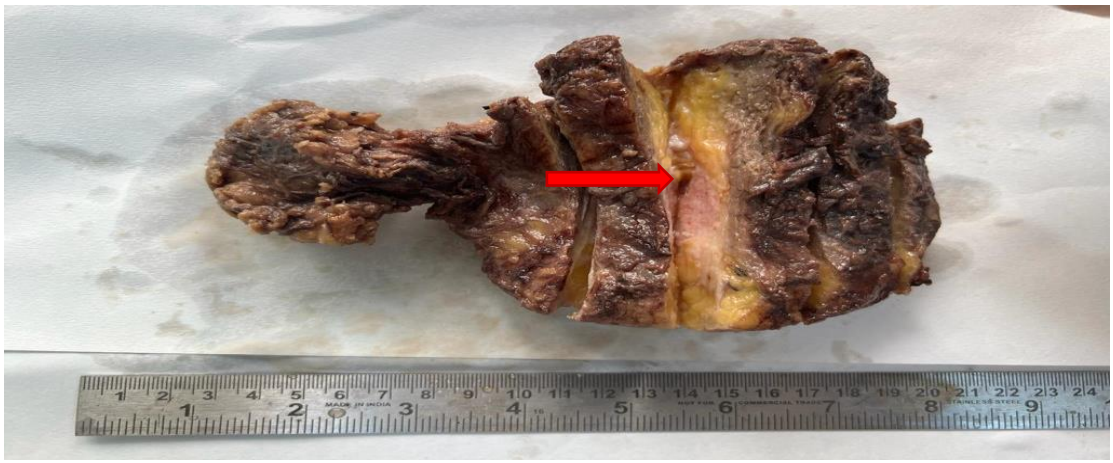


Figure 4: Cut surface showing grey white homogenous solid tumor with foci of haemorrhage B/2204/22

HISTOPATHOLOGY:

Usually, the tumors are architecturally arranged in cords, clusters, and trabeculae, while some tumors are characterized by a predominantly solid or syncytial infiltrative pattern with little associated stroma. In some cases, epithelial differentiation may appear as tubular structures with central lumina in tumor cell groups. Occasionally, areas with single-file infiltration or targetoid features are seen, but these lack the cytomorphological characteristics of invasive lobular carcinoma. The carcinoma cells also have a variable appearance. The cytoplasm is often abundant and eosinophilic. Nuclei may be regular, uniform, or highly pleomorphic with prominent, often multiple nucleoli. Mitosis may be either absent or extensive. In 80% of cases, foci of associated ductal carcinoma in situ (DCIS) will be present. Associated DCIS is often high-grade comedo type, but all other patterns may be seen.

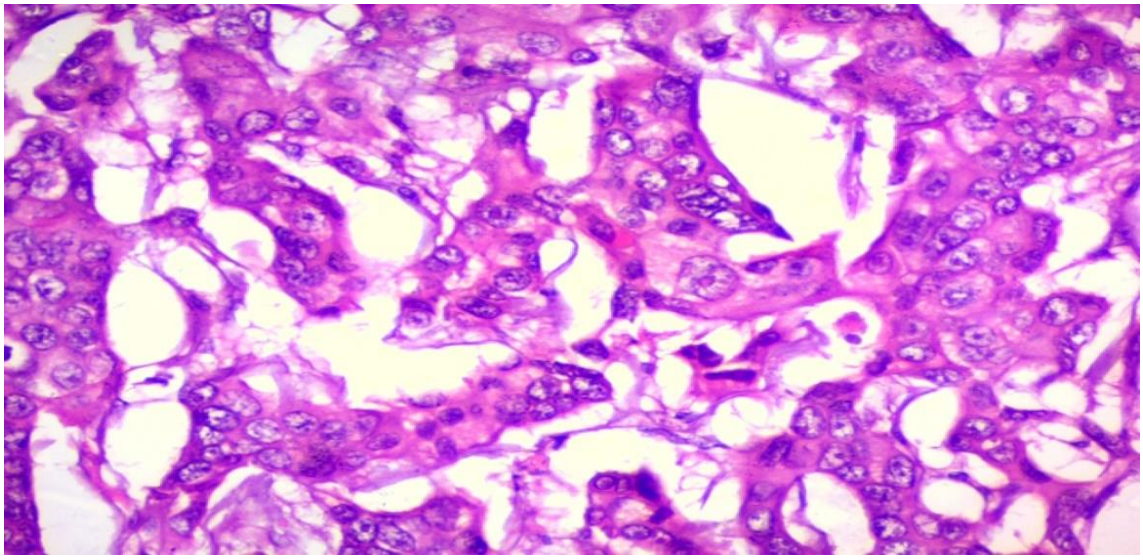


Figure 5: Microscopy (40X): showing tumor cells are arranged nests and in sheets, individual cells are round to oval with pleomorphic vesicular nuclei having

LOBULAR CARCINOMA:[20]

It comprises 5-15% of breast cancers. They are usually present with focal in situ lobular carcinomas and gross appearance is often irregular with poorly defined margins. The tumor cells are small to moderately sized cells usually non-cohesive with cells arranged in Indian file pattern.

TUBULAR CARCINOMA:[20]

Usually comprises 2% of breast cancers and are usually smaller in size (<2 cm). These tumors carry a better prognosis as they are less aggressive, increased use of mammography. Most lesions tend to be in T1 stage, and 90% of tumor express ER positivity. The most consistent microscopic features are the open Lumina lined by single layer of epithelial cells.

MUCINOUS CARCINOMA: [20]

The tumor is also known as colloid, mucoid or gelatinous carcinoma. Focal mucinous differentiation may be seen in 2% of cases of infiltrating duct carcinoma. Microscopically, the tumor consists of small islands of normal epithelial cells set within extensive lakes of extracellular mucin. The cells are small, with darkly staining nuclei and little nuclear pleomorphism. The proportions of mucin and neoplastic epithelium vary among cases, but the magnitude of the overall mucinous component is known to remain constant.

CRIBRIFORM CARCINOMA[20]

This neoplasm has only been recognized as a special type of breast carcinoma in the last two decades because of its distinctive morphologic pattern and excellent prognosis.

Grossly, tumors form a firm mass with a stellate configuration, measuring between 1 and 3cm in diameter. Microscopically, it consists almost entirely (>90%) of an invasive cribriform pattern. The cells are arranged as islands and an often angulated. The tumor cells are small and show a low or moderate degree of nuclear pleomorphism. Cribriform intraductal carcinoma is observed in about 80% of cases.

INVASIVE PAPILLARY CARCINOMA[20]

It is a sporadic tumor and comprises less than 1-2% of invasive breast cancers and is characterized by a relatively good prognosis. Invasive papillary carcinomas are diagnosed predominantly in postmenopausal patients. Grossly, these are demarcated soft tumor measuring between 1-3 cm in diameter. Microscopically, we can note papillary structures with the central fibrovascular core. The nuclei of the tumor cells are typical of intermediate grade, with amphophilic cytoplasm and an increased number of mitoses. In more than 75% of cases, micropapillary or cribriform ductal carcinoma in situ is present.

DUCTAL CARCINOMA IN SITU[20]

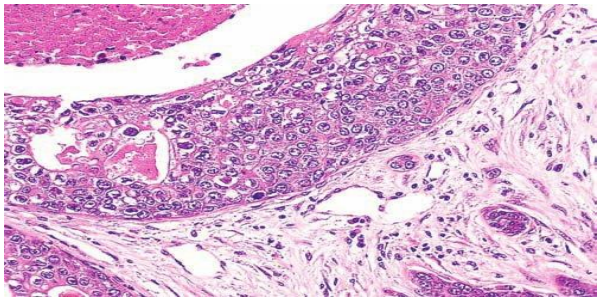


Figure 6: Ductal carcinoma in situ, poorly differentiated, high-grade type with a comedo pattern. The proliferating malignant cells are large and pleomorphic, and a central area of necrosis can be seen at the top of the figure.

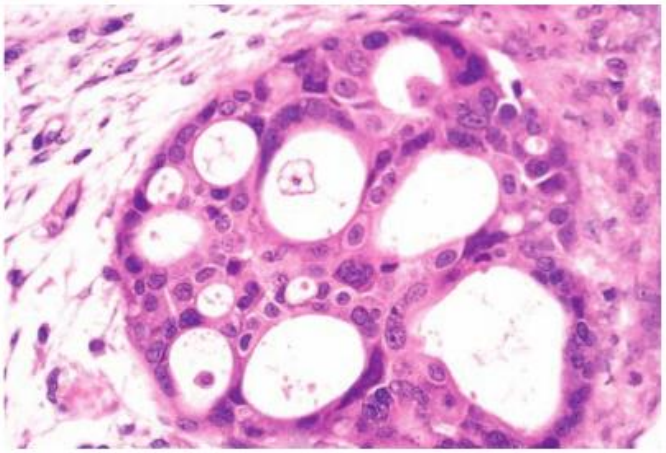


Figure 7: Ductal carcinoma in situ, poorly differentiated, high-grade type. This example shows pseudo cribriform architecture and no necrosis. However, the malignant cells are large and pleomorphic, with coarse, clumped nuclear chromatin.

TUBULAR ADENOMA[20]:

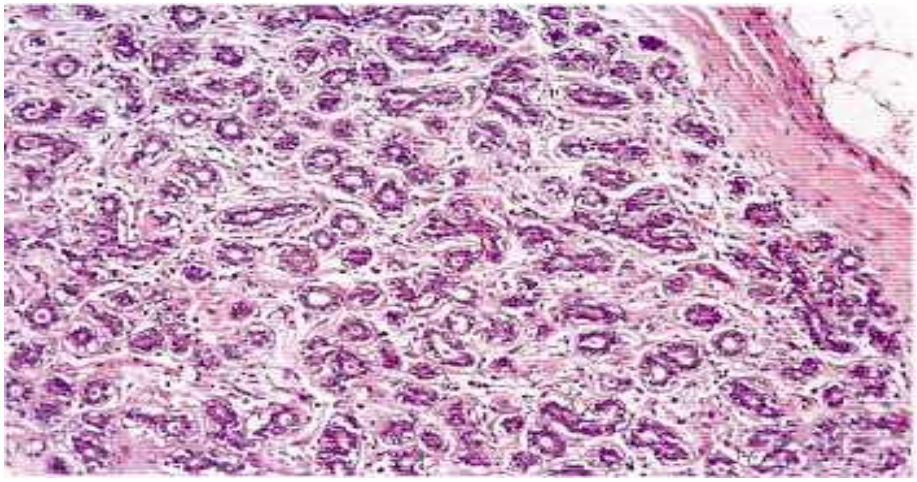


Figure 8: Tubular adenoma.

NEUROENDOCRINE (ARGYROPHILIC) DUCTAL CARCINOMA IN SITU[20]:

A solid pattern of DCIS with neuroendocrine histologic features has been recognized. Such lesions are usually seen in elderly patients, who often present with a blood-stained nipple discharge. The involved glandular elements are often markedly distended. The proliferating cells have a polygonal, oval, or spindle morphologic appearance and granular eosinophilic cytoplasm with intervening fibrovascular cores and septa. Rosettes and ribbons may be evident, as may mucin production and microglandular spaces (87). In some examples, the component cells are a mixture of spindle cells and argyrophilic signet ring cells. Because of the frequent lack of overt cytologic atypia, this type of DCIS can easily be misdiagnosed as benign. Small foci of invasive carcinoma are present in many cases, and in one series, the invasion was associated with endocrine DCIS in 20 of 34 cases. In another 18 cases, nearby papillomas were colonized by endocrine DCIS. "Solid papillary carcinoma" of the breast would appear to be related to, if not identical with, neuroendocrine DCIS.[20]

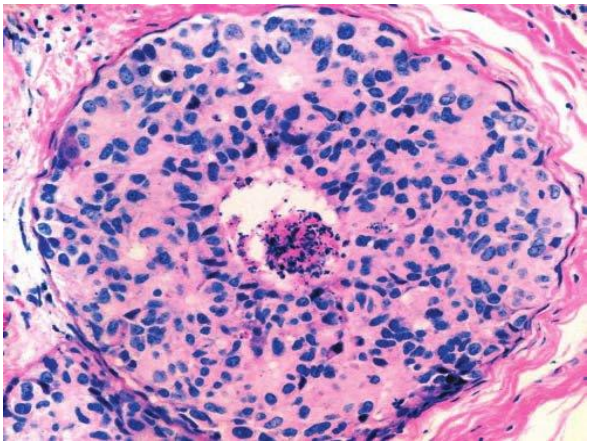


Figure 9: Ductal carcinoma in situ, endocrine type. The cells are pleomorphic, and there is central necrosis. Pseudo rosettes are formed by proliferating cells.

METAPLASTIC CARCINOMA[20]:

A metaplastic carcinoma is a heterogeneous group of malignancies characterized by carcinoma with dominant areas of spindle cell, squamous, or mesenchymal differentiation (chondroid, osseous), which may appear benign or malignant. Metaplastic carcinomas account for less than 1% of all invasive mammary carcinomas.

MIXED TYPE CARCINOMA[20]:

For a tumor to be typed as ductal NOS, it must have a non-specialized pattern in over 50% of its mass, as judged by a thorough examination of representative sections. Suppose the ductal NOS pattern comprises between 10% and 49% of the tumor, the rest being of a recognized particular type. In that case, it will fall into one of the mixed groups: mixed ductal and particular type or mixed ductal and lobular carcinoma.

PLEOMORPHIC CARCINOMA[20]:

Pleomorphic carcinoma is a rare variant of high-grade ductal NOS carcinoma characterized by the proliferation of pleomorphic and bizarre tumor giant cells comprising >50% of the

tumor cells in a background of adenocarcinoma or adenocarcinoma with spindle and squamous differentiation. The patients range in age from 28 to 96 years, with a median of 51 years. Most patients present with a palpable mass; in 12% of cases, a metastatic tumor is the first manifestation of the disease. The mean size of the tumor is 5.4 cm. Cavitation and necrosis occur in large tumors. In most cases, giant tumor cells account for more than 75% of tumor cells. Mitotic figures exceed 20 per 10 high power fields. All these tumors qualify as grade 3 carcinomas. The intraepithelial component displays a ductal arrangement and is often high-grade with necrosis. Lymphovascular invasion is present in 19% of the cases. Generally, these carcinomas are BCL 2, ER, and PR negative. However, two-thirds of these pleomorphic carcinomas are TP53 positive, and one-third are S-100 protein positive. All are positive for CAM 5.2, EMA, and pan-cytokeratin (AE1/AE3, CK1). A majority (68%) are aneuploid, with 47% being triploid. A high S- phase (>10%) is found in 63%. Axillary node metastases are present in 50% of the patients, with the involvement of 3 or more nodes in most cases. Many patients present with advanced disease.

GRADING OF INVASIVE CARCINOMAS[17]:

Invasive ductal carcinomas and all other invasive tumors are routinely graded based on an assessment of tubule/gland formation, nuclear pleomorphism, and mitotic counts. Many have demonstrated a significant association between histological grade and survival in invasive breast carcinoma. It is now recognized as an influential prognostic factor and should be included as a component of the minimum data set for histological reporting of breast cancer. Assessment of histological grade has become more objective with modifications of the Pateley and Scarff method, first by Bloom and Richardson and, more recently, by Elston and Ellis.

METHOD OF GRADING[17,20]:

Nottingham–Bloom–Richardson (NBR) histologic grading system

Three tumor characteristics are evaluated.

I. Tubule formation as an expression of glandular differentiation,

- Score 1: tubular formation of more than 75% of the tumor.
- Score 2: tubular formation 10 to 75% of the tumor.
- Score 3: tubular formation of less than 10% of the tumor.

II. Nuclear pleomorphism,

- Score 1: nuclei with minimal or mild variation in size and shape.
- Score 2: nuclei with moderate variation in size and shape.
- Score 3: nuclei with marked variation in size and shape.

III. Mitotic counts.

- Score 1: 0-5 /10HPF.
- Score 2: 6-10/ 10HPF.
- Score 3: >11 /10HPF.

A numerical scoring of 1-3 ensures that each factor is assessed individually. When evaluating tubules and glandular acini, only structures exhibiting clear central lumina are counted; cut-off points of 75% and 10% of epithelial/tumor area are used to allocate the score. Nuclear pleomorphism is assessed by reference to the regularity of nuclear size and shape of normal epithelial cells in adjacent breast tissue. The increasing irregularity of nuclear outlines and the number and size of nucleoli are helpful additional features in allocating scores of pleomorphism. Evaluation of mitotic figures requires care, and observers must count only defined ones; hyperchromatic and pyknotic nuclei are ignored since they are more likely to represent apoptosis than proliferation. The total number of mitosis per 10 high-power fields is counted. Field selection for mitotic screening should be made from the peripheral leading

edge of the tumor. If there is heterogeneity, regions exhibiting a higher frequency should be selected. Only the field with a representative tumor burden should be assessed. The three values are added together to produce scores of 3 to 9, and the corresponding grade is assigned as follows:

Grade 1 – Well differentiated: 3-5 points.

Grade 2 – Moderately differentiated: 6-7 points.

Grade 3 – Poorly differentiated: 8-9 points.

TNM CLASSIFICATION OF TUMOURS OF THE BREAST:[17]

The American Joint Committee on Cancer (AJCC) staging system 8th edition provides a strategy for grouping patients concerning prognosis. Therapeutic decisions are formulated in part according to staging categories but primarily according to lymph node status, Estrogen and progesterone receptor levels in tumor tissue, menopausal status, and the patient's general health.

T – PRIMARY TUMOR

T_X - Primary tumor cannot be assessed

T_0 - No evidence of primary tumor

T_{is} - Carcinoma in situ

T_{is} (DCIS) - Ductal carcinoma in situ

T_{is} (LCIS) - Lobular carcinoma in situ

T_{is} (Paget) - Paget's disease of the nipple is not associated with invasive carcinoma and carcinoma in situ (DCIS and LCIS) in the underlying breast parenchyma.

T_1 - Tumor 2 cm or less in greatest dimension

T_{1mi} - Microinvasion 0.1 cm or less in the greatest dimension

T_{1a} - More than 0.1 cm but not more than 0.5 cm in the greatest dimension

T_{1b} - More than 0.5 cm but not more than 1 cm in the greatest dimension

T_{1c} - More than 1 cm but not more than 2 cm in the greatest dimension

T_2 - Tumor more than 2 cm but not more than 5 cm in greatest dimension

T_3 - Tumor more than 5 cm in greatest dimension

T_4 - Tumor of any size with direct extension to the chest wall or the skin (Ulceration or skin nodules)

N – REGIONAL LYMPH NODES

N_X - Regional lymph nodes cannot be assessed (e.g., previously removed)

N_0 - No regional lymph node metastasis

N_1 - Metastasis in movable ipsilateral level I, II axillary lymph node(s)

N_2 - Metastasis in ipsilateral level I, II axillary lymph node(s) that are clinically fixed or matted; or in clinically detected ipsilateral internal mammary lymph node(s) in the absence of clinically evident axillary lymph node metastasis

N_{2a} - Metastasis in axillary lymph node(s) fixed to one another (matted) or other structures

N_{2b} - Metastasis only in clinically detected internal mammary lymph node(s) and in the absence of clinically detected axillary lymph node metastasis

N_3 - Metastasis in ipsilateral infraclavicular (level III axillary) lymph node(s) with or without level I, II axillary lymph-node involvement; or in clinically detected ipsilateral internal mammary lymph node(s) with clinically evident level I, II axillary lymph-node metastasis; or metastasis in ipsilateral supraclavicular lymph node(s) with or without axillary or internal mammary lymph node involvement

N_{3a} - Metastasis in infraclavicular lymph node(s)

N_{3b} - Metastasis in internal mammary and axillary lymph nodes

N_{3c} - Metastasis in supraclavicular lymph node(s)

M – DISTANT METASTASIS

M_0 - No distant metastasis

M_1 - Distant metastasis

PREFIXES:

y: preoperative radiotherapy or chemotherapy

r: recurrent tumor stage

Table 1 AJCC Anatomic Stage Groups:[17]

When T	When N	When M	Stage
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[26]:

The pioneer molecular classification was pursued by Perou et al. at the beginning of this century. Using complementary DNA microarrays representing 8102 human genes, they first

characterized a set of 65 surgical specimens of breast tumors from 42 individuals and found that the tumors could be classified into subtypes distinguished by pervasive differences in their gene expression profiling (GEP). With further studies and refinement,3,4 the authors proposed a classification scheme that divided breast cancer into four intrinsic molecular subtypes: luminal A, luminal B, v-erb-b2 (ERBB2)/human epithelial growth factor receptor 2 (HER2) gene-overexpressing (HER2þ), and basal-like. The luminal carcinomas characteristically express estrogen receptors (ER) with variable cell proliferation. Her2 overexpression is the hallmark of ERBB2-overexpressing tumors that also lack ER and progesterone receptor (PR) expression. Basal-like carcinoma fails to express ER, PR, or HER2 (triple-negative carcinoma; TNBC), instead expressing basal cell markers, such as cytokeratin (CK) 5/6 and/or epidermal growth factor receptor (EGFR). These subtypes demonstrated distinct histologic patterns, clinical features, and prognosis. The development seemed quite exciting. However, adopting the GEP test by general pathology laboratories was difficult because of its technical complexity and cost inefficacy. Therefore, alternative ways were sought to simulate the GEP results. Cheang et al. identified a novel immunohistochemical (IHC) panel, including 6 IHC markers, and found it could recapitulate the biologic subgroups of breast cancer derived from full GEP. Schnitt later summarized the IHC diagnosing criteria of the intrinsic classification as follows: (1) luminal A: ER and/or PR, HER2, and low Ki-67 (,14%); (2) luminal B: ER and/or PR, HER2 neu and high Ki-67 (>14%); (3) HER2: ER, PR, and HER2; and (4) basal-like (BLBC): ER, PR, HER2 (triple negative), plus CK 5/6, and EGFR. The 2013 European St Gallen Consensus adopted the criteria with minor modifications: increasing Ki-67 to 20% or more and decreasing PR to 20% or less for better separations.9 The intrinsic molecular subtyping has set a landmark in the development of breast cancer classification. However, it is not perfect. First, it only classifies breast cancer into four sustainable types, which is oversimplified in reflecting the

molecular complexity of the underlying tumor. Each type is still heterogeneous, with variable prognoses and diversified treatment responses. Second, it could be surrogated in large part by IHC. Therefore, its application does not gain popularity in routine practice. However, it does form a foundation for the further exploration of prognostic and predictive molecular assays. After identifying intrinsic molecular subtyping, assays have been developed and are currently more mature in the luminal cancers guiding a patient's clinical management.

Table 2: Molecular classification of breast carcinoma.

Molecular classification		Estrogen receptor	Progesterone Receptor	Her2neu	Ki67%	Treatment of choice
Luminal A		Positive	Positive	Negative	Low	Hormone and chemotherapy.
Luminal B	**Her 2 Negative**	Positive	Negative	Positive	Low	Chemotherapy and hormonal therapy. Her2 targeted therapy.
	Her 2 Positive	Positive	Positive/ Negative	Positive	High	
Her2 Positive		Negative	Negative	Positive	High	Chemotherapy and Her 2 targeted therapy.
TNBC		Negative	Negative	Negative	High	Chemotherapy.

IMMUNOHISTOCHEMISTRY OF BREAST CANCERS:

Immunohistochemistry plays a significant role in the pathology of breast disease, benign or malignant. Myoepithelial markers help distinguish benign lesions from malignant lesions. The most common immunohistochemical markers used in breast cancers, which are also used as prognostic and therapeutic markers, include ER, PR, human epidermal growth factor

receptor-2 (HER-2/Neu), Ki-67, p53, and E-cadherin. In addition, markers of angiogenesis and apoptosis are also important.

TABLE 3: SUMMARIZES VARIOUS MARKERS USED IN BREAST CARCINOMAS.[18]

MARKER	STAINING PATTERN	USEFUL FOR
Smooth muscle actin	Cytoplasmic staining	Myoepithelial differentiation
Calponin	Cytoplasmic staining	Myoepithelial differentiation
p63	Nuclear	Myoepithelial differentiation
Smooth muscle myosin heavy chain	Cytoplasmic	Myoepithelial differentiation
CD10	Membranous staining	Myoepithelial differentiation
S100	Cytoplasmic	Myoepithelial differentiation
High-molecular-weight cytokeratin's (14 and 5/6)	Cytoplasmic	To distinguish invasive carcinoma from benign proliferations; expressed by lobular carcinomas
Cytokeratin 8	Peripheral cytoplasmic	Ductal carcinoma cells
Cytokeratin 8	Perinuclear staining	Lobular carcinoma cells
CK 7 and 20	Mammary origin of a metastatic carcinoma	Epithelial carcinomas.
E-cadherin	Membranous staining	Usual ductal carcinomas
Hormone receptors Estrogen receptor, progesterone receptor	Nuclear	Identify subtypes, Mammary origin
HER-2/Neu	Membranous staining	Identified subtypes
Gross cystic disease fluid protein 15	Cytoplasmic	Mammary origin of a metastatic carcinoma
Mammaglobin A	Cytoplasmic	Mammary origin of a metastatic carcinoma
Carcinoembryonic antigen, CEAD-14 clone	Cytoplasmic	Evaluation of metastatic mammary carcinoma

PROGNOSTIC AND PREDICTIVE FACTORS:

With new treatment modalities, the management of breast carcinoma has changed enormously over the years. Prognostic information plays a crucial role in predicting the possible outcome of these patients and also helps in choosing suitable treatment options for these patients.

Previously they were classified into three categories based on the contribution of these factors in assessing prognostic and treatment outcomes. However, with the advent of new modalities and progress in molecular pathology, these factors are now divided into two types, those related to the extent of carcinoma (tumor burden or stage) and those related to the underlying biology of cancer.[19]

TUMOR SIZE:

Tumor size is one of the most potent and vital predictors of the behavioral outcome of breast cancer.1 Only 10% to 20% of the patients show metastasis to lymph nodes when the size of the tumor is less than 1 cm, and 90% of the patients show 10-year disease-free survival when size is less than 1 cm and are node negative which clearly shows lesser the tumor size better the prognosis.[20] Both gross and microscopy measurement of the tumor has to be done and correlated, with microscopy measurement of an invasive component having its upper hand. Size is less important when it comes to HER-2/Neu positive and ER negative carcinomas, as these carcinomas show metastasis even when the size is relatively small.[21]

NODAL STATUS:

Axillary lymph node status has consistently been shown to be the most

a significant predictor of overall survival and disease outcome in breast cancer.70% to 80%, the 10-year disease survival rate was noted when no lymph node was involved. With one to

three positive lymph nodes, the rate dropped to 35% to 40% and 10% to 15% when more than ten lymph nodes were involved.[20] The absolute number of involved nodes is also of prognostic importance, which is one of the parameters in the Nottingham prognostic index. Lymphatic drainage in breast carcinomas is unique in that it drains to the first one or two lymph nodes, called the sentinel node, which colored dyes can identify. Biopsy from the clinically palpable nodes is required to distinguish metastasis from reactive nodes. A few critical points need to be considered while grossing lymph nodes; all the uninvolved lymph nodes are to be entirely submitted for histologic examination. Small nodes can be wholly embedded, but larger nodes should be appropriately assessed, and multiple sections to be given to avoid false negative results. Sentinel lymph node biopsy has gained importance in recent times as an alternative to axillary dissection, which has shown high specificity and sensitivity in predicting nodal status.[22,23]

INVASIVE CARCINOMA VERSUS CARCINOMA IN SITU:

Women with invasive carcinoma invariably have a poor prognosis compared to those with carcinoma in situ.[20]

LOCALLY ADVANCED DISEASE:

Tumor, when infiltrating skin or underlying muscle, makes the process of complete surgical excision difficult and hence has a high recurrence rate. However, due to various screening measures and awareness programs, such cases have dramatically reduced over the past years.[20]

HISTOLOGIC GRADE:

All invasive ductal carcinomas have to be graded. Three parameters

Nuclear grade, tubule formation, and mitotic rate classify invasive carcinomas into three groups, as described earlier, that is highly correlated with disease-free and overall survival. The AJCC Cancer staging Manual has recommended the Nottingham combined Histologic grade, that is, the Elston Ellis modification to the Scarff Bloom Richardson Grading system. The grading has been described earlier.[24,25]

HISTOLOGIC TYPE:

Favourable prognosis has been well established in certain invasive carcinomas like mucinous, tubular, papillary, lobular, and adenoid cystic compared to invasive carcinomas of no particular type.9 Alternatively, women with micropapillary carcinoma or metaplastic carcinoma are known to have a poorer prognosis. It's histology that has the upper hand over molecular status in tumors of unique subtypes, particularly low-grade Adenosquamous carcinoma and adenoid cystic carcinoma, especially in young women.[17,25]

MOLECULAR PROGNOSTIC PARAMETERS IN BREAST CARCINOMA:

Molecular markers are available to better understand breast carcinogenesis and cancer progression as a guide to treatment. Immunohistochemical analysis of prognostic and predictive factors like ER, PR, Ki-67, HER-2/neu, and p53 are increasingly employed by pathologists.[27]

HORMONE RECEPTORS:

A crucial development in treating breast carcinoma has been the realization that the presence of hormone (Estrogen and progesterone) receptors in the tumor tissue correlates well with response to hormone therapy and chemotherapy.[28] The normal breast epithelium contains receptors for Estrogen and progesterone. The interaction between these receptors and hormones stimulates the cells to proliferate and differentiate. About 60-70% of breast

carcinomas express these receptors. So, ER/PR-positive tumors may be stimulated to increase by interacting with circulating endogenous hormones. Some drugs have developed that interface with hormone binding in ER/PR-positive tumors, thereby inhibiting tumor cell proliferation, prolonging patient survival, and even reducing the size of existing tumors. Thus, patients with Estrogen receptor-positive tumors, whether determined biochemically or immunohistochemically, have longer survival rates than others.[29]

Around 80% of breast carcinomas that are ER and PR-positive respond to hormonal manipulation, whereas only about 40% of those with either ER or PR alone respond. ER-positive cancers are less likely to respond to chemotherapy. Conversely, cancers that fail to express either ER or PR have less than a 10% likelihood of responding to hormonal therapy but are more likely to respond to chemotherapy.[29]

HER-2/neu:

HER-2/neu (c-erbB-2) is an oncogene that encodes a transmembrane glycoprotein with tyrosine kinase activity known as p185, which belongs to the family of epidermal growth factor receptors.[28] Her-2/neu overexpression is associated with poorer survival, but the importance is it is a predictor of response to agents that target this transmembrane protein.[30]

PROLIFERATION MARKERS:

Mitotic counts can measure proliferation (e.g., as part of histologic grading) by immunohistochemical detection of cellular proteins produced during the cell cycle (e.g., cyclins, Ki-67) by flow cytometry (as the S-phase fraction) or by thymidine labeling index.

Carcinomas with high proliferation rates have a poorer prognosis but may respond better to chemotherapy.[30]

p^{53}: It is a tumor suppressor gene normally involved in suppressing the cell cycle. It is mutated and overexpressed in about 50% of breast cancers and these genetic alterations are strongly associated with an increased tumor proliferation rate and poor clinical outcomes.[29]

PROGNOSTIC FACTORS IN THE PATIENT MANAGEMENT-NOTTINGHAM PROGNOSTIC INDEX (NPI):

The original NPI used three factors. Pathological tumor size, lymph node status, and histologic tumor grade. These three factors were assessed together and were used as a prognostic index. The total of these is calculated, and the higher the value poorer the prognosis. A cut-off value of 3.4 and 5.4 was used, and accordingly, the patients were divided into three groups. These three subsets are: Group 1 with a good prognosis (score up to 3.4) with a five-year survival rate predicted to be 80%; group 2 with a moderate prognosis (score 3.4 to 5.4) with 42% five-year survival; and group 3 with poor prognosis (Score greater than 5.4) with 13% five-year survival.[39,40]

It's calculated with the formula:

NPI= pathological size of the tumor in cm x 0.2 + lymph node stage (1, 2 or 3) + histologic grade (1, 2 or 3)

Table 4: Predicted ten-year breast cancer-specific survival and categories of the Nottingham Prognostic Index

NPI	Score	Cancer-specific ten-year Survival
I (Excellent)	≤2.4	96%
II (Good)	>2.4 but ≤3.4	93%
III (Moderate)	>3.4 but ≤5.4	78%
IV (Poor)	>5.4	44%

SMA (SMOOTH MUSCLE ACTIN):

Fibroblast synthesizes extracellular matrix, collagen fibers, glycoproteins, and glycosaminoglycans that constitute the stroma. Fibroblasts greatly influence the tumor microenvironment in malignancies by synthesizing vascular endothelial growth factor A (VEG FA) and C-X-C Motif chemokine ligand 12 (CXCL12). Because of these properties, fibroblasts form another subpopulation of hyperactivated fibroblasts called cancer-associated fibroblasts (CAF).[5] Cancer-associated fibroblasts are highly heterogeneous and enhance cell migration and metabolism of epithelial tumor cells. The most commonly used immunohistochemical marker for CAF is α-SMA which detects the expression of smooth muscle actin in tumor stroma.[31]

Smooth muscle actins (SMA) are a group of proteins that play an essential role in cell motility, structure, integrity, and wound repair. It is an actin isomer in smooth muscle cells, myofibroblasts, and blood vessels. Its expression correlates with the activation of fibroblast to myofibroblast. Contractile properties of this myofibroblast are known to be associated with increased expression of alpha-smooth muscle actin. Carcinoma cells that transform into mesenchymal cells are also known to express alpha-smooth muscle actin.[32-34]

In a study by Catteau X et al., breast carcinoma expression of alpha SMA varied from 0 to 46 %, with a median of 15.1% and a standard deviation of 7.5. Higher expression of myofibroblast was found in 51.5%, 61%, and 24% of tumors in grades 3, 2, and 1, respectively.[11] In contrast, no significant relationship was observed between SMA stromal expression with age, histological type, and lymph node metastasis. 12 In another study, SMA expression in the tumor centre was 76%. The tumor margin showed positivity of 46%.[13] Expression was associated with large tumor size and high grade. A study by Catteau X et al. showed a poor disease-free survival (PFS) rate in the group of node-negative tumors with strong SMA stromal expression.[35]

Cancer-associated fibroblasts have been considered a therapeutic target in cancer. In recent studies, pancreatic cancer models suggested that a specific subset (r-restraining-p-promoting) of CAF exhibits cancer-restraining roles.[38]

MATERIALS AND METHODS

MATERIAL AND METHODS

- **STUDY AREA:**

  The present study was carried out in the Department of Surgery and Department of Surgical Oncology at R.L Jalappa Hospital and Research Hospital attached to Sri Devaraj Urs Medical College, Tamaka, Kolar

- **STUDY POPULATION:**

  All Infiltrating ductal Breast carcinoma patients coming to the Department of Surgery and Department of Surgical Oncology.

- **STUDY DESIGN:**

  An observational study (Cross-sectional analytical study)

- **SAMPLE SIZE:**

  Prevalence has been taken as 46 % from the study by Catteau X et al. -Smooth Muscle Actin[35] expression on stromal cells of breast carcinoma.

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

  The sample size required for the cross-sectional study was 100 for breast carcinoma with 95% confidence.

- **TIME FRAME TO ADDRESS THE STUDY:**

  Two years (January 2021 to November -2022).

- **INCLUSION CRITERIA:**

  Women with infiltrating ductal carcinoma (NOS) type who underwent Modified radical mastectomy surgery.

- **EXCLUSION CRITERIA:**

  Women subjected to neoadjuvant radiotherapy/chemotherapy before radical mastectomy, recurrent tumors, women who received chemotherapy for other cancer over the past five (5) years, diagnosed male breast cancer patients.

- **STUDY COURSE:**

  Paraffin blocks and slides were retrieved from the Department of Pathology. Clinical information, obtained from histopathological diagnosis was obtained from medical records and pathology reports. All the haematoxylin and eosin slides were screened for histological type and Immunohistochemistry.

- **COLLECTION OF DATA:**

  One hundred cases of Infiltrating Ductal Carcinoma were diagnosed and operated on at R L Jalappa Hospital and Research Centre from January 2018 to November 2022.

  All breast cancer cases confirmed by histopathological examination were included in the study. After anonymizing the patient's demographic details, clinical information, tumor size, and axillary lymph node status, paraffin blocks and slides were collected from archives of the department. All the haematoxylin and eosin-stained slides were screened for histological type, tumor grade, and nodal metastasis.

  Selected appropriate slides for alpha-smooth muscle actin immunohistochemistry using appropriate positive and negative controls.

METHODOLOGY:

IMMUNOHISTOCHEMICAL EXAMINATION:

The Immunohistochemistry (IHC) was performed on 3-µm thick sections from 10% formalin-fixed paraffin-embedded tissues, according to the peroxidase –anti peroxidase method. Will run Positive and negative controls simultaneously.

Antigen	Clone	Species	Producer	Control	Stain
N-Terminal decapeptide of alpha-smooth muscle isoform of actin and conjugated to KLH.	1A4	Mouse	Diagnostic Biosystems	Leiomyoma	Cytoplasm

THE IHC PROCEDURE INCLUDES THE FOLLOWING STEPS

1. Sections are 3-5µm thickness, floated onto an organosilane-coated slide, and left on a hot plate at 60^{o} overnight.
2. Deparaffinization using Xylene I and II—15 min each
3. Dexylinisation using absolute alcohol I and II—1 min each
4. Dealcoholizing using 90% and 70% alcohol—1 min each
5. Washing with distilled water.
6. Antigen Retrieval technique: Microwave power 10 for 6 minutes in TRIS EDTA buffer of pH-9.0 for two cycles.
7. Distilled water rinsing for 5 minutes. Transfer to TBS (Tris buffer solution pH- 7.6) - 5 minutes x 3 times-wash.
8. Peroxidase block- Thirty (30) minutes to block endogenous peroxidase enzyme.
9. TBS buffer for 5 minutes, washing three times.
10. Power block for 15 min to block the non-specific reaction with other tissue antigens.
11. Cover sections with primary antibody for 45min
12. Rinse with TBS (Tris buffer pH- 7.6) for 5 min x 3 times wash with gentle agitation.

13. Super enhancer added for 20 min to enhance the reaction between primary and secondary antibodies.
14. TBS wash 5min for three times to wash unbounded antibodies
15. Super sensitive poly HRP was added for 30 min to elongate the chain and added DAB resulted in chromogen development within 5 mins.
16. TBS wash for 5 min x 3 times.
17. Tap water wash x 5 min, then Counterstain with Hematoxylin
18. Dehydrate, clear and mount with DPX

Antigen detection in tissues and cells is detected by a multi-step process using the HRP method.

DOCUMENTATION AND INTERPRETATION OF DATA

All slides were revived and histopathological data such as tumor size, grade of the tumor, lymph node metastasis was interpreted and documented. These slides were stained with IHC marker Alpha smooth muscle actin and stromal expression scoring was done. Positivity in the cytoplasm of stromal cells was considered positive and score given as follows:

Score 0- Less than 10% of the stromal cells.

Score 1- 11-50% of the stromal cells.

Score 2- 51-80% of the stromal cells.

Score 3- >80% of stromal cells showing positivity.

All the slides stained with Estrogen receptor and progesterone receptor and HER-2/neu, Ki67 were retrieved from the archives from the immunohistochemistry lab, and scoring was done as per the CAP-ASCO (College of American Pathologists -American Society of Clinical Oncology).

Table 5: IHC of ER, PR – Allred Scoring in breast carcinoma [60]

Score for percentage of positive tumor cells (PS)		Score for average intensity of staining (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	>66%		

Allred Score = PS+IS.

HER-2/neu membrane staining in tumor cells will be scored from 0 to 3 according to 2018 ASCO guidelines[61].

Table 6: IHC Scoring of Her2neu in breast carcinoma:

Scoring	ASCO guidelines 2018
Score 0	No staining is observed or Membrane staining that is incomplete and is faint/barely perceptible and in ≤10% of tumor cells.
Score 1+	Incomplete membrane staining that is faint/ barely perceptible and in >10% of tumor cells.
Score 2+	Weak to moderate complete membrane staining observed in >10% of tumor cells.
Score 3+	Circumferential membrane staining that is complete, intense and in >10% of tumor cells.

Ki 67 was scored as per Kan Yilmaz G et al[62] and as follows

Table 7 : IHC scoring of Ki67 in breast carcinoma:

<10%	Low
10-15%	Borderline
>15%	High

STATISTICAL ANALYSIS:

Data entry was done using M.S. Excel and statistically analyzed using Statistical package for social sciences (SPSS Version 16) for M.S. Windows. Conducted descriptive statistical analysis to explore the distribution of several categorical and quantitative variables. Categorical variables were summarized with n (%), while mean ± S.D summarized quantitative variables. All results were presented in tabular form and are also shown graphically using a bar diagram or pie diagram as appropriate. The difference in the two groups was tested for Statistical Significance, and categorical variables were tested by chi-square. A P-value less than 0.05 are considered to be statistically significant.

OBSERVATION AND RESULTS

RESULTS

Microscopic Images:

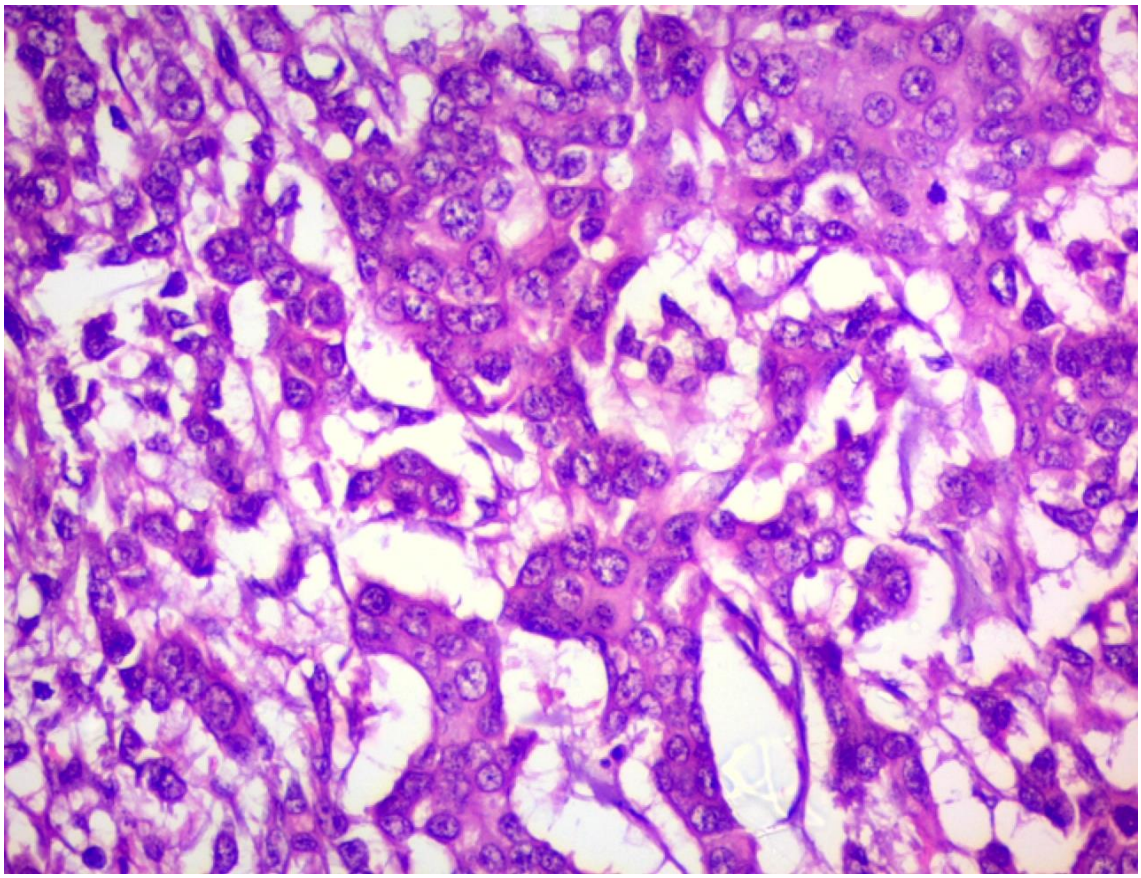


Figure 10: Invasive ductal carcinoma Breast (Not otherwise specified) Grade 1 (H & E)-40x.

Microscopy of IDC Breast: Pleomorphic tumor cells arranged in tubules and in sheets

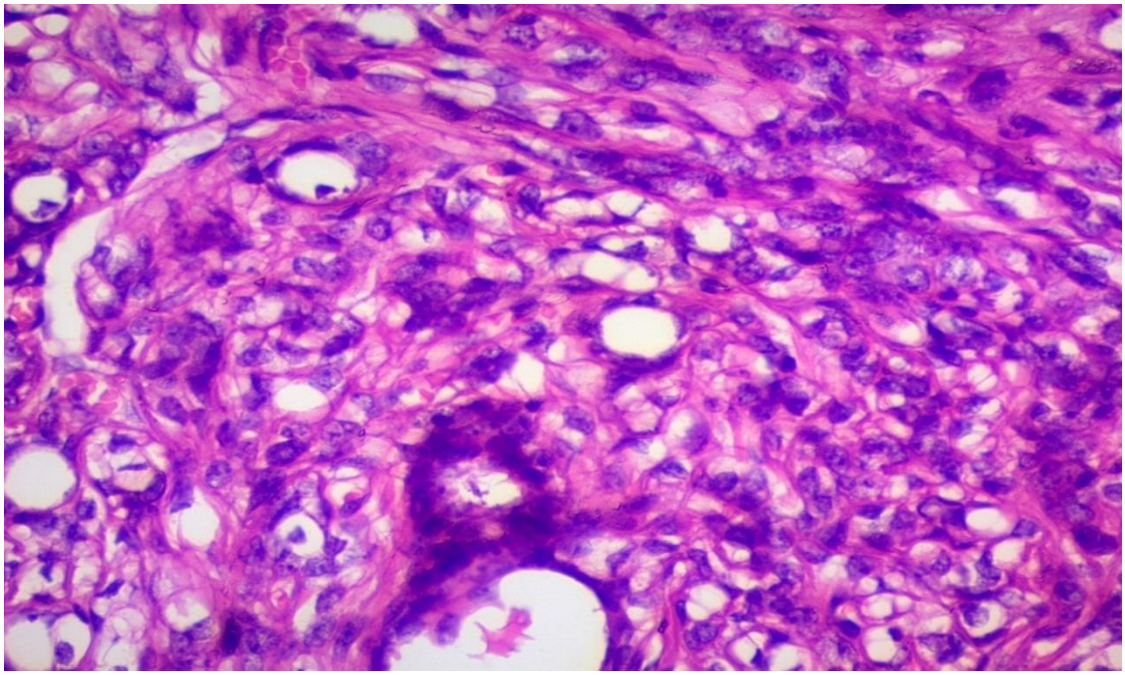


Figure 11: Invasive ductal carcinoma Breast – Grade 2 (H & E) – 40x

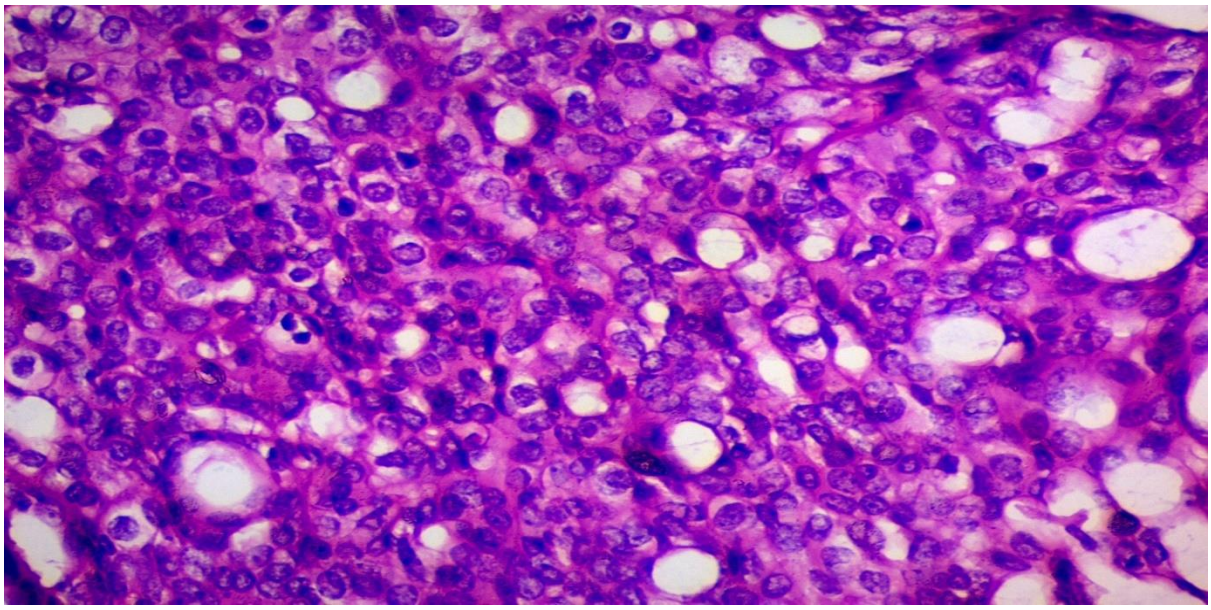


Figure 12 : Invasive ductal carcinoma Grade 3(H & E) – 40x

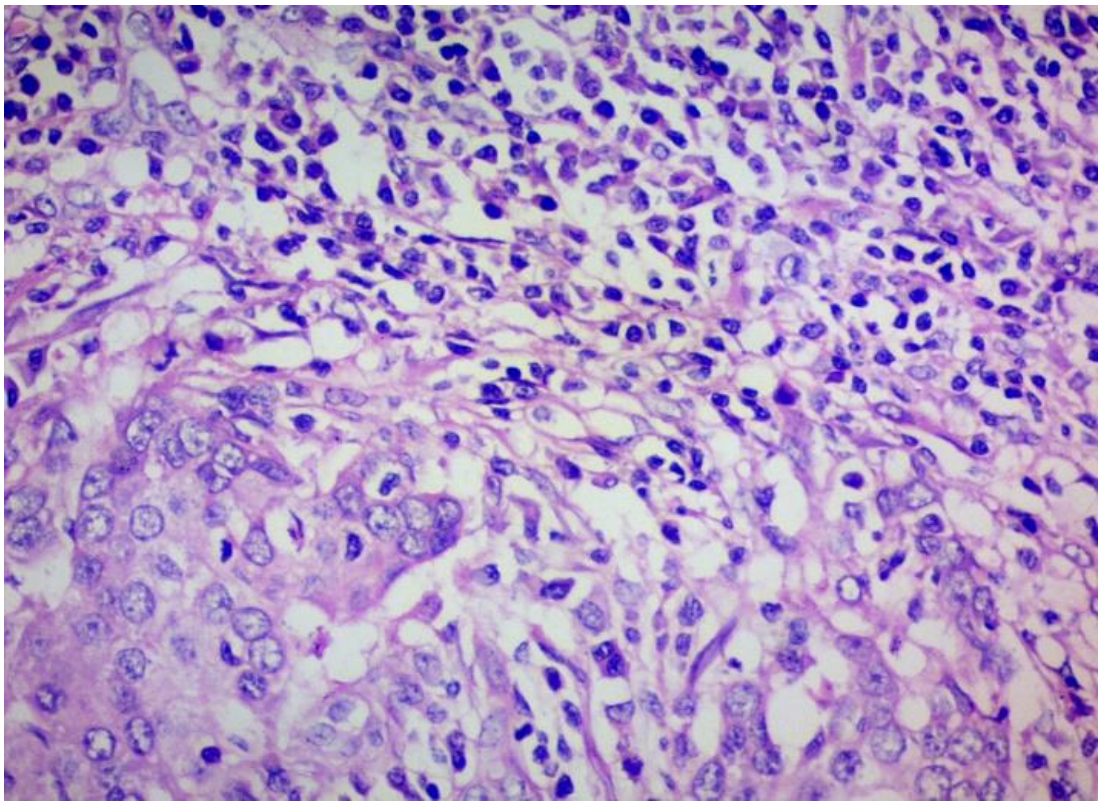


Figure 13: Invasive ductal carcinoma deposits in lymph node (H & E) – 40x

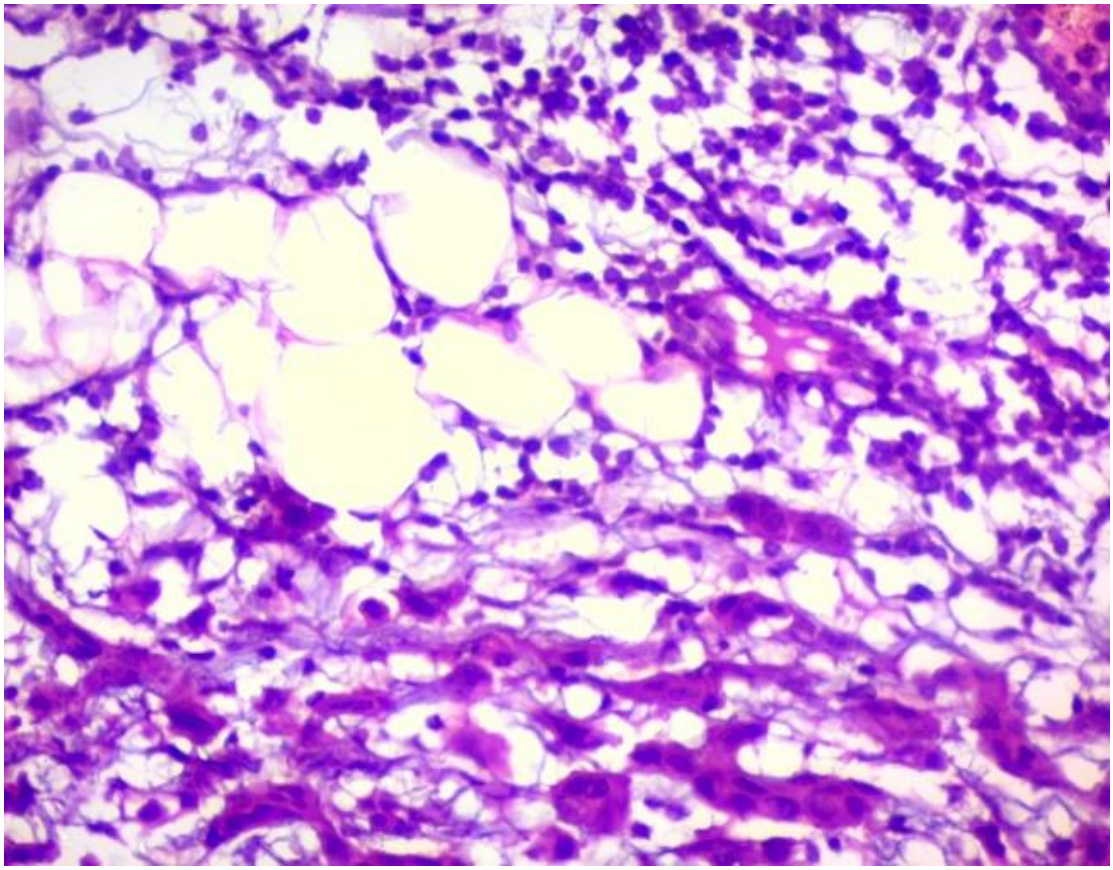


Figure 14: Extra nodal extension in lymph node (H & E) – 40x

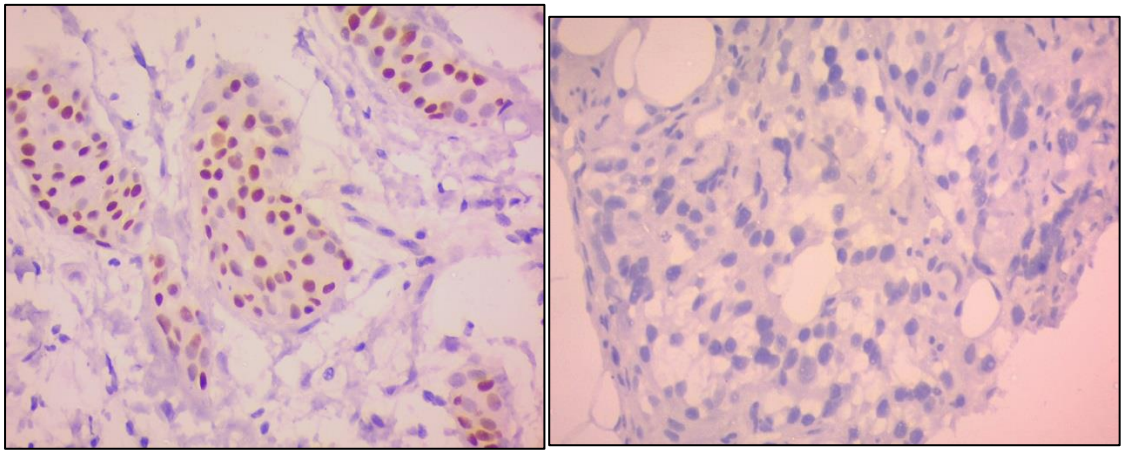


Figure 15: ER Positive and negative IHC-40x

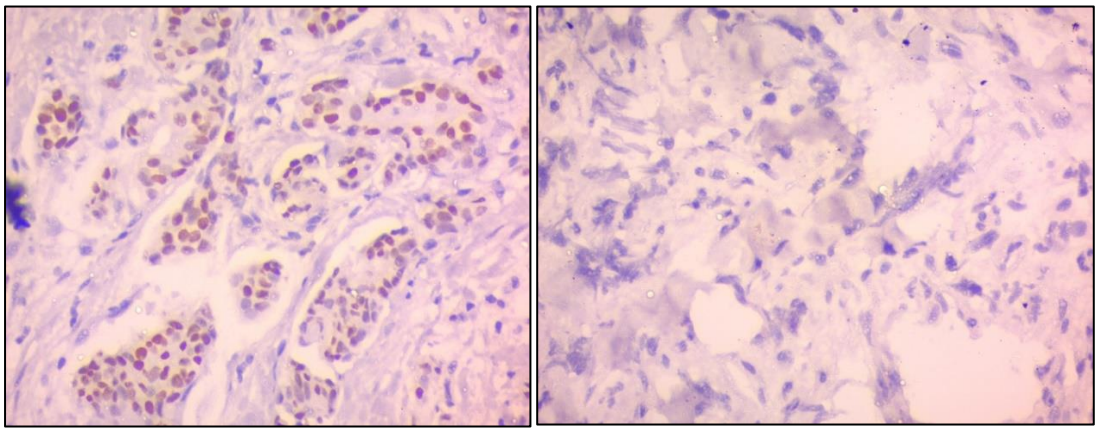


Figure 16: PR Positive and negative IHC-10x

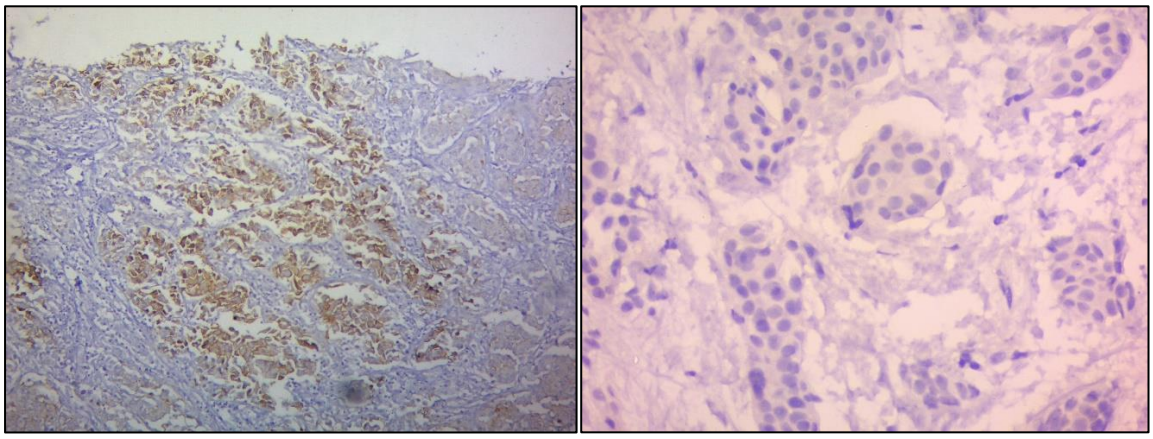


Figure 17: HER-2neu Positive and negative IHC-10x

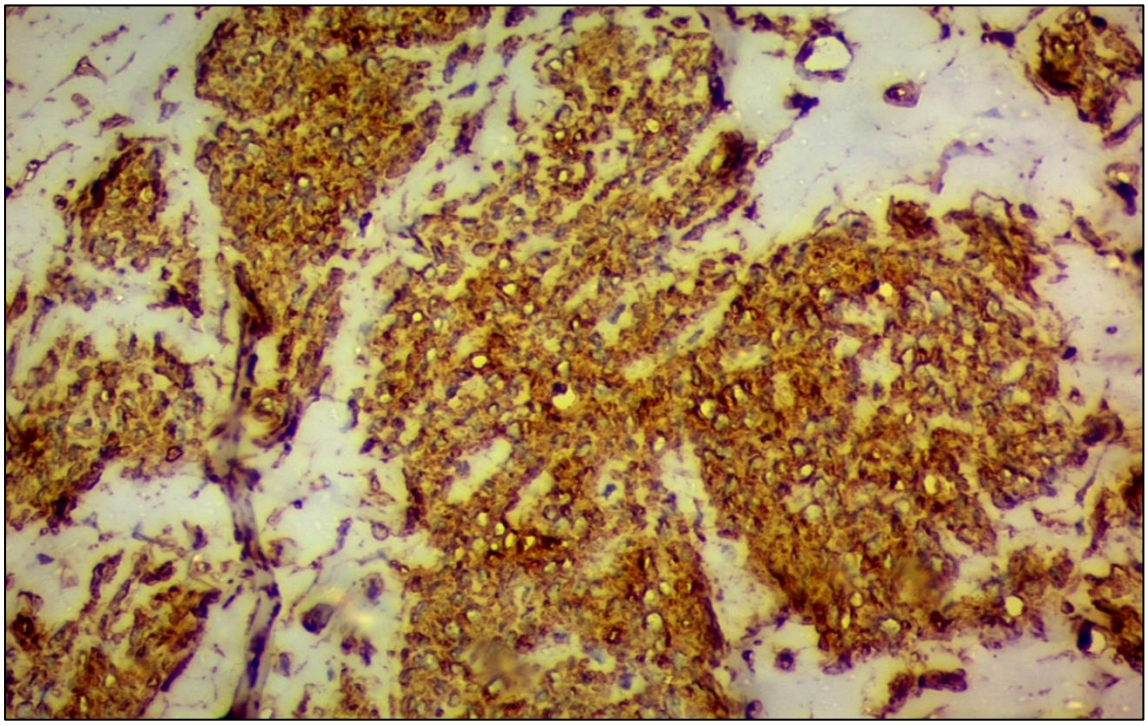


Figure 18: Alpha SMA Control-Leiomyoma-Showing strong cytoplasmic positivity-40x

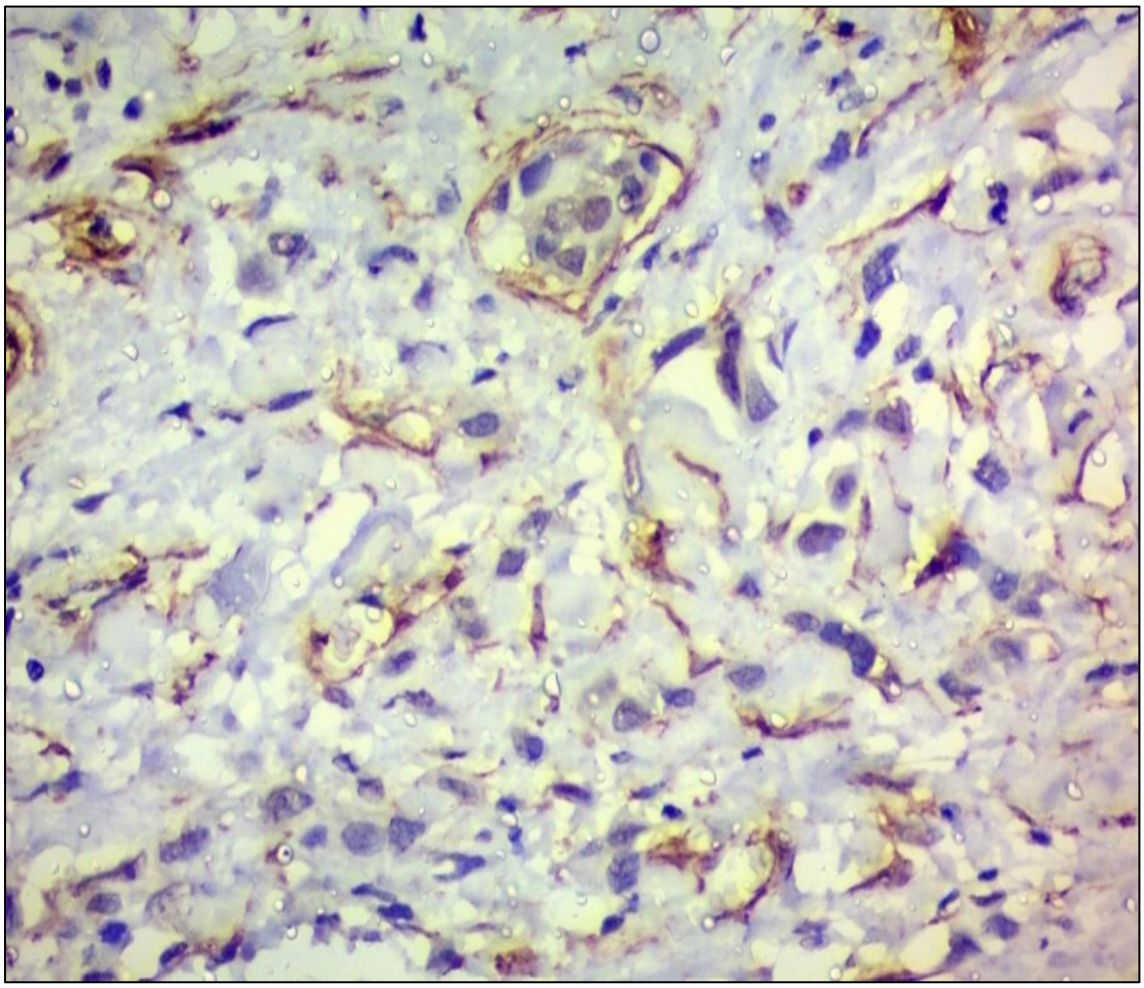


Figure 19: IDC- Alpha-SMA IHC – 40x (Cytoplasmic positivity in stromal cells <10 - Score 0)

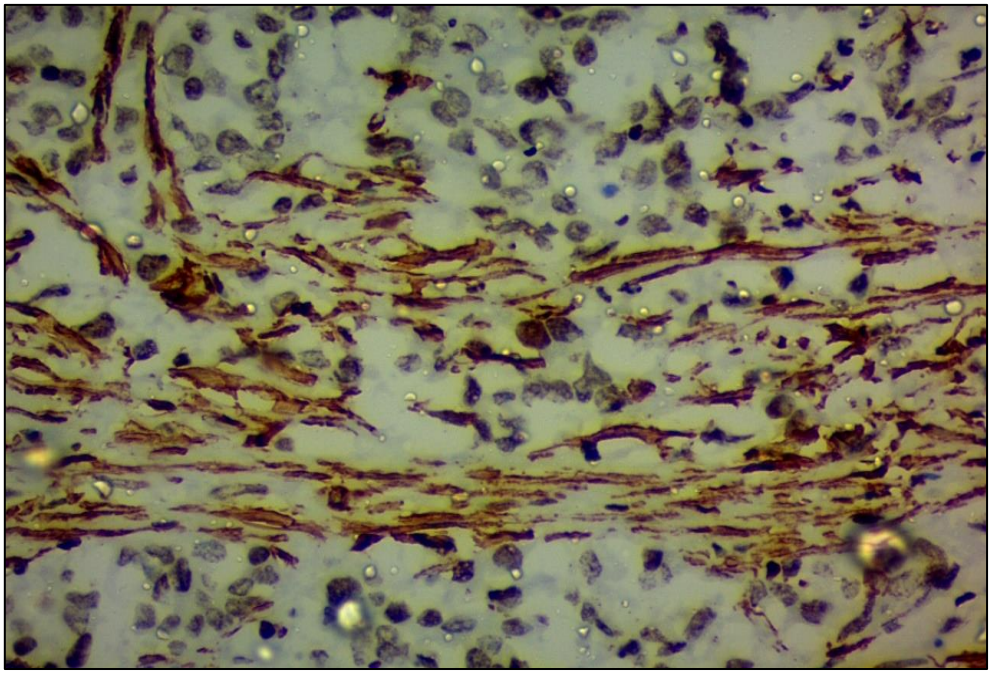


Figure 20: IDC- Alpha-SMA IHC – 40x (Cytoplasmic positivity in stromal cells 11-50% Score 1)

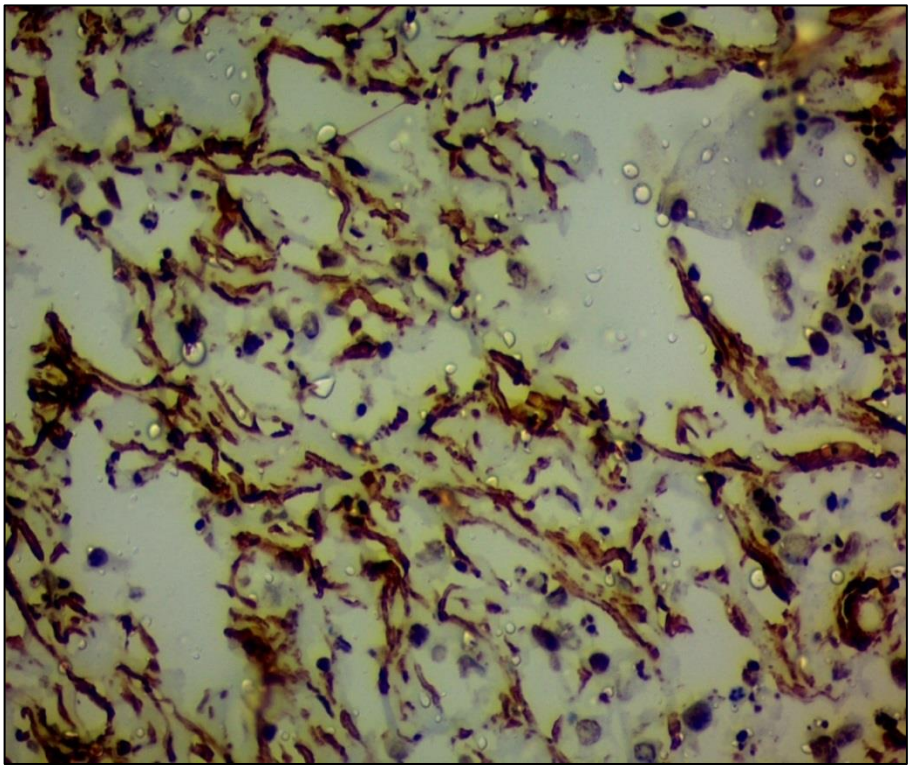


Figure 21: IDC-Alpha-SMA IHC – 40x (Cytoplasmic positivity in stromal cells 51-80% Score 2)

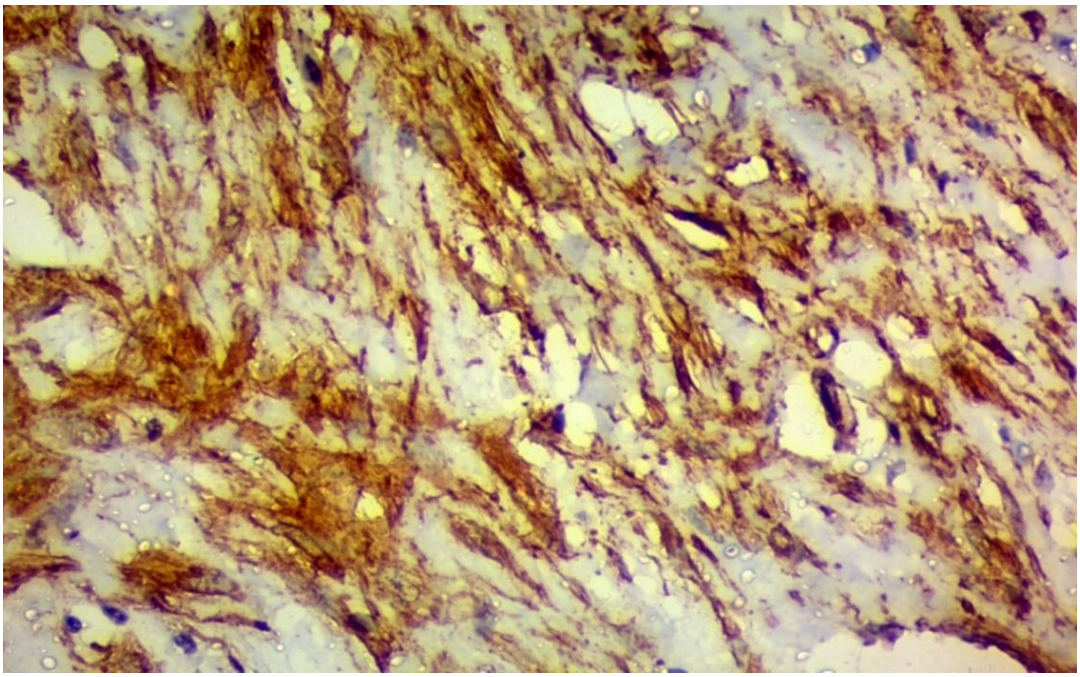


Figure 22: IDC-Alpha-SMA IHC– 40x (Cytoplasmic positivity in stromal cells >80% Score 3)

Table 8: Distribution of patients based on the age group

		Frequency	Percent
Age Group	28-40 years	20	20.0%
	41-50 years	23	23.0%
	51-60 years	33	33.0%
	61-70 years	13	13.0%
	>71 years	11	11.0%
	Total	100	100.0%

The majority of the patients belonged to the age group between 51 to 60 years [33%], followed by 41 to 50 years [23%], 28 to 40 years [20%], 61 to 70 years [13%], and more than 71 years [11%].

Graph 1: Distribution of patients based on the age group

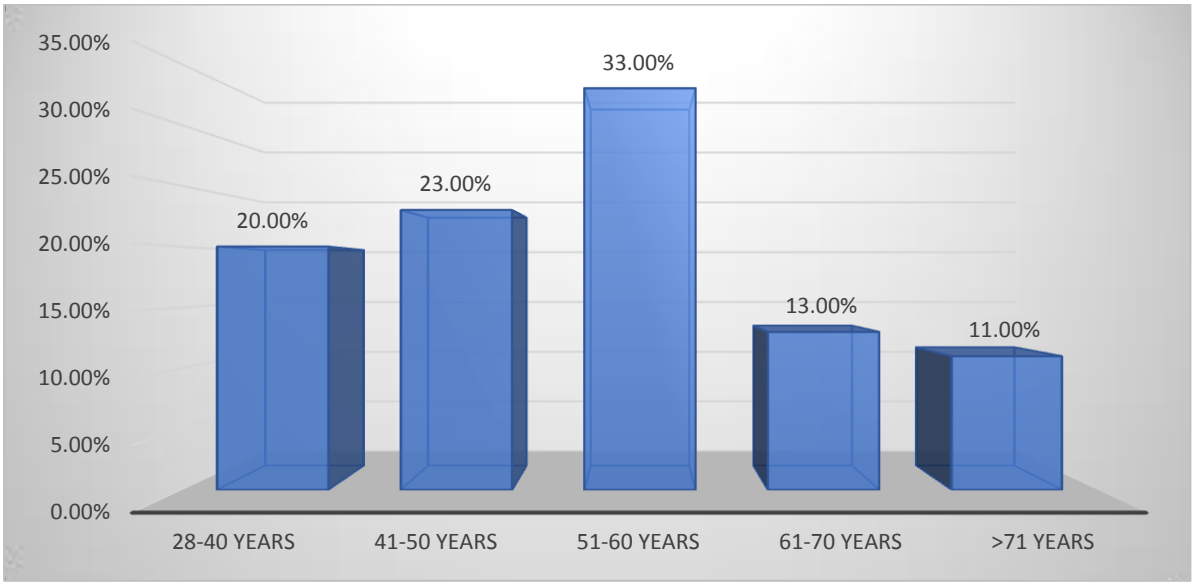


Table 9: Distribution of patients based on the tumor size

		Frequency	Percent
Tumor size (cm)	T1 (<2 cms)	9	9.0%
	T2 (2-5 cms)	62	62.0%
	T3 (>5 cms)	29	29.0%
	Total	100	100.0%

Tumor size was less than 2 centimetres in 9% of patients, 2 to 5 centimetres in 62% of patients, and more than 5 centimetres in 29% of patients.

Graph 2: Distribution of patients based on the tumor size

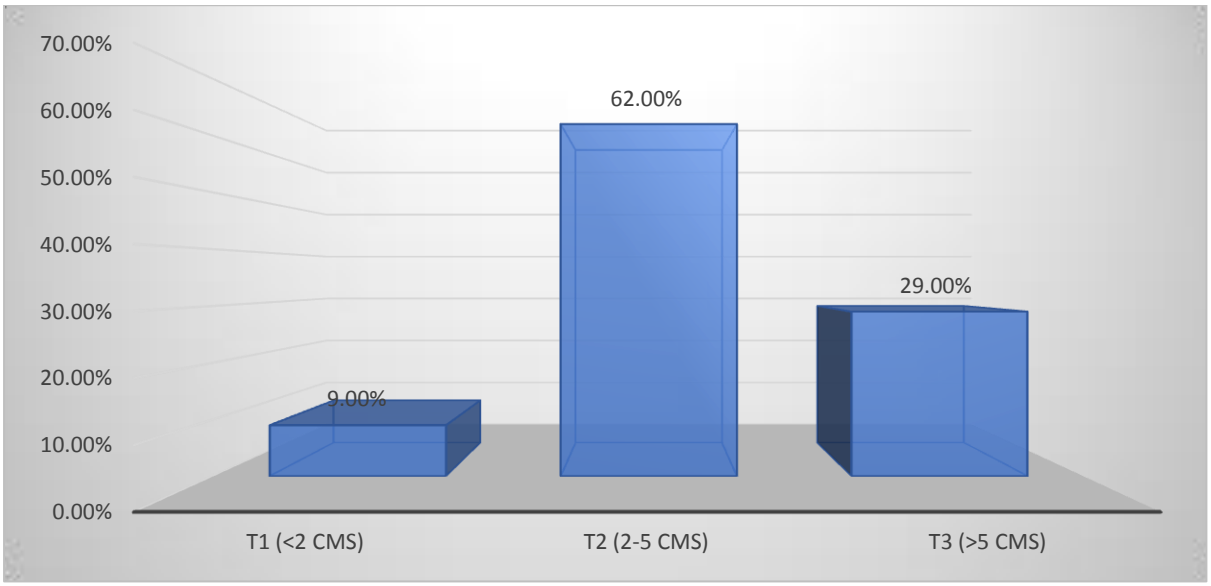


Table 10: Distribution of patients based on the tumor grade

		Frequency	Percent
Tumor Grade	I	58	58.0%
	II	30	30.0%
	III	12	12.0%
	Total	100	100.0%

Grade I tumor was seen in 58% of patients, grade II was seen in 30% of patients, and grade III was seen in 12% of patients.

Graph 3: Distribution of patients based on the tumor grade

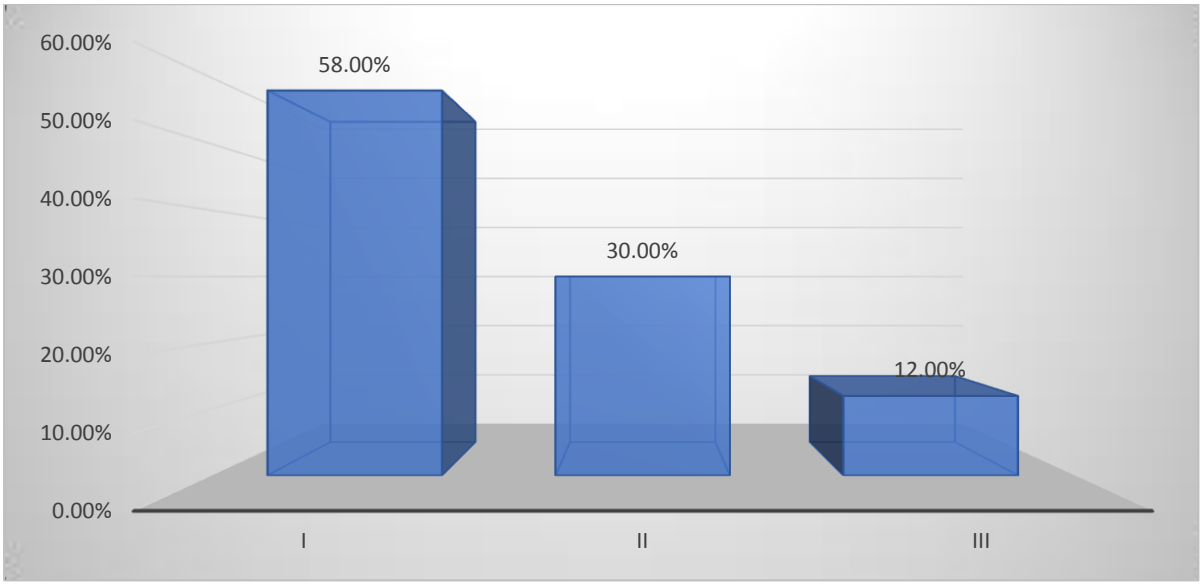


Table 11: Distribution of patients based on Lymph node

		Frequency	Percent
Lymph node	Positive	50	50.0%
	Negative	50	50.0%
	Total	100	100.0%

Graph 4: Distribution of patients based on Lymph node status

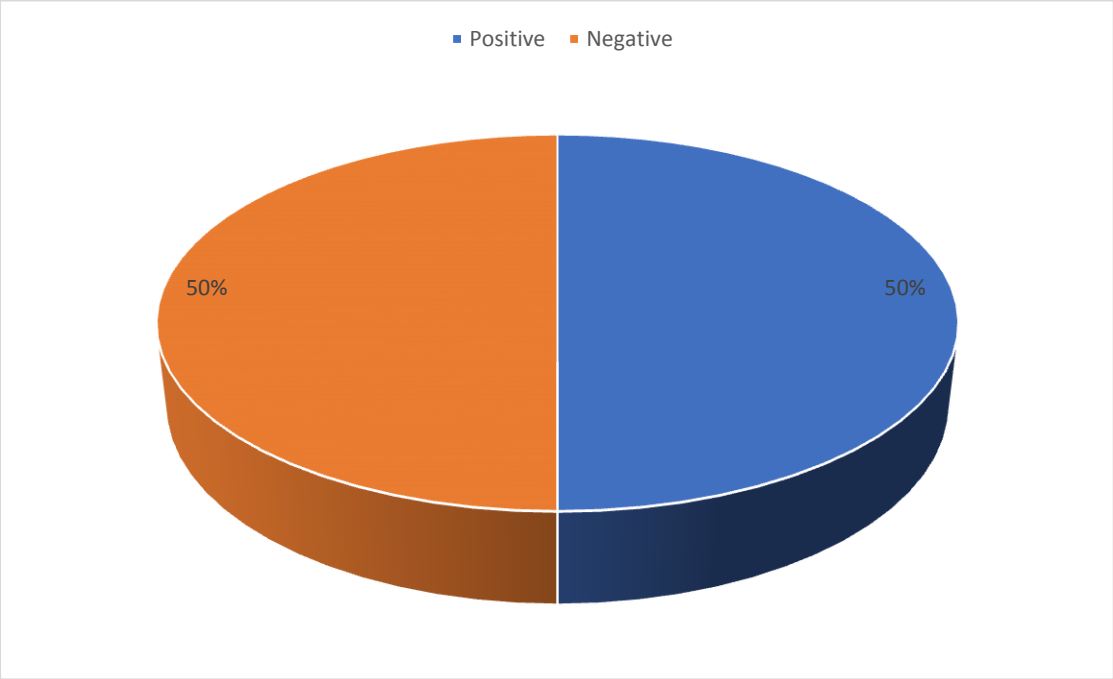


Table 12: Distribution of patients based on the extranodal extension

		Frequency	Percent
Extra nodal extension	Present	11	11.0%
	Absent	89	89.0%
	Total	100	100.0%

The extranodal extension was present in 11% of patients.

Graph 5: Distribution of patients based on the extranodal extension

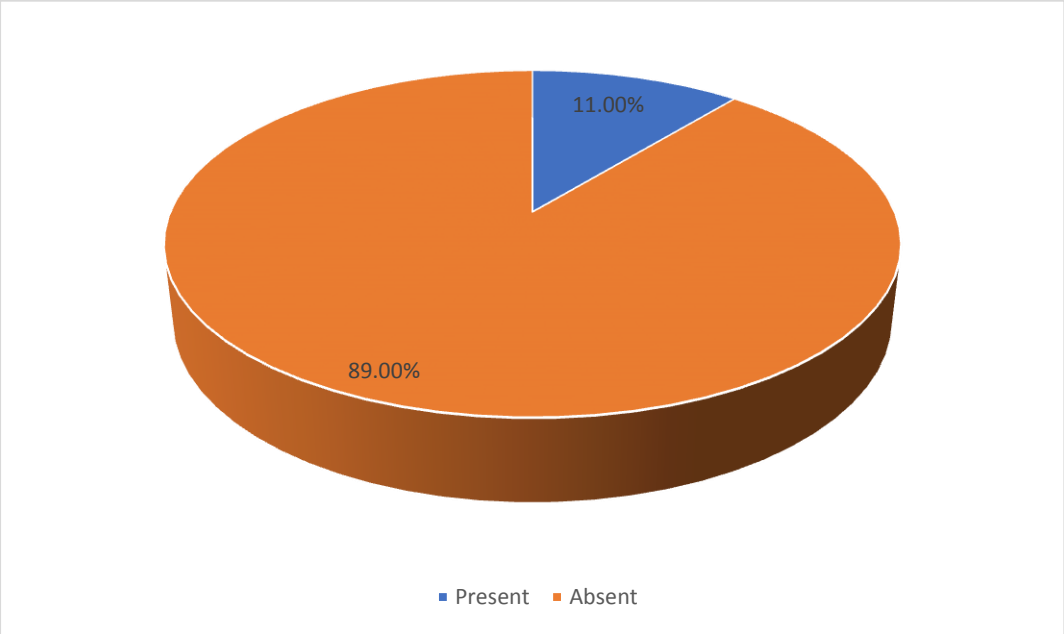


Table 13: Distribution of patients based on the TNM staging

		Frequency	Percent
T	1	7	7.0%
	2	54	54.0%
	3	25	25.0%
	4	14	14.0%
N	0	50	50.0%
	1	24	24.0%
	2	14	14.0%
	3	12	12.0%
	Total	100	100.0%
M	M1	1	1.0%
	M0	99	99.0%
	Total	100	100.0%

Table 14: Distribution of patients based on the cancer stage

		Frequency	Percent
Stage	I	7	7.0%
	II	50	50.0%
	III	42	42.0%
	IV	1	1.0%
	Total	100	100.0%

Stage I tumor was seen in 7% of patients, Stage II was seen in 50% of patients, Stage III was seen in 42% of patients, and Stage IV was seen in 1% of patients.

Graph 6: Distribution of patients based on the cancer stage

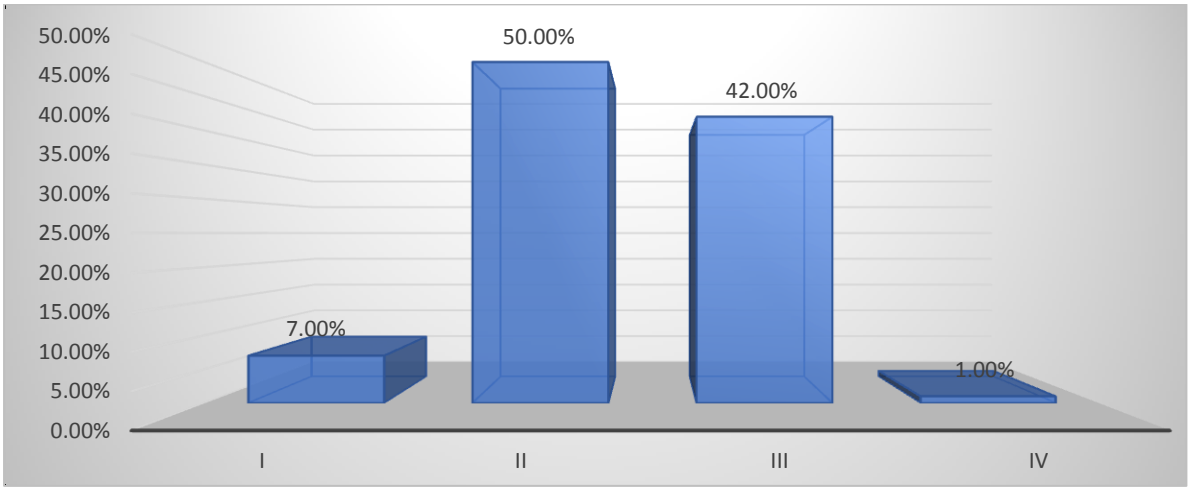


Table 15: Distribution of patients based on the NPI in breast carcinoma

		Frequency	Percent	5 Year Survival
NPI in breast carcinoma	Excellent	15	15.0%	96%
	Good	36	36.0%	93%
	Moderate	32	32.0%	78%
	Poor	17	17.0%	44%
	Total	100	100.0%	

The five-year survival rate was 96% in 15% of patients, 93% in 36%, 78% in 32%, and 44% in 17% of patients.

Graph 7: Distribution of patients based on the NPI in breast carcinoma

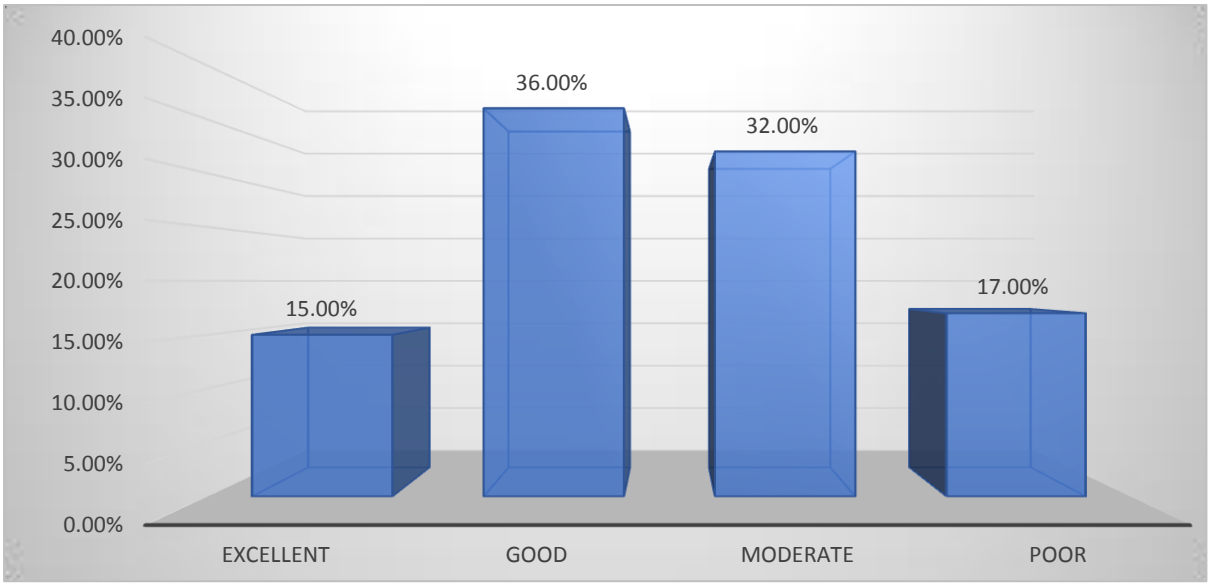


Table 16: Distribution of patients based on the ER status

		Frequency	Percent
ER	Positive	54	54.0%
	Negative	46	46.0%
	Total	100	100.0%

ER was positive in 54% of patients.

Graph 8: Distribution of patients based on the ER status

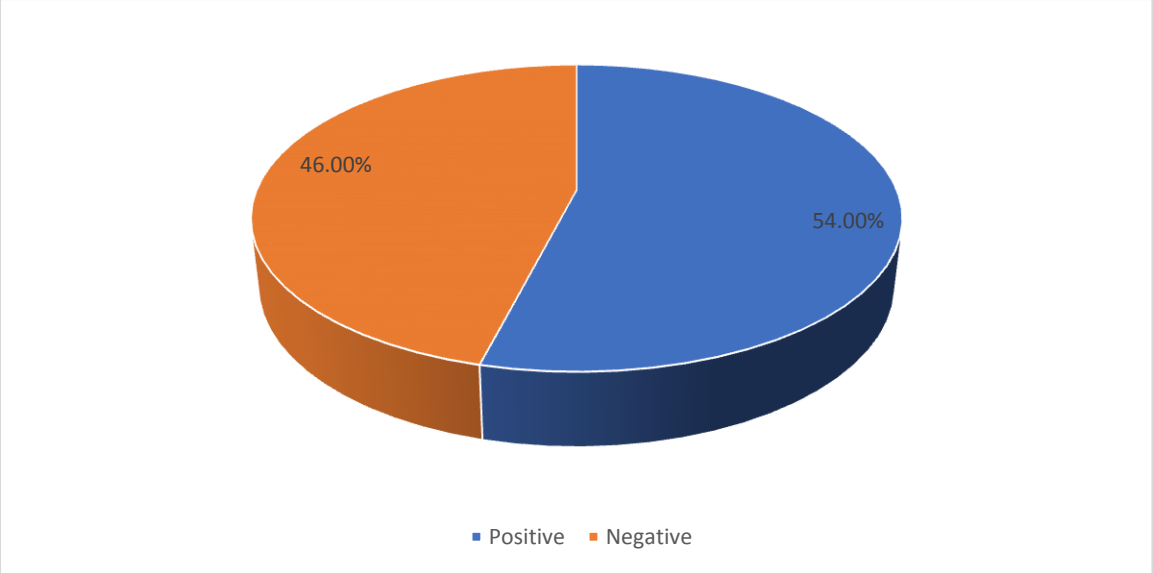


Table 17: Distribution of patients based on the PR status

		Frequency	Percent
PR	Positive	52	52.0%
	Negative	48	48.0%
	Total	100	100.0%

PR was positive in 52% of patients.

Graph 9: Distribution of patients based on the PR status

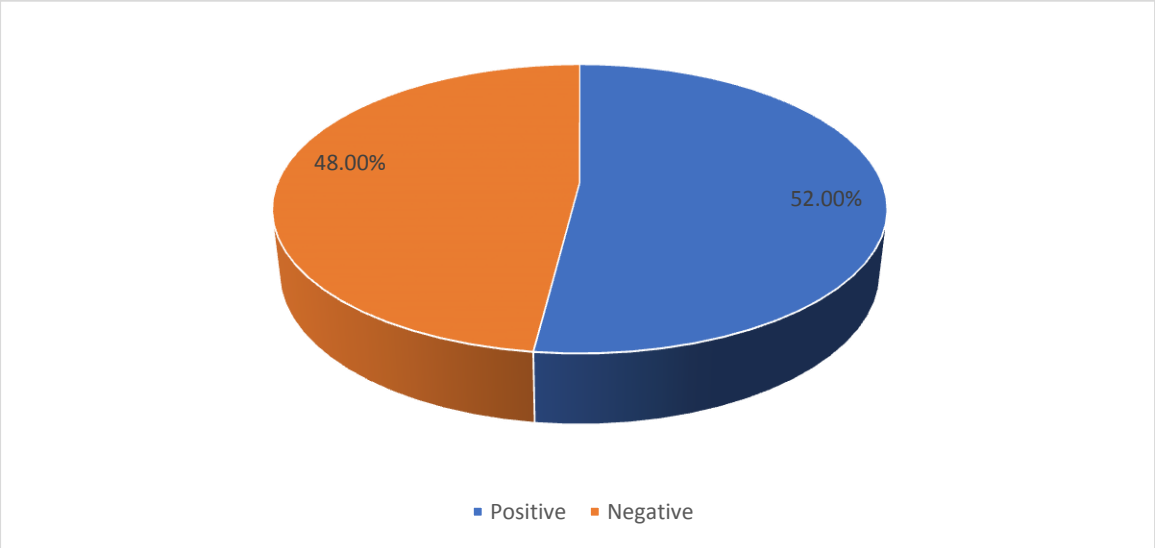


Table 18: Distribution of patients based on the Her 2 neu status

		Frequency	Percent
Her 2 neu	Positive	30	30.0%
	Negative	70	70.0%
	Total	100	100.0%

Her 2 neu was positive in 30% of patients.

Graph 10: Distribution of patients based on the Her 2 neu status.

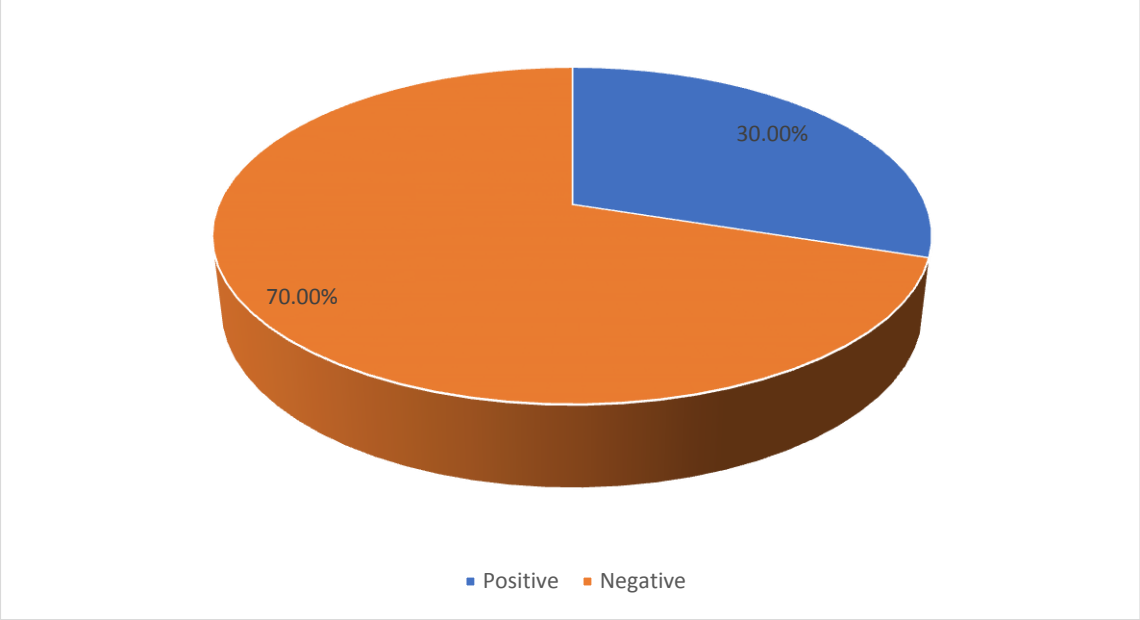


Table 19: Distribution of patients based on the Ki67 status

		Frequency	Percent
Ki67	<14 %	47	47.0%
	>14%	53	53.0%
	Total	100	100.0%

Ki67 was <14 % in 47% of patients and > 14% in 53%.

Graph 11: Distribution of patients based on the Ki67 status

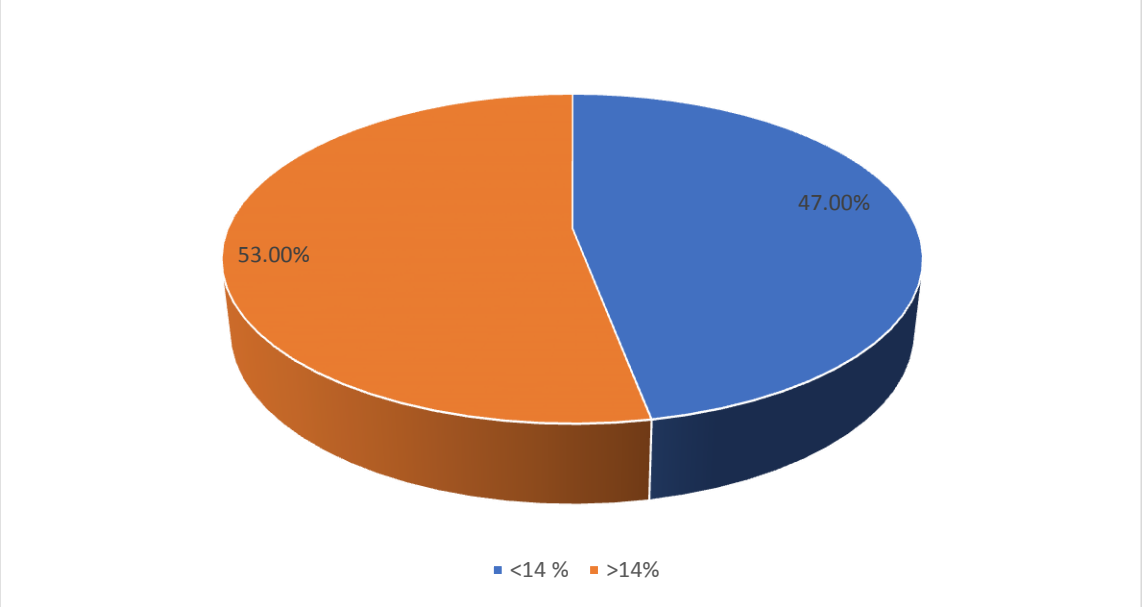


Table 20: Distribution of patients based on the molecular typing

		Frequency	Percent
Molecular typing	Her2 Positive	12	12.0%
	Luminal A	38	38.0%
	Luminal B	20	20.0%
	TNBC	30	30.0%
	Total	100	100.0%

Molecular typing showed Her2 positive in 12% of patients, Luminal A in 38% of patients, Luminal B in 20% of patients, and TNBC in 30% of patients.

Graph 12: Distribution of patients based on the molecular typing

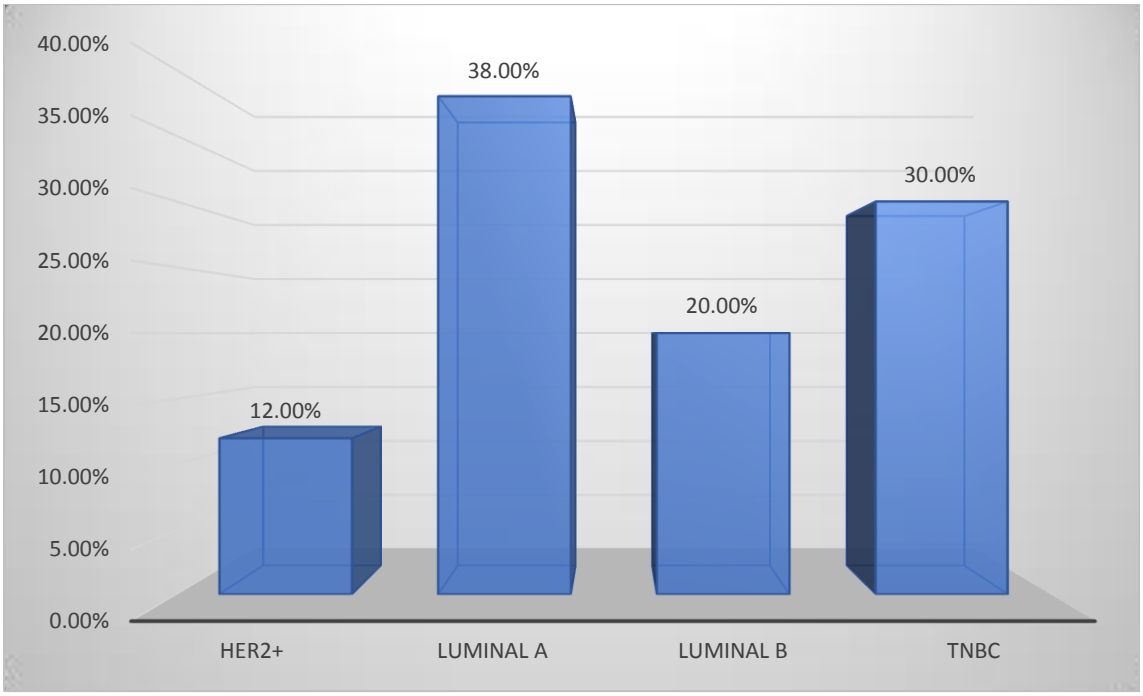


Table 21: Distribution of patients based on the stromal alpha SMA score

			Frequency	Percent
Stromal Alpha SMA Score	0	No Staining	3	3.0%
	1	Weak Staining	9	9.0%
	2	Moderate Staining	32	32.0%
	3	Strong Staining	56	56.0%
	Total		100	100.0%

According to the stromal alpha SMA score, staining was absent in 3% of patients, weak staining was seen in 9% of patients, moderate staining was seen in 32% of patients, and strong staining was seen in 56% of patients.

Graph 13: Distribution of patients based on the stromal alpha SMA score.

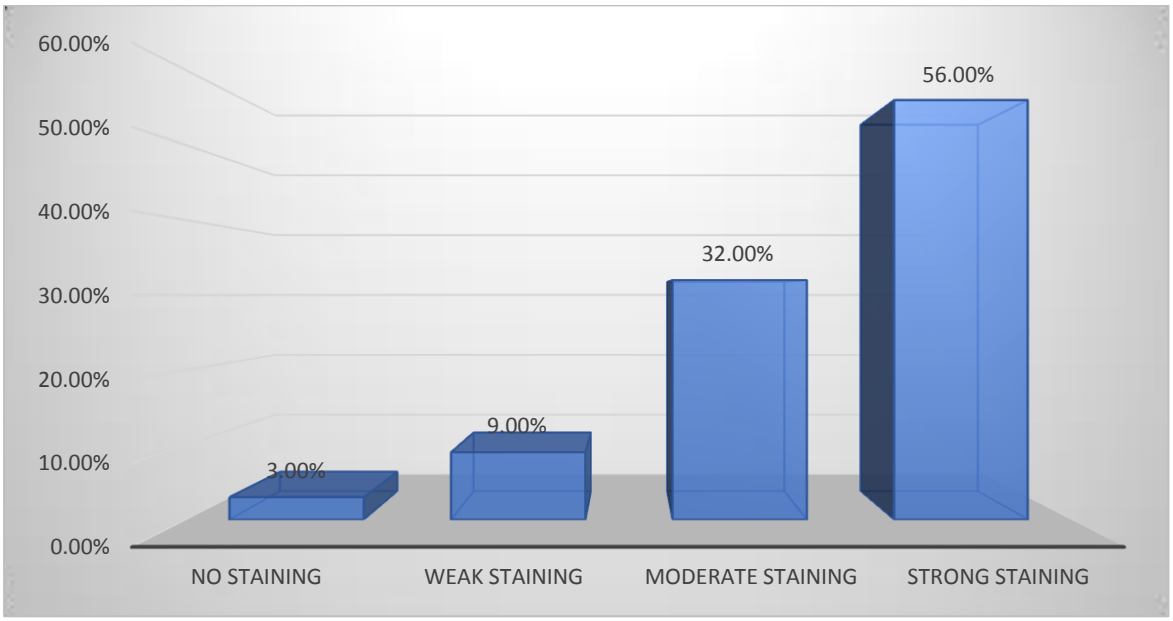


Table 22: Distribution of patients based on the age & stromal alpha SMA score

			Stromal Alpha SMA Score				Total
			0- No Staining	1- Weak Staining	2- Moderate Staining	3- Strong Staining	
Age Group	28-40 years	n	0	4	6	10	20
		%	0.0%	44.4%	18.8%	17.9%	20.0%
	41-50 years	n	1	1	10	11	23
		%	33.3%	11.1%	31.3%	19.6%	23.0%
	51-60 years	n	2	3	11	17	33
		%	66.7%	33.3%	34.4%	30.4%	33.0%
	61-70 years	n	0	1	1	11	13
		%	0.0%	11.1%	3.1%	19.6%	13.0%
	>71 years	n	0	0	4	7	11
		%	0.0%	0.0%	12.5%	12.5%	11.0%
Total		n	3	9	32	56	100
		%	100.0%	100.0%	100.0%	100.0%	100.0%

Chi-Square: 12.74, P Value: 0.38, Statistically not significant

The association between age and stromal alpha SMA score was found to be statistically not significant.

Graph 14: Distribution of patients based on age & stromal alpha SMA score.

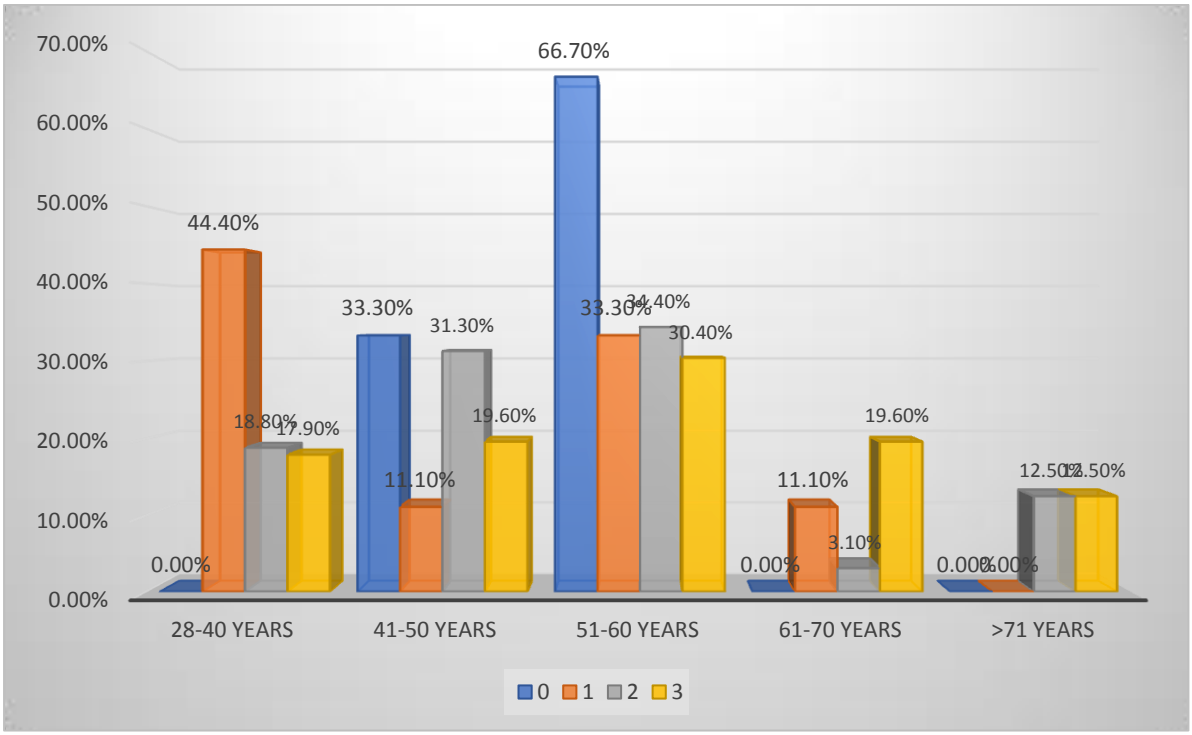


Table 23: Distribution of patients based on the Tumor size & stromal alpha SMA score

			Stromal Alpha SMA Score				Total
			0- No Staining	1- Weak Staining	2Moderate Staining	3- Strong Staining	
Tumor size (cm)	T1 (<2cms)	n	0	1	1	7	9
		%	0.0%	11.1%	3.1%	12.5%	9.0%
	T2(2-5 cms)	n	2	3	25	32	62
		%	66.7%	33.3%	78.1%	57.1%	62.0%
	T3 (>5 cms)	n	1	5	6	17	29
		%	33.3%	55.6%	18.8%	30.4%	29.0%
Total		n	3	9	32	56	100
		%	100.0%	100.0%	100.0%	100.0%	100.0%

Chi-Square: 8.46, P Value: 0.20, Statistically not significant

Among patients with strong staining alpha SMA score, tumor size was <2 cm in 12.5% of patients, 2 to 5 cm in 57.1% of patients, and >5 cm in 30.4% of patients. The association between tumor size and stromal alpha SMA score was found to be statistically not significant.

Graph 15: Distribution of patients based on the Tumor size & stromal alpha SMA score

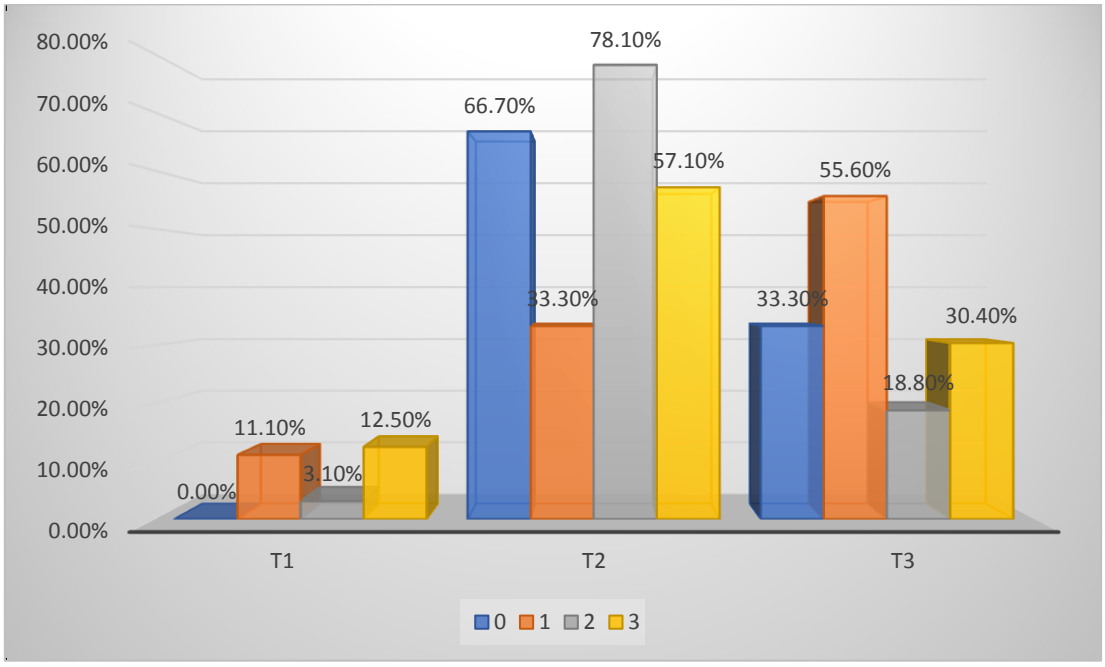


Table 24: Distribution of patients based on the Lymph node status & stromal alpha SMA score

			Stromal Alpha SMA Score				Total
			0- No Staining	1- Weak Staining	2- Moderate Staining	3- Strong Staining	
Lymph node status	Positive	n	2	3	18	27	50
		%	66.7%	33.3%	56.3%	48.2%	50.0%
	Negative	n	1	6	14	29	50
		%	33.3%	66.7%	43.8%	51.8%	50.0%
Total		n	3	9	32	56	100
		%	100.0%	100.0%	100.0%	100.0%	100.0%

Chi-Square: 1.90 P-Value: 0.05, Statistically significant

Among patients with strong staining alpha SMA score, Lymph node status was positive in 48.2%.

The association between Lymph node status and stromal alpha SMA score was found to be statistically significant.

Graph 16: Distribution of patients based on the lymph node status & stromal alpha SMA score.

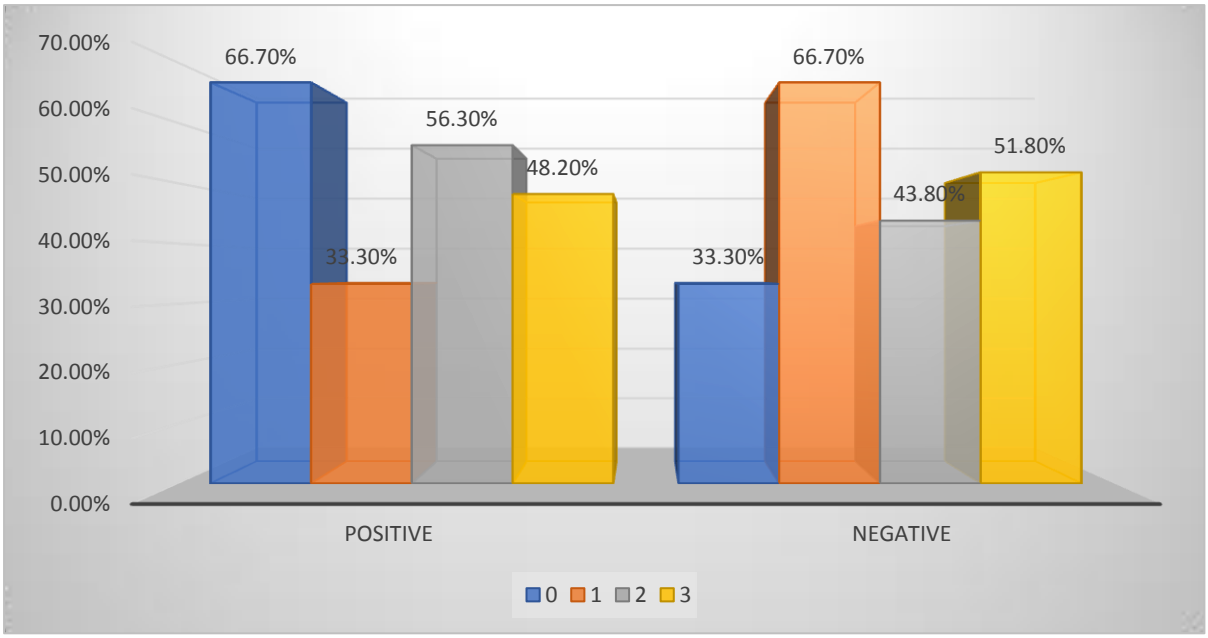


Table 25: Distribution of patients based on the extranodal extension & stromal alpha SMA score

			Stromal Alpha SMA Score				Total
			0- No Staining	1- Weak Staining	2- Moderate Staining	3- Strong Staining	
Extra nodal extension	Present	n	0	1	2	8	11
		%	0.0%	11.1%	6.3%	14.3%	11.0%
	Absent	n	3	8	30	48	89
		%	100.0%	88.9%	93.8%	85.7%	89.0%
Total		n	3	9	32	56	100
		%	100.0%	100.0%	100.0%	100.0%	100.0%

Chi-Square: 1.72, P Value: 0.63, Statistically not significant

Among patients with strong staining alpha SMA scores, Extra nodal extension was positive in 11 % in which eight cases are showing strong expression (73 %). The association between Extra nodal extension and stromal alpha SMA score was found to be statistically not significant.

Graph 17: Distribution of patients based on the extranodal extension & stromal alpha SMA score

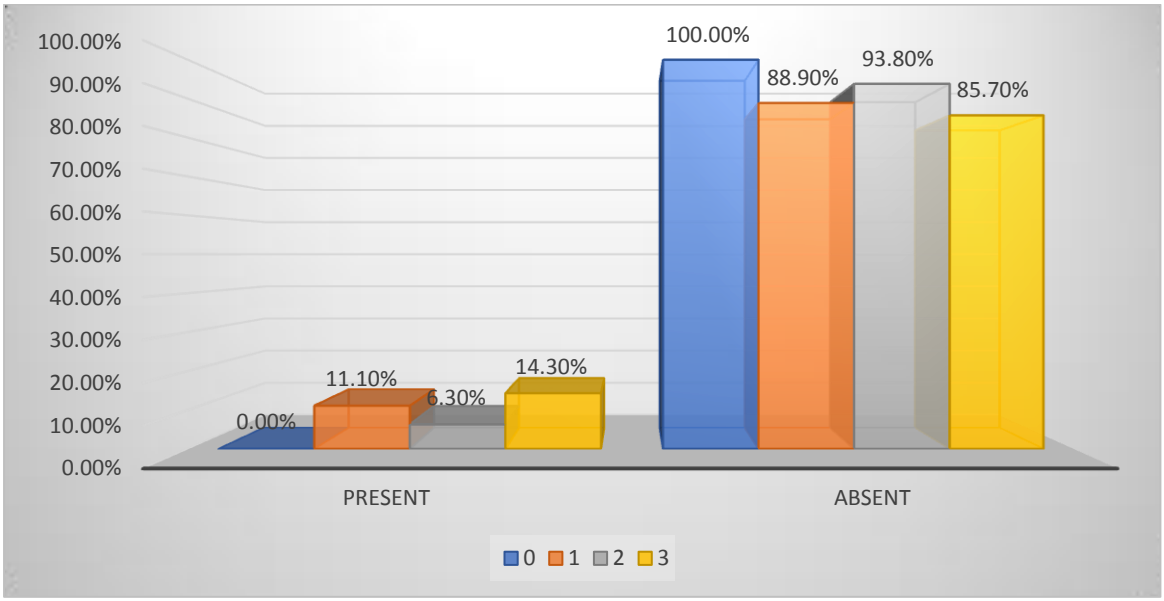


Table 26: Distribution of patients based on the tumor grade & stromal alpha SMA score

			Stromal Alpha SMA Score				Total
			0- No Staining	1- Weak Staining	2- Moderate Staining	3- Strong Staining	
Tumor Grade	I	n	1	5	24	28	58
		%	33.3%	55.6%	75.0%	50.0%	58.0%
	II	n	2	2	6	20	30
		%	66.7%	22.2%	18.8%	35.7%	30.0%
	III	n	0	2	2	8	12
		%	0.0%	22.2%	6.3%	14.3%	12.0%
Total		n	3	9	32	56	100
		%	100.0%	100.0%	100.0%	100.0%	100.0%

Chi-Square: 8.29, P Value: 0.021, Statistically significant

Among patients with strong staining alpha SMA score, a Grade I tumor was seen in 50% of patients; grade II was seen in 35.7%, and grade III was seen in 14.3% of patients.

The association between tumor grade and stromal alpha SMA score was found to be statistically significant.

Graph 18: Distribution of patients based on the tumor grade & stromal alpha SMA score

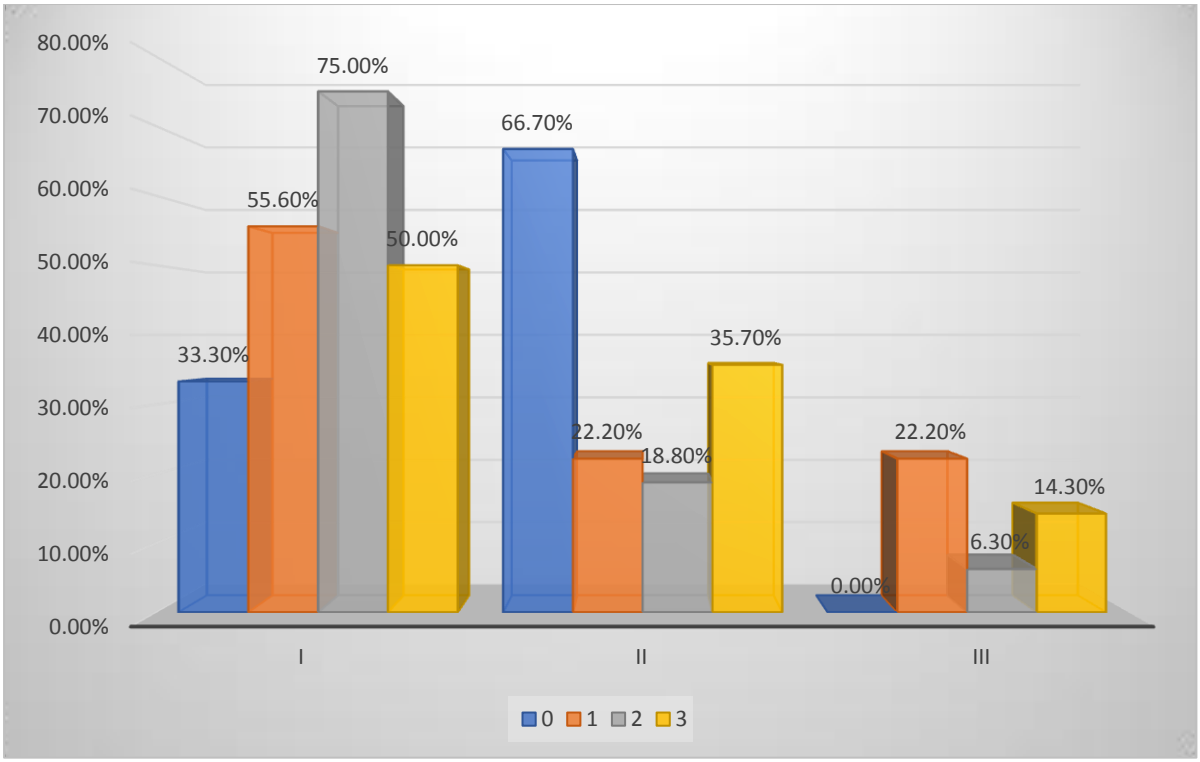


Table 27: Distribution of patients based on the tumor stage & stromal alpha SMA score

			Stromal Alpha SMA Score				Total
			0- No Staining	1- Weak Staining	2- Moderate Staining	3- Strong Staining	
Stage	I	n	0	1	2	4	7
		%	0.0%	11.1%	6.3%	7.1%	7.0%
	II	n	2	5	15	28	50
		%	66.7%	55.6%	46.9%	50.0%	50.0%
	III	n	1	3	15	23	42
		%	33.3%	33.3%	46.9%	41.1%	42.0%
	IV	n	0	0	0	1	1
		%	0.0%	0.0%	0.0%	1.8%	1.0%
Total		n	3	9	32	56	100
		%	100.0%	100.0%	100.0%	100.0%	100.0%

Chi-Square: 6.93, P Value: 0.05, Statistically significant

Among patients with strong staining alpha SMA score, Stage I tumor was seen in 7.1% of patients, Stage II was seen in 50% of patients, Stage III was seen in 41.1% of patients, and Stage IV was seen in 1.8% of patients.

The association between tumor stage and stromal alpha SMA score was found to be statistically significant.

Graph 19: Distribution of patients based on the tumor stage & stromal alpha SMA score

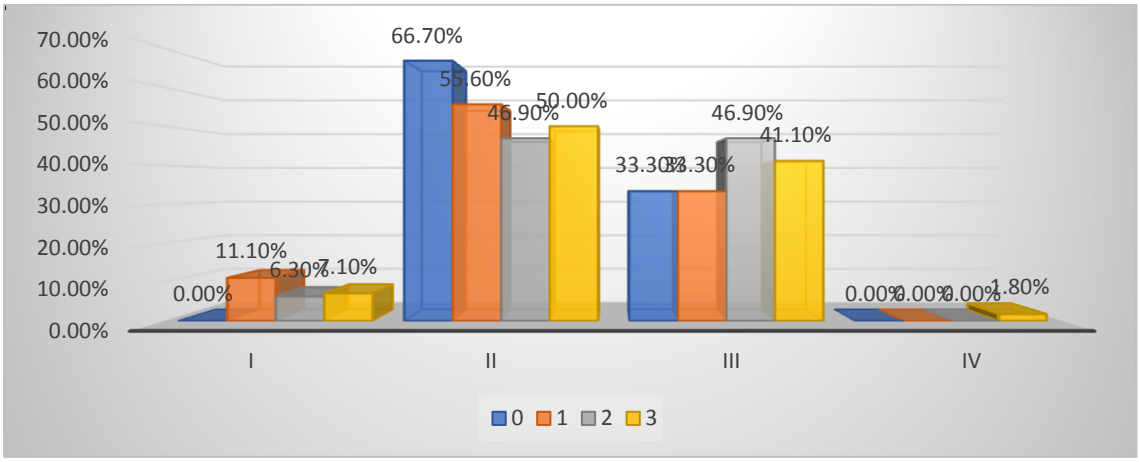


Table 28: Distribution of patients based on the ER status & stromal alpha SMA score

			Stromal Alpha SMA Score				Total
			0- No Staining	1- Weak Staining	2- Moderate Staining	3- Strong Staining	
ER	Positive	n	0	3	19	32	54
		%	0.0%	33.3%	59.4%	57.1%	54.0%
	Negative	n	3	6	13	24	46
		%	100.0%	66.7%	40.6%	42.9%	46.0%
Total		n	3	9	32	56	100
		%	100.0%	100.0%	100.0%	100.0%	100.0%

Chi-Square: 8.66, P Value: 0.02, Statistically significant

Among patients with strong staining alpha SMA score, ER was positive in 57.1%.

The association between ER status and stromal alpha SMA score was found to be statistically significant.

Graph 20: Distribution of patients based on the ER status & stromal alpha SMA score.

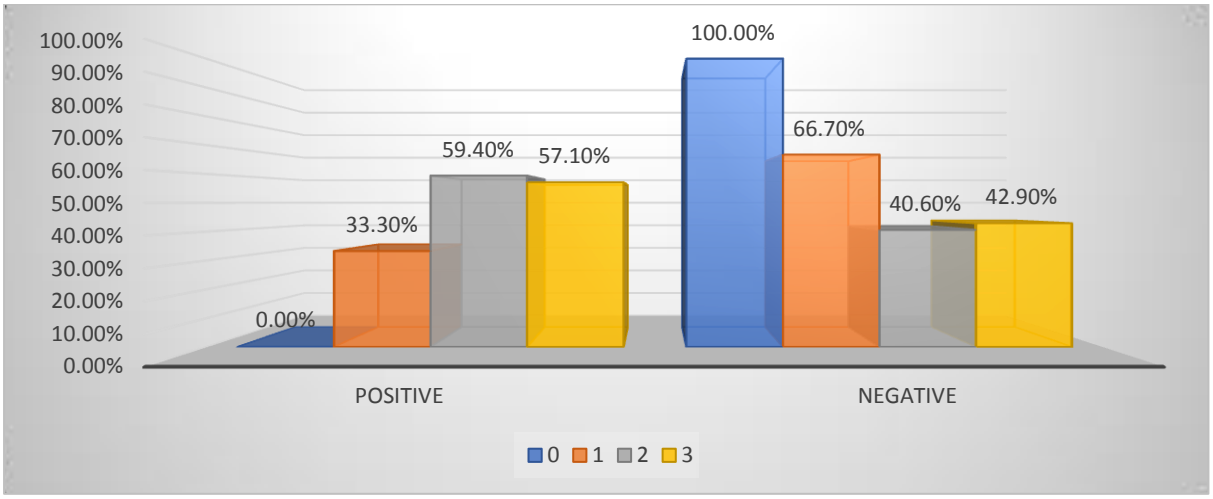


Table 29: Distribution of patients based on the PR status & stromal alpha SMA score

			Stromal Alpha SMA Score				Total
			0- No Staining	1- Weak Staining	2- Moderate Staining	3- Strong Staining	
PR	Positive	n	0	1	19	32	52
		%	0.0%	11.1%	59.4%	57.1%	52.0%
	Negative	n	3	8	13	24	48
		%	100.0%	88.9%	40.6%	42.9%	48.0%
Total		n	3	9	32	56	100
		%	100.0%	100.0%	100.0%	100.0%	100.0%

Chi-Square: 10.56, P Value: 0.01, Statistically significant

Among patients with strong staining alpha SMA scores, PR was positive in 57.1%.

The association between PR status and stromal alpha SMA score was found to be statistically significant.

Graph 21: Distribution of patients based on the PR status & stromal alpha SMA score.

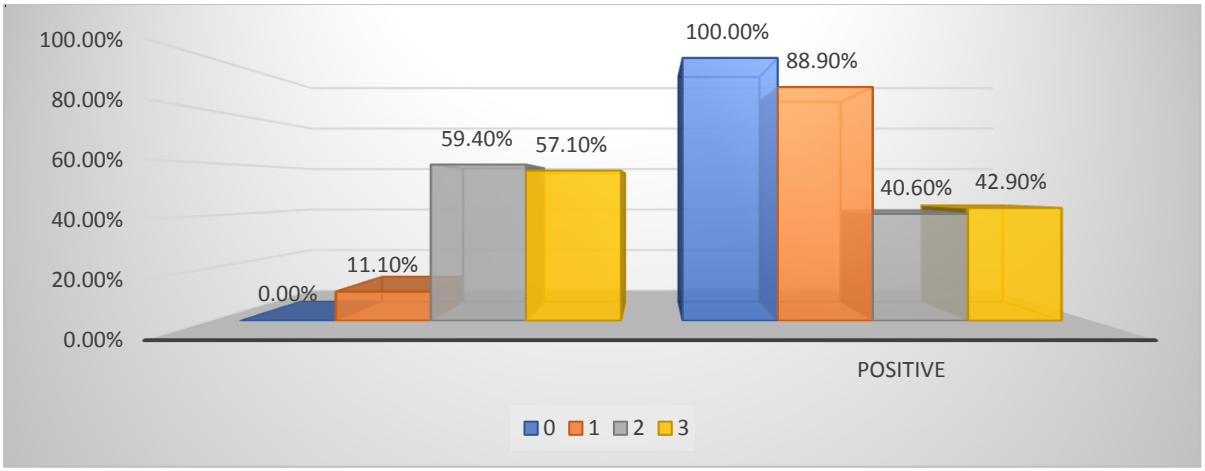


Table 30: Distribution of patients based on the Her 2 neu & stromal alpha SMA score

			Stromal Alpha SMA Score				Total
			0- No Staining	1- Weak Staining	2- Moderate Staining	3- Strong Staining	
Her 2 neu	Positive	n	1	4	10	15	30
		%	33.3%	44.4%	31.3%	26.8%	30.0%
	Negative	n	2	5	22	41	70
		%	66.7%	55.6%	68.8%	73.2%	70.0%
Total		n	3	9	32	56	100
		%	100.0%	100.0%	100.0%	100.0%	100.0%

Chi-Square: 1.20, P Value: 0.75, Statistically not significant

Among patients with strong staining alpha SMA score, Her 2 neu was positive in 26.8%. The association between Her 2 neu and stromal alpha SMA score was found to be statistically not significant.

Graph 22: Distribution of patients based on the Her 2 neu & stromal alpha SMA score.

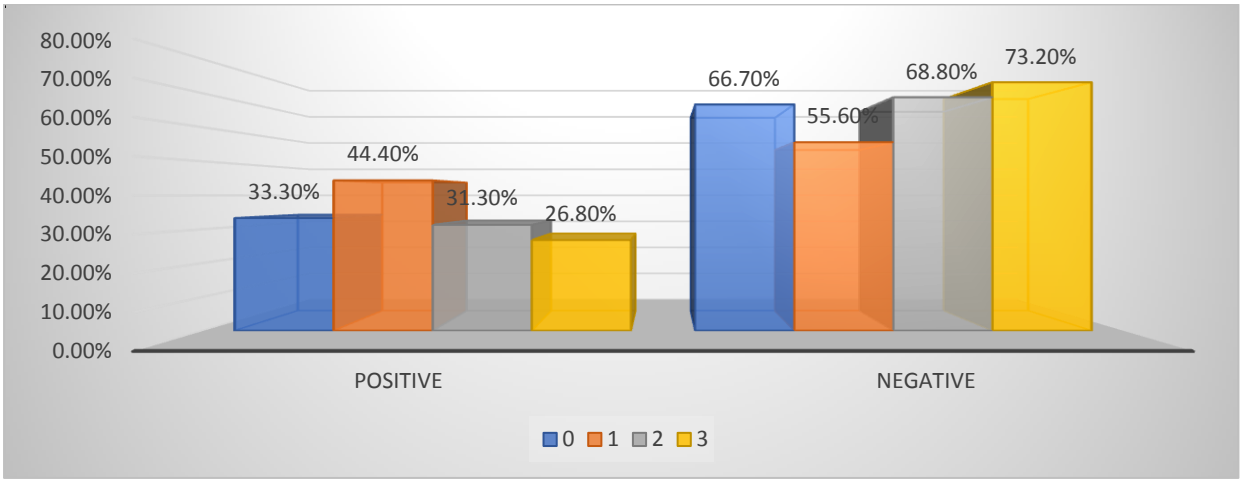


Table 31: Distribution of patients based on the Ki67 & stromal alpha SMA score

			Stromal Alpha SMA Score				Total
			0- No Staining	1- Weak Staining	2- Moderate Staining	3- Strong Staining	
Ki67	<14 %	n	0	4	15	28	47
		%	0.0%	44.4%	46.9%	50.0%	47.0%
	>14%	n	3	5	17	28	53
		%	100.0%	55.6%	53.1%	50.0%	53.0%
Total		n	3	9	32	56	100
		%	100.0%	100.0%	100.0%	100.0%	100.0%

Chi-Square: 2.88, P Value: 0.40, Statistically not significant

Among patients with strong staining alpha SMA score, Ki67 was <14% in 50% of patients and >14% in 50% of patients. The association between Ki67 and stromal alpha SMA score was found to be statistically not significant.

Graph 23: Distribution of patients based on the Ki67 & stromal alpha SMA score.

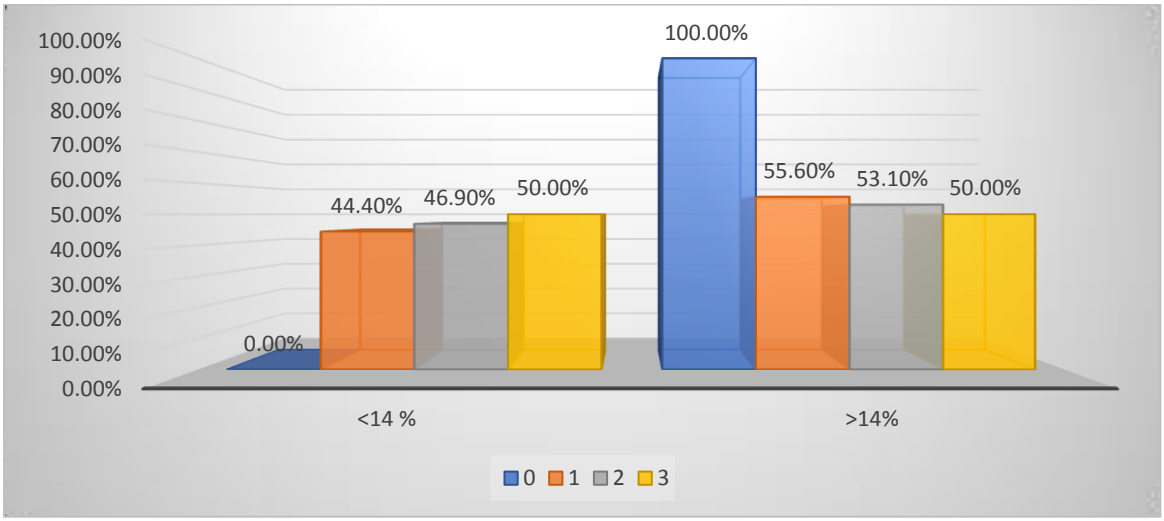


Table 32: Distribution of patients based on the Molecular typing & stromal alpha SMA score

			Stromal Alpha SMA Score				Total
			0- No Staining	1- Weak Staining	2- Moderate Staining	3- Strong Staining	
Molecular typing	Her 2 Positive	n	1	3	3	5	12
		%	33.3%	33.3%	9.4%	8.9%	12.0%
	Luminal A	n	0	3	13	22	38
		%	0.0%	33.3%	40.6%	39.3%	38.0%
	Luminal B	n	0	1	7	12	20
		%	0.0%	11.1%	21.9%	21.4%	20.0%
	TNBC	n	2	2	9	17	30
		%	66.7%	22.2%	28.1%	30.4%	30.0%
Total		n	3	9	32	56	100
		%	100.0%	100.0%	100.0%	100.0%	100.0%

Chi-Square: 9.08, P Value: 0.043, Statistically significant

Among patients with strong staining alpha SMA score, Molecular typing showed Her2 positive in 8.9% of patients, Luminal A in 39.3% of patients, Luminal B in 21.4% of patients, and TNBC in 30.4% of patients.

The association between Molecular typing and stromal alpha SMA score was found to be statistically significant.

Graph 24: Distribution of patients based on the Molecular typing & stromal alpha SMA score

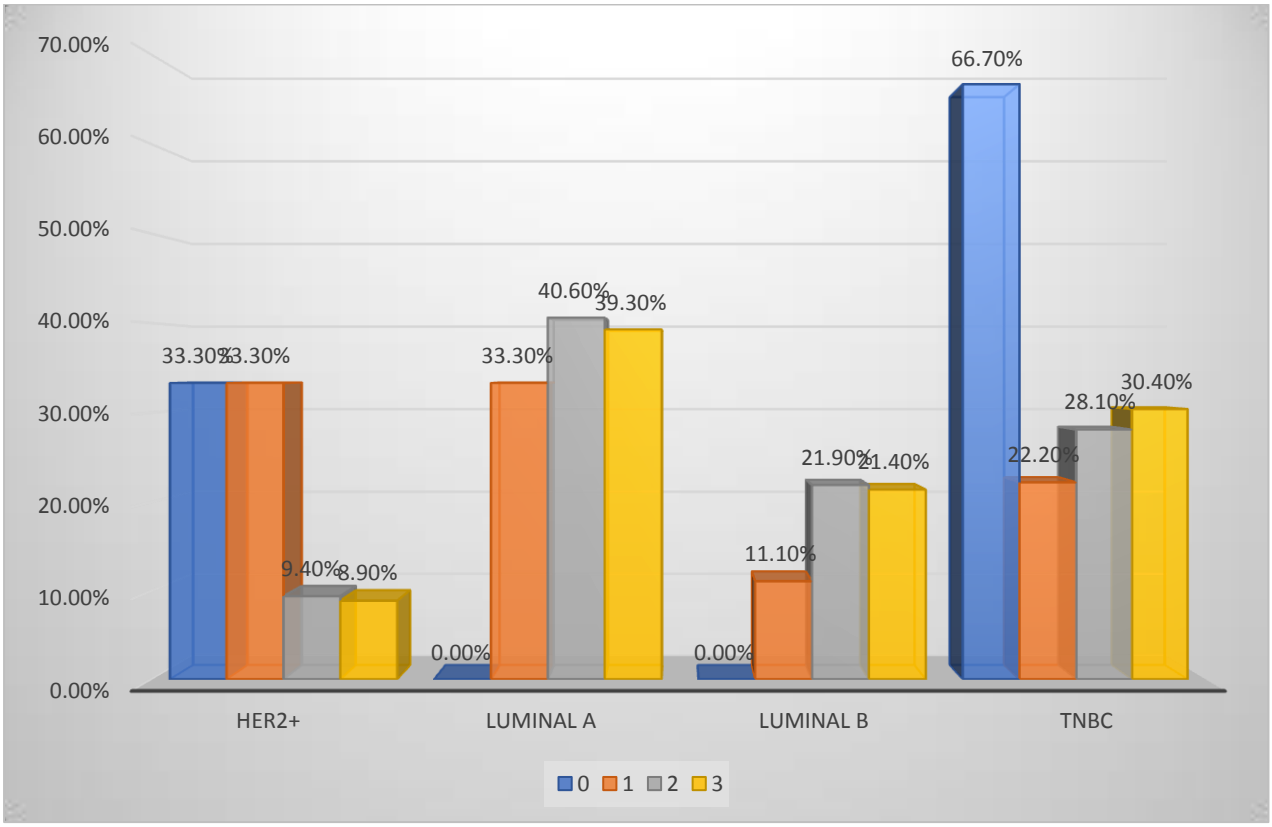


Table 33: Distribution of patients based on the NPI & stromal alpha SMA score

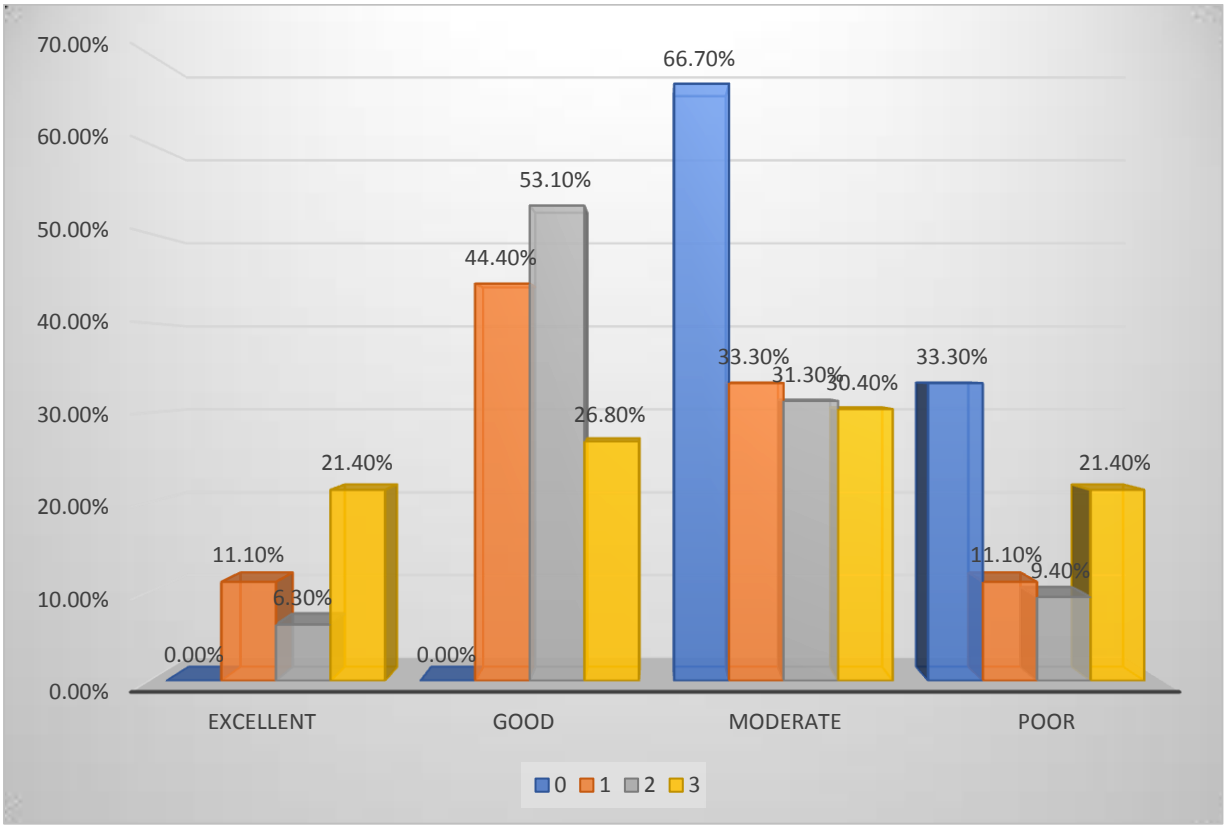
			Stromal Alpha SMA Score				Total
			0- No Staining	1- Weak Staining	2- Moderate Staining	3- Strong Staining	
NPI in breast carcinoma	Excellent	n	0	1	2	12	15
		%	0.0%	11.1%	6.3%	21.4%	15.0%
	Good	n	0	4	17	15	36
		%	0.0%	44.4%	53.1%	26.8%	36.0%
	Moderate	n	2	3	10	17	32
		%	66.7%	33.3%	31.3%	30.4%	32.0%
	Poor	n	1	1	3	12	17
		%	33.3%	11.1%	9.4%	21.4%	17.0%
Total		n	3	9	32	56	100
		%	100.0%	100.0%	100.0%	100.0%	100.0%

Chi-Square: 12.48, P Value: 0.18, Statistically not significant

Among patients with strong staining alpha SMA score, the Five-year survival rate was 96% in 21.4%, 93% in 26.8%, 78% in 30.4%, and 44% in 21.4% of patients.

The association between NPI and stromal alpha SMA score was found to be statistically not significant.

Graph 25: Distribution of patients based on the NPI & stromal alpha SMA score.



DISCUSSION

DISCUSSION

Immunohistochemistry (IHC) is used to characterize intracellular proteins or various cell surfaces in all tissues. Individual markers or panels of various markers can be used to characterize various tumor subtypes, confirm tissue of origin, distinguish metastatic from the primary tumor and provide additional information which may be necessary for diagnosis, prognosis, predicting response to therapy, or evaluating residual tumor post-treatment. A growing list antibodies and antigen retrieval techniques will contribute to the broader utility of immunohistochemistry for solving diagnostic problems or determining prognosis and response to therapy in breast pathology. Smooth muscle actin (SMA) has long been used as a myoepithelial marker in breast pathology diagnosis as a sensitive marker for myoepithelial differentiation. This is because any cell with a substantial expression of actin is positive for SMA (myofibroblasts and blood vessels are positive for SMA).[54]

Al-Khayat ZAY et al., in 2016, conducted a study on confirmed breast cancer cases. The patient's mean age was 48±5 (Range: 28-83) years; 57% were ≤50 years old. ER and PR were 58.8% and 49.1%, respectively. Hormone receptors (ER and PR) correlated significantly with the age and grade of the tumor.[41]

Thiagarajan M et al. conducted a study in 2015 that studied 60 cases of breast carcinoma. The mean age of the patients was 51.17 years, with a standard deviation of 9.859 years. The youngest patient with breast cancer was 32 years

of age. About 61.7 % of the cases were ER-positive, and another 70% were PR-positive. In this study group, 40 patients (66.7%) were in the postmenopausal age group, and 20 patients (33.3%) were in the premenopausal age group. In most cases, tumor size ranged from 2- 5cm (68.3%). Compared with tumor grade, ER negativity increased significantly with increasing tumor grade (G). In Grade 1, ER-negative was 5.4%. Grade 2 ER-negative was 43.2%, and Grade 3 ER-negative was 51.4%. The p-value was 0.006.[42]

A study by Rao C et al. comprised 126 invasive breast cancer specimens between 2009 and 2011 and showed that 67% of patients were 50 years or younger. Histological types were invasive ductal carcinoma, not otherwise specified (58.7%), and overall (15.9%) were grade 3. ER was positive in 36.5%, Her2neu was over-expressed in only three cases, and 50 % were triple negative (ER, PR, and Her2neu negative). ER and PR positivity decreased with an increase in tumor grade. There was a significant association between tumor size and ER positivity. [43]

Pathak TB et al. in 2011, conducted a study on ER and PR expression in breast carcinoma. Out of 136 cases, there were 131 (96%) cases of infiltrating ductal carcinoma with a mean age of 48yrs. Most cases were grade III (21%) and grade I (20%). ER and PR expression were seen in 28% and 19%, respectively. In grade I, 16 (59%) and 10 (37%) cases out of 27 were ER and PR positive, respectively. In grade II, 21 (26%) and 15 (19%) out of 80 cases were ER and

PR positive, respectively. In grade III, 1 (3%) and 1 (3%) case were positive for ER and PR, respectively. [44]

Abdalla FBE, et al. conducted a similar study in 2012. Of the 62 patients, disease in 16% was of the lobular type, 69% had invasive ductal, and 15% had other carcinoma types; 47 out of 62 cases had lymph node involvement. Positive hormonal receptor expression was more common among those with lymph node-negative than lymph node-positive tumors. ER and PR-positive patients appeared to have better survival than ER and PR-negative patients. 58% and 51% of all tumors exhibited positive epithelial nuclear staining for ER and PR, respectively. Three cases (4.8%) were positive for ER only and negative for PR; The other 32 cases were found to be positive for both the ER and PR tests. Accordingly, a total of 27 (43.5%) of the patients tested negative for both the ER and the PR.[27]

Wludarski SCL et al. A 2011 study tested 255 invasive breast carcinomas for ER and PR. In this study, 172 samples were considered positive, 83 were negative for ER, 164 were considered positive, and 91 were negative for PR. Overall positivity for ER and PR was 98.8% (252/255 samples) and 98.4% (251/255 samples), respectively. For positive results, the concordance was 100.0% for both ER and PR. For negative results, the concordance was 96.4% for ER and 95.6% for PR. [45]

Dayal A et al. conducted a similar study in which out of 80 cases, 69 (86%) cases were of Infiltrating Ductal Carcinoma with a mean age of 53. ER & PR

expression was seen in 56.9% and 35.5%, respectively. ER, PR expressing tumors tended to have lower grade I with a significant p-value of 0.05. Lymph node metastases were found to be significantly associated with PR-positive status with a p-value of 0.03. [46]

Dong W et al. conducted a study in 2008 to assess the impact of the tumor detection method in predicting breast cancer survival. Out of 5481 cases of primary breast cancer, tumors were more common in women 60 years or older (41.1%, age ≥ 60 years; 34.9%, age 50-59 and 24.0%, age < 50 years). Screen-detected tumors were of significantly smaller size and lower nuclear grade, had fewer axillary lymph nodes involved, and were less likely to have invaded intramammary lymphatic or vascular tissue. 81.0% of the screen-detected tumors were ER-positive, compared with 67.3% of symptom-detected tumors. [47]

Helin HJ et al. have conducted a study on 232 cases of breast carcinoma for ER&PR expression by immunohistochemical study. Of the invasive ductal carcinomas, 72% were ER-positive, and 55% were PR positive. The invasive lobular, intraductal, tubular, and mucinous carcinomas were the most frequent ER-positive tumor types, whereas comedo and medullary carcinomas only rarely contained ER. PR was most frequently present in intraductal, tubular, and mucinous carcinomas. Better differentiated tumors with lower histologic grades were significantly associated with a high prevalence of immunohistochemically determined ER and PR ($P < 0.0001$).[48]

Koonmee S et al. conducted a study in 2006 on 294 cases of breast carcinoma. The mean and median ages were 52.21 years and 53 years, respectively. The smaller number of patients was in the <35-year age group, which was 3.40%. 89.83% of the patients were invasive ductal carcinoma, and 50.93% were in histological grading II. The ER & PR positives were 53.1% and 42.26%, respectively. The proportions of ER+ PR+, ER+PR-, ER-PR+, and ER-PR- were 4.7, 20.5, and 29.4, respectively. [49]

Mirumalini S et al. studied 15 cases of breast carcinoma, which found ER and PR to be positive in all the cases with differential grading of I, II & III. ER, positivity is seen in grade I (100%), grade II (53.3%), and grade III (40%). PR positivity is seen in grade I (100%), grade II (53%), and grade III (41%). As the tumor grade increases, receptors' expression is reduced. [50]

Ozmen V et al. The median age of 128 patients was 48 years, and 55% were premenopausal. Most patients had invasive ductal (81%) and histologic grade III (81%) breast cancer. Hormonal receptor status changed in 36 patients (28%). ER & PR receptor positivity rates at diagnosis and after NAC was 44–32.8% and 43–29.7%, respectively. Negative-to-positive change in HR status was observed in five patients. The 5-year overall survival was 76% in patients whose HR status converted to negative, compared with 91% in patients who remained HR-positive ($p<0.05$). [51]

Nigam JS, Yadav P, and Sood N. conducted a study on 328 cases of breast carcinoma in India. The most common age of presentation was in the 4th & 5th

decade of life, and the median age of presentation was 49 years of age (range 19-88 years). The majority of the patient (79%, 259/328) had a presentation more than ten years after the last childbirth, and 54.6% (179/328) of patients were postmenopausal. Histopathology showed that IDC, NOS was the most typical variant comprising 81.40% (267/328) of cases, followed by medullary carcinoma (10.36%, 34/328) and mucinous carcinoma (2.74%, 9/328). Other variants included infiltrating lobular carcinoma (2.44%, 8/328) and mixed ductal-lobular type (6/328, 1.83%). The immunohistochemical markers—ER, PR, and Her2neu, were evaluated in 142 cases. Triple-negative (ER, PR, and Her2Neu negative) were found to be the most specific group comprising 39.4% (56/142) of all the cases, followed by ER and PR, both positive (34.50%, 49/142) and triple-positive (ER, PR, and Her2neu positive; 26.06%, 37/142). [52]

Yu K et al. have conducted a study on breast cancer patients with estrogen receptor-negative/ progesterone receptor-positive tumors: being younger and getting less benefit from adjuvant tamoxifen treatment. Among all 1,836 consecutive patients with operable primary breast cancer, 798 cases were with ER+/PR+ tumors and 205 with ER-/PR+ tumors. Patients with ER-/PR+ tumors were younger than those with ER+/PR+ tumors (P = 0.021) and were mainly premenopausal (P = 0.013). ER-/PR+ patients were related to more involved lymph nodes and later stages. In the absence of TAM treatment, the ER+/PR+

group had a similar survival to the ER-/PR+ group in terms of 5-year disease-free survival (DFS) and overall survival (OS). [53]

STROMAL ALPHA SMA SCORE

In this study, according to the stromal alpha SMA score, staining was not seen in 3% of patients, weak staining was seen in 9% of patients, moderate staining was seen in 32% of patients, and strong staining was seen in 56% of patients.

In Yamashita M et al.[55], there was a relatively wide variation in the expression of alpha-SMA with the percentage of the area from 0.68 to 28.15% (mean 8.48 ± 5.40%). However, the values in the metastasis group were significantly higher, namely ranging from 6.5 to 28.15% (mean 13.47 ± 6.38%) in comparison to 0.68–21.64% (mean 7.35 ± 4.52%) in the no metastasis group when the invasive breast cancer patients were divided into two groups, those with a high a-SMA (n = 25) and those with a low a-SMA (n = 35) when the mean value of 8.48% was used as the cut-off point.

In Mohamed D et al.[37]**,** there were significant differences between Fibroblast activation protein expression in the tumor centre and tumor margin, as there were 24 cases that were negative for Fibroblast activation protein expression in the tumor centre. Thirty cases showed low expression, while 46 cases showed stromal Fibroblast activation protein high expression. On examination of the tumor margin, 44 cases were negative, 21 showed low expression, and 35 showed high stromal Fibroblast activation protein expression. Also, there were

significant differences between α-SMA expression in the tumor centre and tumor margin, as there were 24 cases were negative for expression of α-SMA in the tumor centre, and 76 cases showed positive expression. In contrast to tumor margin, 54 cases were negative, and 46 showed positive expression. Fibroblast activation protein expression in the tumor centre was positively correlated with histopathological types, tumor size, grade, and lymph node metastasis.

In contrast to tumor margin, Fibroblast activation protein expression was negatively correlated with tumor size and lymph node metastasis. On examination of α-SMA expression in the tumor centre, there was a positive correlation between tumor size and grade. In contrast, there was a negative correlation with tumor size, grade, and lymph node metastasis in the tumor margin.

Julia T et al. (2013) reported that Fibroblast activation protein expression in breast cancer stroma is heterogeneous and may correlate with clinicopathologic parameters such as size, grade, axilla nodal involvement, and tumor histological types, especially TNBC. So, Fibroblast activation protein may represent a potentially targetable therapy, especially for tumors that lack targeted therapy, such as TNBC[56.]

Henry et al. (2007) concluded that Fibroblast activation protein and α-SMA expressing CAFs in the tumor margin are of importance during the early invasion and metastasis, high Fibroblast activation protein expression and

positive α-SMA expression in the tumor margin are correlated with small tumor size, low lymph node metastasis, and low histological grade cases. Once the invasive carcinoma is established, other factors affect clinical outcomes. [57]

AGE

In this study, the majority of the patients belong to the age group of 51 to 60 years [33%], followed by 41 to 50 years [23%], 28 to 40 years [20%], 61 to 70 years [13%] and more than 71 years [11%]. In Yamashita M et al.,[55] mean age was 56 years in the non-metastasis group and 57.5 years in the metastasis group. The mean age was 54.4 years in high alpha SMA expression and 57.6 years in low alpha SMA expression, with a p-value of 0.37.

In Tse GMK et al.,[58] age range was 22–89 years (mean age 47 years). Excised tumours were in the size range 0.1–2.2 cm (mean size 0.71 cm).

TUMOR SIZE

In this study, Tumour size was less than 2 centimetres in 9% of patients, 2 to 5 centimetres in 62% of patients, and more than 5 centimetres in 29% of patients. In Yamashita M et al.,[55] tumor size was <5 cm in 76% and >5 cm in 24% of patients with high alpha SMA expression, and tumor size was <5 cm in 88.5% and >5 cm in 11.5% of patients in low alpha SMA expression with a p-value of 0.34. The size of tumor was not associated with myofibroblast differentiation.

TUMOR GRADE

In this study, among patients with strong staining alpha SMA score, a Grade I tumor was seen in 50% of patients, grade II was seen in 35.7% of patients, and grade III was seen in 14.3% of patients. The association between tumor size and stromal alpha SMA score was statistically significant.

Ours is the one of the studied where we have compared tumor grade with alpha smooth muscle actin expression in stroma of breast carcinoma. It shows that as increase in tumor grade associated with increase in formation of cancer associated fibroblasts (CAF) in the stroma which affect the prognosis of the patient.

EXTRA NODAL EXTENSION

In this study, among patients with strong staining alpha SMA score, Lymph node status was positively seen in 48.2% of cases. The association between tumor size and stromal alpha SMA score was found to be statistically not significant.

STAGE

In this study, among patients with strong staining alpha SMA score, Stage I tumor was seen in 7.1% of patients, Stage II was seen in 50% of patients, Stage III was seen in 41.1% of patients, and Stage IV was seen in 1.8% of patients.

The association between tumor size and stromal alpha SMA score was statistically significant. In Yamashita M et al.,[55] 36% belonged to stage 1, 40% to stage 2, and 24% to stage 3 in high alpha SMA expression. 48.5% belonged to stage 1, 40% to stage 2, and 11.4% to stage 3 in low alpha SMA expression with a p-value of 0.38.

NPI IN BREAST CARCINOMA

In this study, among patients with strong staining alpha SMA score, the Five-year survival rate was 96% in 21.4% of patients, 93% in 26.8% of patients, 78% in 30.4% patients, and 44% in 21.4% of patients. The association between tumor size and stromal alpha SMA score was found to be statistically not significant.

ER

In this study, among patients with strong staining alpha SMA score, ER was positive in 57.1% of patients. The association between tumor size and stromal alpha SMA score was found to be statistically significant. In Yamashita M et al.,[55] ER was positive in 65% of the non-metastasis group and 35% in the metastasis group. ER was positive in 64% of patients with high alpha SMA expression, and ER was positive in 62.8% of patients with low alpha SMA expression with a p-value of 0.34.

PR

In this study, among patients with strong staining alpha SMA scores, PR was positive in 57.1% of patients. The association between tumor size and stromal alpha SMA score was statistically significant.

Her2neu

In this study, among patients with strong staining alpha SMA score, Her 2 neu was positive in 26.8% of patients. The association between tumor size and stromal alpha SMA score was found to be statistically not significant. In Yamashita M et al.,[55] Her 2 was positive in 20% of the non-metastasis group and 36% in the metastasis group. Her 2 was positive in 24% of patients in high alpha SMA expression, and Her 2 was positive in 22.8% of patients in low alpha SMA expression with a p-value of 0.91.

Ki67

In this study, among patients with strong staining alpha SMA score, Ki67 was <14% in 50% and >14% in 50% of patients. The association between tumor size and stromal alpha SMA score was found to be statistically not significant.

Molecular typing

In this study, among patients with strong staining alpha SMA score, Molecular typing showed HER+ in 8.9% of patients, Luminal A in 39.3% of patients, Luminal B in 21.4% of patients, and TNBC in 30.4% of patients. The association between tumor size and stromal alpha SMA score was statistically significant.

In Singh A et al., [36] IHC assays performed on tumor samples confirmed that alpha SMA protein localizes, particularly in adjacent stromal cells, mainly comprised of the stromal myofibroblast.

In Tamiolakis D et al., [59]**,** Myoepithelial differentiation was determined by alpha-smooth muscle actin (alpha-SMA) expression. They concluded that IDCs with diffuse fibrosis is associated with a myoepithelial immunophenotype of carcinoma cells.

If the expression of alpha-SMA had been quantified, these patients would have been regarded as high-risk patients and, therefore, might have received more intensive therapy. The myofibroblast represents a good candidate for such a prognostic factor. Moreover, modulating myofibroblast behaviour may open up a new area for targeted therapy.

CONCLUSION

CONCLUSION

Myofibroblasts can be an important prognostic factor for a poor clinical prognosis as stromal alpha smooth muscle expression is correlated with grade and triple negative breast cancer (TNBC). Thus, patients with a high level of myofibroblasts (or their marker, alpha-SMA) should be considered for more aggressive treatments and more frequent monitoring for the development of metastatic disease.

SUMMARY

SUMMARY

- According to the stromal alpha SMA score, staining was not seen in 3% of patients, weak staining was seen in 9% of patients, moderate staining was seen in 32% of patients, and strong staining was seen in 56% of patients.
- Most of the patients belonged to the age group of 51 to 60 years, followed by 41 to 50 years & 28 to 40 years. The association between age and stromal alpha SMA score was found to be statistically not significant.
- Tumor size was less than 2 centimetres in 9% of patients, 2 to 5 centimetres in 62% of patients, and more than 5 centimetres in 29% of patients. The association between tumor size and stromal alpha SMA score was found to be statistically not significant.
- Grade I tumor was seen in 58% of patients, grade II was seen in 30% of patients, and grade III was seen in 12% of patients. The association between tumor grade and stromal alpha SMA score was statistically significant.
- The extranodal extension was present in 11% of patients. The association between Extra nodal extension and stromal alpha SMA score was found to be statistically not significant.
- Stage I tumor was seen in 7% of patients, Stage II was seen in 50% of patients, Stage III was seen in 42% of patients, and Stage IV was

seen in 1% of patients. The association between tumor stage and stromal alpha SMA score was statistically significant.

- The five-year survival rate was 96% in 15% of patients, 93% in 36%, 78% in 32%, and 44% in 17% of patients.
- ER was positive in 54% of patients, PR was positive in 52%, and Her 2 neu was positive in 30% of patients. The association between ER, PR and stromal alpha SMA score was found to be statistically significant.
- Ki67 was <14 % in 47% of patients and > 14% in 53%. The association between Ki67 and stromal alpha SMA score was found to be statistically not significant.
- Molecular typing showed Her2 positive in 12% of patients, Luminal A in 38% of patients, Luminal B in 20% of patients, and TNBC in 30% of patients. The association between Molecular typing and stromal alpha SMA score was statistically significant.

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ANNEXURES

ANNEXURE - I
INFORMED CONSENT FORM

STUDY TITLE: Immunohistochemical Expression of Alpha Smooth Muscle Actin in Stroma of Infiltrating Ductal Carcinoma of Breast and Its Correlation with Histopathological and Hormonal Factors- A Laboratory Observational Study.

I,_____have read or have been read to me the patient information sheet and understand the purpose of the study, the procedure that will be used, the 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.

Name and signature / thumb impression (subject) Date: Place:

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

ANNEXURE –II

PATIENT INFORMATION SHEET

STUDY TITLE: Immunohistochemical Expression of Alpha Smooth Muscle Actin in Stroma of Infiltrating Ductal Carcinoma of Breast and Its Correlation with Histopathological and Hormonal Factors- A Laboratory Observational Study.

PLACE OF STUDY: Sri Devaraj Urs Medical College attached to R.L Jalappa Hospital and Research Centre, Tamaka, Kolar.

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

All the cost incurred for collection of data, Performing the immunohistochemistry tests, analysis, printing publication will be borne by the post graduate student (Dr Vajja Nagaraju)

For any clarification you are free to contact the investigator.

PRINCIPAL INVESTIGATOR: Dr. Vajja Nagaraju, Mobile No: +91981165388

ANNEXURE III

PATIENT PROFORMA:

Name :	Hospital Number:	Age :

- Anonymised Sample No:
- Chief complaint :
- History of presenting illness :
- Past history :
- Family History:
- Menstrual history :
- Clinical diagnosis with TNM staging :
- Type of surgery:
- Side: Right / Left Biopsy Number:

GROSS FEATURES:

- Specimen Size: Skin: Tumor proper measurement:
- Histopathological diagnosis:
- Histological Grading:

- **MICROSCOPY :**
- Extent of tumor : Skin
  Nipple:
  Skeletal Muscle:
- **Lymph-Vascular Invasion:**
- **Pathologic Staging (pTNM):**
  Primary Tumor (Invasive Carcinoma)-
  Regional Lymph Nodes -
  Distant Metastasis (M) -
- Modified Bloom –Richardson grading : _____
- ER scoring : _____
- PR scoring : _____
- Her 2 neu scoring : _____
- Ki 67 : _____
- Alpha smooth muscle actin scoring: _____

KEY TO MASTER CHART

T – T staging according to 8th TNM Staging of breast carcinoma.

N – N staging according to 8th TNM Staging of breast carcinoma.

M – M staging according to 8th TNM Staging of breast carcinoma.

P – Positive lymph node status.

N –Negative lymph node status.

NPI – Nottingham Prognostic Index.

IDC – Infiltrating Ductal Carcinoma of breast.

LVI-Lymphovascular invasion.

EN-Extra nodal extension.

ER – Estrogen Receptor protein.

PR -Progesterone Receptor protein.

Her2 neu – Human Epidermal Growth Factor Receptor 2 neu protein.

Alpha-SMA stromal score- Alpha smooth muscle actin stromal expression score.

Neg –Negative.

TNBC – Triple Negative Breast carcinoma.

Her2+ - Her 2 enriched.

Hosp No – 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	Diagnosis	Proforma	Blocks collect	Blocks cut	Slides	Tumor proper	ER	PR	Her 2 neu	Ki67	Molecular typing	STROMAL ALPHA SMA SCORE
1	2018	1536	45	588294	4.5	I	0	0	3	x	x	IIB	2.8	IDC	Yes	Yes	Yes	Yes	A,B	5+	7+	Neg	<14 %	Luminal A	2
2	2018	1917	59	601433	4.5	I	1 of 12	0	1	1a	x	IIIA	2.8	IDC	Yes	Yes	Yes	Yes	G,H	Neg	Neg	Neg	>14%	TNBC	2
3	2018	1918	65	608018	3.8	I	0	0	2	0	x	IIA	2.8	IDC	Yes	Yes	Yes	Yes	H,J	7	6	1	<14 %	Luminal B	3
4	2018	1972	42	612034	10	I	2 of 16	0	3	3a	x	IIIC	4.2	IDC	Yes	Yes	Yes	Yes	A,B	Neg	Neg	Neg	>14%	TNBC	3
5	2018	1987	50	611791	12	I	3 of 10	0	3	1a	x	IIIA	5.4	IDC	Yes	Yes	Yes	Yes	F,G,H,J	Neg	Neg	Neg	>14%	TNBC	2
6	2018	2028	55	609669	3	I	0 of 10	0	2	0	x	IIA	2.6	IDC	Yes	Yes	Yes	Yes	A,B	5	Neg	Neg	<14%	Luminal A	1
7	2018	2086	37	618289	4	I	1 of 23	0	2	1	x	IIB	2.8	IDC	Yes	Yes	Yes	Yes	A,B	Neg	Neg	Neg	>14%	TNBC	1
8	2018	2101	60	616799	3	I	0 of 4	0	2	0	x	IIA	2.6	IDC	Yes	Yes	Yes	Yes	F,G	Neg	Neg	3	>14%	HER2+	2
9	2018	2334	60	603691	4.5	II	2 of 8	0	4	1	x	IIIB	3	IDC	Yes	Yes	Yes	Yes	J,K	Neg	Neg	Neg	<14%	TNBC	2
10	2018	2373	41	600105	4	I	1 of 4	1	4a	1	x	IIIB	2.8	IDC	Yes	Yes	Yes	Yes	E,F,G,H,L	7	7	Neg	>14%	Luminal B	2
11	2018	2390	58	635438	4	I	0 of 5	0	4	0	x	IIIB	2.8	IDC	Yes	Yes	Yes	Yes	A,B,C,D	5	Neg	Neg	<14%	Luminal A	3
12	2018	2431	55	630252	6	II	10 of 13	1	4b	3	x	IIIC	6.2	IDC	Yes	Yes	Yes	Yes	A,B,C	Neg	Neg	Neg	>14%	TNBC	3
13	2018	2695	70	642200	3	I	0 of 11	0	2	0	x	IIA	2.6	IDC	Yes	Yes	Yes	Yes	A,B	5	Neg	Neg	<14%	Luminal A	3
14	2018	2732	28	647618	3.5	II	7 of 10	0	2	2a	x	IIIA	4.8	IDC	Yes	Yes	Yes	Yes	A,B	Neg	Neg	Neg	>14%	TNBC	3
15	2018	2741	45	608809	5.5	I	5 of 5	0	3	2a	x	IIIA	4.2	IDC	Yes	Yes	Yes	Yes	A,B	Neg	Neg	Neg	>14%	TNBC	0
16	2018	2804	36	650777	3.5	I	1 of 12	0	2	1	x	IIA	2.8	IDC	Yes	Yes	Yes	Yes	A,B	3	3	Neg	<14 %	Luminal A	2
17	2018	2831	38	650825	3	I	0 of 13	0	2	0	x	IIA	2.6	IDC	Yes	Yes	Yes	Yes	A,B,C	7	6	1	<14 %	Luminal B	2
18	2019	31	48	664790	8	II	11/15	4	3	3	x	III	6.6	IDC	Yes	Yes	Yes	Yes	F,G,H	Neg	Neg	Neg	>14%	TNBC	3
19	2019	347	55	678651	4	I	6/13	6	2	2	x	III	4.8	IDC	Yes	Yes	Yes	Yes	G,H,J	Neg	Neg	Neg	>14%	TNBC	2
20	2019	369	82	681338	3	I	0/9	0	2	0	x	II	2.5	IDC	Yes	Yes	Yes	Yes	A,B	3	3	Neg	<14 %	Luminal A	3
21	2019	371	58	681638	4	II	1/14	0	2	1	x	II	4.8	IDC	Yes	Yes	Yes	Yes	B,C,D	Neg	Neg	Neg	<14 %	TNBC	3
22	2019	386	80	682643	2	II	25/25	0	1	3	x	III	5.4	IDC	Yes	Yes	Yes	Yes	A,B	5+	7+	Neg	<14 %	Luminal A	3
23	2019	550	49	694955	12	III	0/13	0	4	0	x	III	4	IDC	Yes	Yes	Yes	Yes	J,K,L	Neg	Neg	3+	>14%	Luminal B	1
24	2019	641	45	690144	3	I	0/6	0	2	0	x	II	4	IDC	Yes	Yes	Yes	Yes	H,J,K,L	5	7	Neg	<14 %	Luminal A	3
25	2019	921	44	706401	2.5	I	0/5	0	2	0	x	II	2.5	IDC	Yes	Yes	Yes	Yes	V,W,X/F,	8	7	Neg	<14 %	Luminal A	2
26	2019	984	53	708238	3.2	I	0/13	0	2	0	x	II	2.64	IDC	Yes	Yes	Yes	Yes	F,G	6	Neg	Neg	<14 %	Luminal A	1
27	2019	1108	52	407606	3.5	I	0/4	0	2	0	x	II	2.7	IDC	Yes	Yes	Yes	Yes	G	7	6	Neg	<14 %	Luminal A	2
28	2019	1252	52	717035	2.2	II	1/5	0	2	1	x	II	3.44	IDC	Yes	Yes	Yes	Yes	F,G	3	3	Neg	<14 %	Luminal A	2
29	2019	1373	75	726027	4	I	1/1	0	2	1	x	II	2.8	IDC	Yes	Yes	Yes	Yes	G,H	7	6	Neg	<14 %	Luminal A	2
30	2019	1392	40	728557	4	III	1/5	0	2	1	x	II	4.8	IDC	Yes	Yes	Yes	Yes	F,G,H	Neg	Neg	3	<14 %	HER2+	3
31	2019	1410	75	726902	6	III	0/6	0	3	0	x	II	5.2	IDC	Yes	Yes	Yes	Yes	F,G	10	7	3	>14%	Luminal B	2
32	2019	1454	50	713964	3.5	I	0/14	0	2	0	x	II	2.7	IDC	Yes	Yes	Yes	Yes	G,H	Neg	Neg	Neg	>14%	TNBC	2
33	2019	1490	55	730817	3	II	21/25	0	2	3	x	III	5.6	IDC	Yes	Yes	Yes	Yes	F,G	Neg	Neg	Neg	>14%	TNBC	3
34	2019	1599	80	736905	9	III	1/7	0	3	1	x	III	6.8	IDC	Yes	Yes	Yes	Yes	G,J,K	6	6	Neg	<14 %	Luminal A	3
35	2019	1643	53	735987	2.5	III	1/5	0	2	1	x	II	5.5	IDC	Yes	Yes	Yes	Yes	F,G	6	5	Neg	>14%	Luminal B	3
36	2019	1744	40	734649	9	II	0/6	0	3	0	x	II	4.8	IDC	Yes	Yes	Yes	Yes	F,G	Neg	Neg	Neg	>14%	TNBC	1
37	2019	1745	60	740099	4	II	0/9	0	2	0	x	II	2.8	IDC	Yes	Yes	Yes	Yes	F,G	Neg	Neg	Neg	>14%	TNBC	3
38	2019	2257	41	761094	2.2	III	7/10	0	2	2a	x	III	6.4	IDC	Yes	Yes	Yes	Yes	G,H	5	4	3	<14 %	Luminal A	2
39	2019	2275	55	684653	1.5	I	0/3	0	1	0	x	I	2.3	IDC	Yes	Yes	Yes	Yes	H,J	8	4	3	>14%	Luminal B	3
40	2019	2390	54	767363	7	II	14/20	0	3	3	x	III	6.4	IDC	Yes	Yes	Yes	Yes	F,G,H	Neg	Neg	Neg	>14%	TNBC	3
41	2019	2476	36	742083	13	II	4/7	1	4	2	x	III	8	IDC	Yes	Yes	Yes	Yes	J,K	Neg	Neg	Neg	<14 %	TNBC	3
42	2019	2523	33	757437	4	II	4/5	0	2	2	x	III	6.8	IDC	Yes	Yes	Yes	Yes	F,G	3	2	2	>14%	Luminal B	3
43	2019	2549	55	746844	11	III	4/11	0	3	2	x	III	8.2	IDC	Yes	Yes	Yes	Yes	A,B	Neg	Neg	2	<14 %	HER2+	1
44	2019	2726	38	759213	2	I	0/4	0	2	1	x	II	2.4	IDC	Yes	Yes	Yes	Yes	F,G	Neg	Neg	3	>14%	HER2+	3
45	2019	2830	42	786531	5	I	2/13	0	3	2	x	I	4	IDC	Yes	Yes	Yes	Yes	E,F	6	6	2	>14%	Luminal B	3
46	2019	2905	60	732092	1.2	II	1/6	1	4	1	x	III	3.2	IDC	Yes	Yes	Yes	Yes	J	Neg	Neg	3	<14 %	HER2+	3
47	2019	2972/397/20	42	797271	3	I	0	0	2	0	x	I	2.6	IDC	Yes	Yes	Yes	Yes	F,G	4	4	Neg	<14 %	Luminal A	2
48	2020	228	33	812928	7	I	0/9	0	3	0	x	III	5.4	IDC	Yes	Yes	Yes	Yes	E,F	Neg	Neg	Neg	>14%	TNBC	2
49	2020	397	42	797271	3	I	0/9	0	2	0	x	I	1.6	IDC	Yes	Yes	Yes	Yes	N	4	4	Neg	<14 %	Luminal A	2
50	2020	480	65	825541	2.4	II	0/12	0	2	0	x	II	3.4	IDC	Yes	Yes	Yes	Yes	F,G	4	6	2	<14 %	Luminal A	3
51	2020	621/624	53	815423	4	II	9/15	0	1	2	x	II	5.8	IDC	Yes	Yes	Yes	Yes	J,K,L	Neg	Neg	3	>14%	HER2+	0
52	2020	889/1260	60	845877	6.5	II	25/25	0	4	3	x	III	6.3	IDC	Yes	Yes	Yes	Yes	P	Neg	Neg	Neg	>14%	TNBC	2
53	2020	910	70	844590	2.5	I	6/15	6	2	3	x	II	4.5	IDC	Yes	Yes	Yes	Yes	M,N	8	5	Neg	>14%	Luminal B	3
54	2020	914	65	837500	4.5	I	0/8	0	2	0	x	II	1.6	IDC	Yes	Yes	Yes	Yes	F,G,S	Neg	4	Neg	<14 %	Luminal A	3
55	2020	935	65	845622	4	I	7/9	0	4	2	x	III	4.8	IDC	Yes	Yes	Yes	Yes	R,S	6	5	Neg	>14%	Luminal A	3
56	2020	1002	39	820441	4.5	I	0/35	0	2	0	x	II	1.9	IDC	Yes	Yes	Yes	Yes	K,L	6	5	Neg	>14%	Luminal B	3
57	2020	1044	56	839107	2	I	3/9	0	2	1	x	II	3.4	IDC	Yes	Yes	Yes	Yes	A,B	Neg	Neg	Neg	>14%	TNBC	2
58	2020	1354	59	857344	3.5	I	0/11	0	2	0	x	II	2.7	IDC	Yes	Yes	Yes	Yes	A,B	6	7	Neg	<14 %	Luminal A	3
59	2020	1407	72	863435	3.5	II	0/13	0	2	0	x	II	2.7	IDC	Yes	Yes	Yes	Yes	L,M,Q,R	Neg	Neg	Neg	<14 %	TNBC	3
60	2020	1445	31	852475	3	II	1/9	0	2	1	x	II	3.6	IDC	Yes	Yes	Yes	Yes	F,G	Neg	7	3	>14%	Luminal B	3
61	2020	1462	73	865603	3	I	0/15	0	2	0	x	II	1.6	IDC	Yes	Yes	Yes	Yes	C,D	6	5	Neg	<14 %	Luminal A	3
62	2020	1476	60	865757	3.3	I	7/9	0	2	1	x	III	4.6	IDC	Yes	Yes	Yes	Yes	B,C	6	5	Neg	<14 %	Luminal A	2
63	2020	1648	45	873038	2.5	II	0	0	2	0	x	II	1.5	IDC	Yes	Yes	Yes	Yes	F	6	7	Neg	<14 %	Luminal A	3
64	2020	1878	36	878950	1.5	I	0	0	2	0	x	I	1.4	IDC	Yes	Yes	Yes	Yes	A,B,C	6	7	Neg	<14 %	Luminal A	1
65	2020	1913	67	879823	6.5	II	0/1	0	3	1	x	III	5.2	IDC	Yes	Yes	Yes	Yes	P,Q	Neg	Neg	2	<14 %	HER2+	3
66	2020	1920	45	882814	8	III	6/22	0	3	1	x	III	3.9	IDC	Yes	Yes	Yes	Yes	B,C	6	7	2	>14%	Luminal B	3
67	2020	2017	50	876387	4.5	II	0/11	0	3	0	x	II	2.89	IDC	Yes	Yes	Yes	Yes	A,B,C	6	7	5	>14%	Luminal B	2
68	2021	33	76	885577	6	II	0/11	0	3	0	x	II	4.2	IDC	Yes	Yes	Yes	Yes	C,H,U	Neg	Neg	Neg	>14%	TNBC	2
69	2021	256	48	892991	7	III	2/33	2	4	1	x	III	6.6	IDC	Yes	Yes	Yes	Yes	F,G	6	7	Neg	<14 %	Luminal A	3
70	2021	410	35	897214	4	I	5/12	0	3	2	x	III	4.8	IDC	Yes	Yes	Yes	Yes	J,K	6	7	5	>14%	Luminal B	2
71	2021	544	37	905154	9	I	0/8	0	2	0	x	IIB	3.8	IDC	Yes	Yes	Yes	Yes	F	Neg	Neg	3	>14%	HER2+	1
72	2021	582	57	903629	4	II	0/10	0	2	0	x	IIA	3.8	IDC	Yes	Yes	Yes	Yes	G	Neg	Neg	Neg	>14%	TNBC	0
73	2021	893	50	923478	1.7	I	0/4	0	1	0	X	II	2.24	IDC	Yes	Yes	Yes	Yes	F	Neg	Neg	Neg	>14%	TNBC	3
74	2021	910	52	923327	3	I	0/16	0	2	0	X	IIA	2.6	IDC	Yes	Yes	Yes	Yes	B	Neg	Neg	Neg	>14%	TNBC	3
75	2021	1408	71	938765	6.2	I	0/25	0	3	0	X	IIB	2.24	IDC	Yes	Yes	Yes	Yes	F	7	6	Neg	<14%	Luminal A	3
76	2021	1452	53	933465	3.5	I	0/7	0	2	0	X	IIA	2.62	IDC	Yes	Yes	Yes	Yes	S	5	Neg	Neg	>14%	Luminal B	2
77	2021	1477	54	936249	10.5	I	2 OF 10	0	2	1	X	IIIA	3.8	IDC	Yes	Yes	Yes	Yes	F	5	5	Neg	>14%	Luminal B	3
78	2021	1540	70	940132	2.5	I	1 OF 10	0	2	1	X	IIIA	3.5	IDC	Yes	Yes	Yes	Yes	C	7	3	Neg	<14%	Luminal A	3
79	2021	1570	54	936249	5	I	13/21	0	3	3A	X	IIIC	6	IDC	Yes	Yes	Yes	Yes	J	Neg	Neg	2	>14%	HER2+	2
80	2021	1586	51	941346	1.5	III	0/10	0	1C	0	X	IA	2.3	IDC	Yes	Yes	Yes	Yes	C	Neg	Neg	Neg	>14%	TNBC	3
81	2021	1678	65	946403	3.5	I	0/4	0	2	0	X	IIA	2.7	IDC	Yes	Yes	Yes	Yes	G	Neg	Neg	3	>14%	HER2+	3
82	2021	1705	65	945963	6	II	1 OF 19	1	4B	1A	X	IIIB	3.3	IDC	Yes	Yes	Yes	Yes	L	Neg	Neg	3	>14%	HER2+	1
83	2021	1852	42	39485	3.5	I	5 OF 21	0	3	1	X	IIIA	4.7	IDC	Yes	Yes	Yes	Yes	G	6	6	3	<14%	Luminal A	2
84	2021	1970	65	39318	14	I	18/21	3	4C	3C	M1	IV	11	IDC	Yes	Yes	Yes	Yes	E	6	2	Neg	>14%	Luminal B	3
85	2021	2155	55	39217	8	I	0/ 10	0	2	0	X	IIB	3.6	IDC	Yes	Yes	Yes	Yes	N	5	5	Neg	<14%	Luminal A	3
86	2022	165	67	58769	5	I	1 OF 17	0	2	0	X	IIA	2.02	IDC	Yes	Yes	Yes	Yes	F	8	7	Neg	<14%	Luminal A	3
87	2022	319	58	62864	3.8	II	0/14	0	2	0	X	IIA	2.6	IDC	Yes	Yes	Yes	Yes	F	6	5	Neg	<14 %	Luminal A	3
88	2022	493	41	65320	3	I	0/11	0	2	0	X	IIIA	2.6	IDC	Yes	Yes	Yes	Yes	G	7	7	Neg	<14%	Luminal A	3
89	2022	618	48	67214	3	I	0/5	0	2	0	X	IIA	2.6	IDC	Yes	Yes	Yes	Yes	A14	Neg	Neg	Neg	>14%	TNBC	3
90	2022	717	37	71915	4.5	I	0/5	0	2	0	X	IIA	1.9	IDC	Yes	Yes	Yes	Yes	F	Neg	4	3	>14%	Luminal B	2
91	2022	857	39	75439	9	I	0/6	0	1	0	X	IA	2.8	IDC	Yes	Yes	Yes	Yes	F	7	6	Neg	<14%	Luminal A	3
92	2022	876	75	77136	5	I	1 OF 13	0	2	1a	X	IIIA	3	IDC	Yes	Yes	Yes	Yes	E,G	7	6	Neg	<14%	Luminal A	2
93	2022	955	38	34745	3.5	II	0/24	0	2	0	X	IIA	4.7	IDC	Yes	Yes	Yes	Yes	F	6	6	3	<14%	Luminal A	3
94	2022	993	52	75084	1.8	II	0/10	0	4	0	X	III	2.36	IDC	Yes	Yes	Yes	Yes	N,P	Neg	Neg	Neg	>14%	TNBC	3
95	2022	1084	54	55208	6.5	III	5 OF 22	1	3	3	X	IIIC	6.3	IDC	Yes	Yes	Yes	Yes	H,J	Neg	Neg	Neg	>14%	TNBC	3
96	2022	1097	68	81315	7	I	7 OF19	0	3	2a	X	IIIA	4.4	IDC	Yes	Yes	Yes	Yes	F,G	6	7	Neg	<14 %	Luminal A	2
97	2022	1167	30	83399	6	I	0/6	0	3	0	X	IIB	2.1	IDC	Yes	Yes	Yes	Yes	J	6	7	5	>14%	Luminal B	3
98	2022	1817	40	114769	2.8	II	3//41	0	3	2a	X	IIIA	4.56	IDC	Yes	Yes	Yes	Yes	G	Neg	Neg	3	>14%	HER2+	2
99	2022	2204	74	130798	6	II	17/21	0	4b	2a	X	IIIB	6.2	IDC	Yes	Yes	Yes	Yes	F,G	6	6	3	<14%	Luminal A	3
100	2022	2214	48	131764	4	III	1 OF 13	0	3	3	X	IIIC	4.8	IDC	Yes	Yes	Yes	Yes	E,F	Neg	Neg	Neg	>14%	TNBC	3