"SIGNIFICANCE OF MAST CELL DENSITY AND MICROVESSEL DENSITY IN BREAST CARCINOMA"

By

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DOCTOR OF MEDICINE

IN

PATHOLOGY

Under the guidance of

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Dr. DIVYARANI M.N.

LIST OF ABBREVIATIONS

MVD : Microvessel Density

MCD : Mast cell Density

CD - Cluster of differentiation

TNF α-Tumor Necrosis Factor Alpha

IL-1 –Interleukin 1

IL-6 –Interleukin 6

IL -4 – Interleukin 4

H and E – Hematoxyllin and Eosin

IHC – Immunohistochemistry

HRP – Horse Radish Peroxidase

DAB – Di-amino Benzidine

DPX – Dibutyl Phthalate Xylene

CA - Carcinoma

SL No –Serial number

VEGF – Vascular Endothelial Growth Factor

PDEGF – Platelet Derived Endothelial Growth Factor

BFGF – Basic Fibroblast Growth factor

TDLU – Terminal Duct Lobular Unit

WHO –World Health Organization

NOS –Not otherwise specified

ER – Estrogen Receptor

PR – Progesterone Receptor

FGF –Fibroblast Growth Factor

IDC – Infiltrating Ductal Carcinoma

ILC – Infiltrating Lobular Carcinoma

LCIS –Lobular carcinoma in situ

DCIS –Ductal carcinoma in situ

PASH – Pseudo angiomatous stromal hyperplasia

EPC – Endothelial precursor cells

SCF –Stem cell factor

PAR 1 & 2- Protease activated receptors

SD – Standard deviation

EDTA – Ethylene diamine tetra acetic acid

TBS –Tris buffer solution

UO – Upper outer

UI – Upper inner

LO –Lower outer

LI – Lower inner

ABSTRACT

Background:

Angiogenesis is a highly regulated process balanced by inhibitors and stimulators of endothelial cell proliferation and endothelial cell migration. Breast cancer remains a leading cause of death in women of reproductive age group. Angiogenesis in breast carcinoma has been extensively studied and proven to play an important role in tumor outcome and patient prognosis. The prognostic relevance of angiogenesis expressed as microvessel density has been reported in various neoplasms. Inflammatory infiltrates like mast cells are recruited and activated by tumor cells. This study has been undertaken to know the role of mast cells and microvessels in breast carcinoma.

Objective of the study:

1) To study the Intratumoral and Peritumoral Micro Vessel Density (MVD) and Mast Cell Density (MCD) in breast carcinoma

2) To Correlate Intratumoral and Peritumoral Microvessel Density (MVD) and Mast Cell Density (MCD) with histological type and grade in breast carcinoma

Methods:

A total of 55 cases were studied from January 2012 to July 2013. All H & E sections were examined and reviewed for histopathological type and grading. 55 cases of breast carcinoma were immunohistochemically stained with endothelial antigen, CD 34 and counterstained by 0.1% toluidine blue to highlight mast cells.

The microvessels were counted in three hotspot area as described by weidner et al and mast cells were counted in the vicinity of microvessels at 200 magnification

Results:

In the present study, out of 55 cases, 54 were females and one was male patient and age ranged from 35 to 85 years. Majority of our cases were infiltrating ductal carcinoma and most of the tumors were in Grade 2.

Intratumoral and peritumoral mean MVD was highest (132 ±18.7 and 48.50 ±14.95) in Grade III tumors respectively and intratumoral and peritumoral mean MCD was highest (3.27±2 and 16.64±11.12) in Grade 1 tumors respectively.

Intratumoral and peritumoral mean MCD was highest (3.13±2.91and 16.62±10.15) in Infiltrating ductal Carcinoma respectively and intratumoral and peritumoral mean MVD was highest (89.71±29.83 and 38.43±17.70) respectively in other tumors (medullary, papillary and metaplastic Carcinoma).

Conclusion:

In the present study, intratumoral and peritumoral mean MVD was highest in grade 3 tumors suggesting that intensity of tumor angiogenesis correlates with tumor grade and intratumoral and peritumoral mean MCD was highest in grade 1(low grade) tumors suggesting the protective effect of mast cells which exerts a cytotoxic effect on the tumor cells.

Key words: Breast carcinoma, MVD, MCD

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INTRODUCTION

Breast (mammary gland) consists of both epithelial and stromal elements giving rise to various neoplastic and non-neoplastic lesions. It is under constant hormonal influence and undergoes various changes during puberty, lactation and menopause.

Breast cancer is the most frequent occurring neoplasm in women worldwide, causing death in women between 35 and 55 years of age. Despite various screening modalities, diagnosis and treatment employed, nearly all women die from the disease each year.[1]

Worldwide incidence of breast cancer comprises of 10.4% of all cancer incidence among women. The estimated number of new cancers in India per year is about 7 lakhs and over 3.5 lakh people die of cancer each year.[2] In India breast cancer is one among the top three cancers. The incidence of breast cancer in Kolar district is around 6.41%.[3]

There are various prognostic factors which predicts the tumor behavior and survival rate in a patient with breast cancer like tumor size, histopathological grade, tumor type, axillary lymphnode status, vascular invasion and estrogen receptor status. Among all these, lymphnode status remains important. Hence these prognostic factors should be identified for benefit from adjuvant chemotherapy and predict better survival.[4,5]

Along with these factors, “Tumor Angiogenesis” (Neo Vascularization) is well established in the literature in various neoplasms. For the tumor to grow and proliferate, tumor cells require nutrition which is provided by angiogenesis along with growth factors like VEGF (vascular endothelial growth factors), PDEGF (platelet derived endothelial cell growth factor) and BFGF (basic fibroblast growth factor) which are produced by tumor and stromal cells. [1]

Most commonly used technique to assess the microvessel density is by quantifying the intratumoral angiogenesis in the tumor. For >10years, Micro Vessel Density (MVD) has been used as a surrogate marker for tumor angiogenesis.[1] Blood microvessels can be highlighted using various endothelial immunohistochemical stains like factor VIII, CD 31, CD34 and rarely CD105 which was used by Weidner.[6]

Neoplastic transformation evokes an immune response which causes inflammatory cell infiltrates like mast cells and macrophages along with fibroblast to accumulate in peritumoral and intratumoral areas. The inflammatory cells are recruited and activated by tumor cells.[7] In various studies, mast cell infiltration has been implicated in tumor growth and proliferation by various growth factors. [8, 9, 10, 11] They are also rich in metalloproteases that contribute to majority of the proteolytic components necessary for tumor invasiveness. [12]

With introduction and advancement of various techniques available for the management of breast cancer, knowledge of the factors that may influence tumor behavior and disease course have become increasingly important in assigning patients for different treatment modalities. [7]

Distinct biological features expressed by tumor associated vasculature may serve as potential prognostic markers of disease progression as well as novel targets for therapeutic intervention.[13]

Hence the study has been undertaken to see whether there is correlation between Mast cell density and Micro vessel density with histological type and grade in breast carcinomas.

OBJECTIVES

1) To study the Intratumoral and Peritumoral Micro Vessel Density (MVD) and Mast Cell Density (MCD) in breast carcinoma

2) To Correlate Intratumoral and Peritumoral MicroVessel Density (MVD) and Mast Cell Density(MCD) with histological type and grade in breast carcinoma

REVIEW OF LITERATURE

HISTORY AND BACKGROUND

Edwin smith surgical papyrus (3000-2500B.C) is the earliest known document to refer to breast cancer. [14] Roman physician Aulus Cornelius Celsus noted that the breast of women were frequent sites of cancer and described breast cancer in his manuscript.[14]

Breast cancer is the most common malignancy in women of reproductive age group. Almost 1.4 million women are diagnosed with breast cancer worldwide and approximately 4.5 lakh deaths are recorded till date. Incidence rates are high in developed countries compared to developing countries (71.7/100,000 and 29.3/100,000 respectively) corresponding mortality rate were (17.1/100,000 and 11.8/100,000). [15]

STRUCTURAL ANATOMY OF BREAST

Breast is a modified apocrine gland which lies between the second and sixth ribs in the vertical axis and between the sternal edge to mid-axillary line in horizontal axis. The supero-lateral quadrant is prolonged to form axillary tail of Spence & may reach up to the apex of axilla. [16, 17,18]

The paired mammary glands rest on the pectoralis muscle on the upper chest wall and consists of about 15-25 independent glandular units called breast lobe or mammary lobes each, almost pyramidal in shape with an apex at the areola. [16, 17,18]

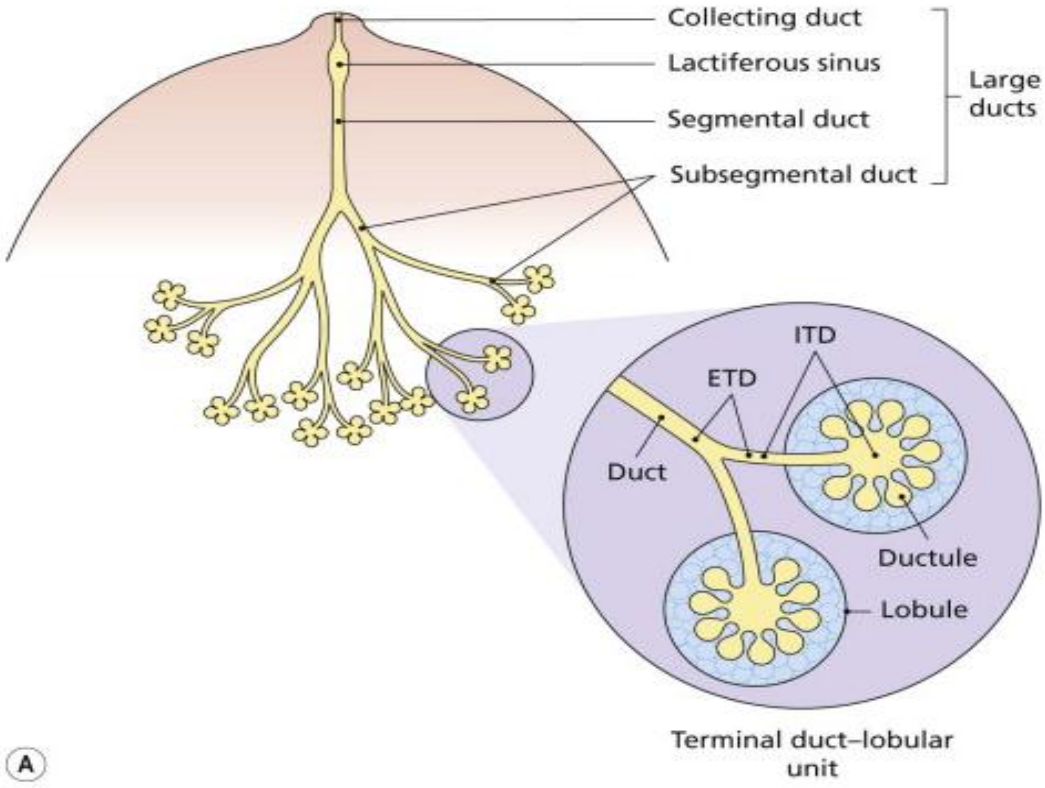


Figure 1 : Structural Anatomy of breast

Each of these lobes represents the morphofunctional unit of the organ consisting of compound tubuloacinar glands. These lobes are embedded in a mass of adipose tissue and are separated by connective tissue of breast. A single large duct,the lactiferous duct drains each lobe via a separate opening on the surface.[16, 17, 18][Figure 1]

Each breast lobe is sub-divided into a variable number of lobules. The lobule with a terminal duct is called “Terminal duct lobular unit”. The overlying skin is lined by keratinized squamous epithelium which continues into ducts and then changes into a double layered epithelium which rests on a continuous basement membrane. The luminal cuboidal or columnar cells which produce milk rest on the flat discontinuous myoepithelial cells which help in milk ejection. [16, 17, 18] [Figure 2]

The interlobular stroma consists of dense fibrous connective tissue with adipose tissue and the intralobular stroma consists of myxomatous stroma with scattered lymphocytes. Areola contains sebaceous glands which open via lactiferous ducts or directly on the surface. [16, 17]

Male breast: remains rudimentary throughout life. It is formed by small ducts without lobules or alveoli. [16, 17, 18]

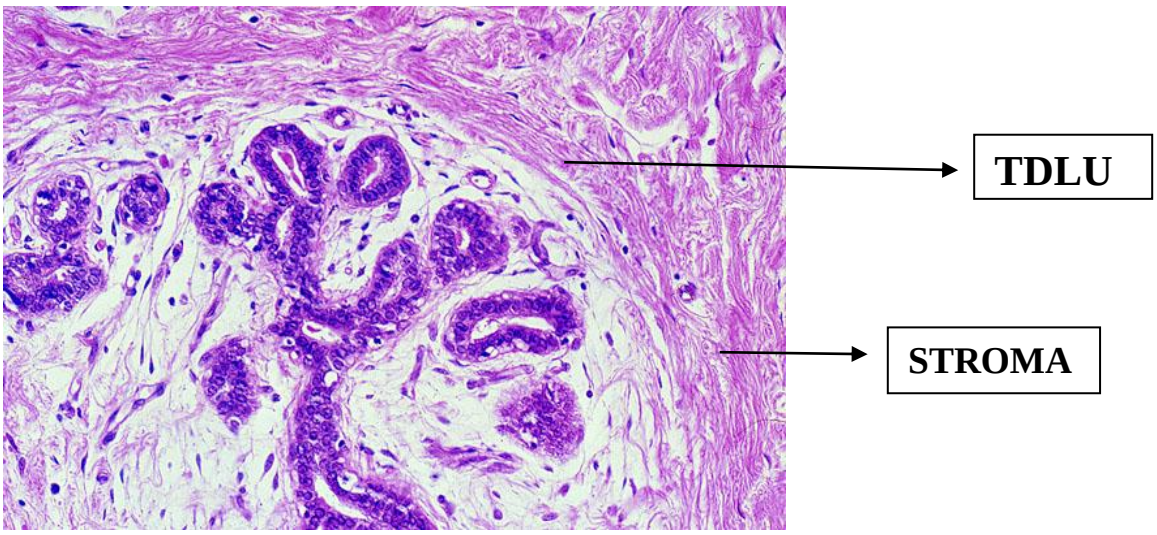


Figure 2 : - Normal Histology of Breast –TDLU(Terminal duct lobular unit)

RISK FACTORS [19]

1. **Inherited Breast cancer**

   Approximately 5 to 10 % of all breast cancers result from autosomal dominant inheritance of a mutated gene, the vast majority being sporadic. Several classes of genes have been identified which are mutated and accumulation of their products Proto-oncogenes, Tumor suppressor genes and mismatch repair genes.

2. **Inherited predisposition**

   BRCA 1
   BRCA 2 } **Tumor Suppressor Gene**

   BRCA 1: It is located on chromosome 17 - Ovary and Breast; It causes 50% to 85% increased in risk of carcinoma breast. Mutation in BRCA 1 is estimated to account for 4% to 5% of all breast cancers and 45 % of hereditary early onset cancer

   BRCA 2 : It is located on chromosome 13, associated with hereditary early onset breast cancer in families. Men with BRCA 2 mutations have increased risk of carcinoma breast.

3. **Familial**

   5-10% have hereditary predisposition to carcinoma breast

4. **Hormonal factors**

   Increased exposure to endogenous estrogen, early age of menarche, nulliparity or late age at menopause, postmenopausal women, obesity increases the risk for breast carcinoma.

5. **Life style and dietary factors**

   Fat intake, Alcohol consumption

6. **Benign breast disease**

   Atypical hyperplasia is associated with a greater risk of carcinomas.

THE WORLD HEALTH ORGANIZATION (WHO) [20]

CLASSIFICATION OF TUMORS OF THE BREAST (2003)

Epithelial Tumors

Invasive ductal carcinoma ,not otherwise specified (NOS)
Mixed type carcinoma
Pleomorphic carcinoma
Carcinoma with osteoclastic giant cells
Carcinoma with choriocarcinomatous features
Carcinoma with melanotic features
Invasive lobular carcinoma
Tubular carcinoma
Invasive cribriform carcinoma
Medullary carcinoma
Mucinous carcinoma and other tumors with abundant mucin
Mucinous carcinoma

Cystadenocarcinoma

Columnar cell mucinous carcinoma

Signet ring cell carcinoma

Neuroendocrine tumors

Solid neuroendocrine carcinoma

Atypical carcinoid tumor

Small cell/oat cell carcinoma

Large cell neuroendocrine carcinoma

Invasive papillary carcinoma

Invasive micropapillary carcinoma

Apocrine carcinoma

Metaplastic carcinoma

Pure epithelial metaplastic carcinoma

Squamous cell carcinoma

Adenocarcinoma with spindle cell metaplasia

Adenosquamous carcinoma

Mucoepidermoid carcinoma

Mixed epithelial/mesenchymal metaplastic carcinomas

Lipid-rich carcinoma

Secretory carcinoma

Oncocytic carcinoma

Adenoid cystic carcinoma

Acinic cell carcinoma

Glycogen-rich clear cell carcinoma

Sebaceous carcinoma

Inflammatory carcinoma

Precursor lesions

Lobular Neoplasia

Lobular carcinoma In situ (LCIS)

Intraductal proliferative lesions

Usual ductal hyperplasia

Flat epithelial hyperplasia

Atypical ductal hyperplasia

Ductal carcinoma insitu(DCIS)

Microinvasive carcinoma

Intraductal papillary neoplasms

Central papilloma

Peripheral papilloma

Atypical papilloma

Intraductal papillarycarcinoma

Intracystic papillary carcinoma

Benign epithelial proliferations

Adenosis , including variants

Sclerosing adenosis

Apocrine adenosis

Blunt duct adenosis

Microglandular adenosis

Adenomyoepithelial adenosis

Radial scar / complex sclerosing lesion

Adenomas

Tubular adenoma

Lactating adenoma

Apocrine adenoma

Pleomorphic adenoma

Ductal adenoma

Myoepithelial lesions

Myoepitheliosis

Adenomyoepithelial adenosis

Adenomyoepithelioma

Malignant myoepithelioma

Mesenchymal Tumors

Haemangioma

Angiomatosis

Hemangiopericytoma

Pseudoangiomatous stromal hyperplasia(PASH)

Myofibroblastoma

Fibromatosis(aggressive)

Inflammatory myofibroblastic tumor

Lipoma

Angiolipoma

Granular cell tumor

Neurofibroma

Schwannoma

Angiosarcoma

Liposarcoma

Rhabdomyosarcoma

Osteosarcoma

Leiomyoma

Leiomyosarcoma

Fibroepithelial tumors

Fibroadenoma

Phyllodes tumor

Benign

Borderline

Malignant

Periductal stromal sarcoma, low grade

Mammary hamartoma

Tumors of Nipple

Nipple adenoma

Syringomatous adenoma

Pagets disease of the nipple

Malignant lymphoma

Diffuse large B cell lymphoma

Burkitt lymphoma

Extranodal marginal zone B –cell lymphoma of MALT type

Follicular lymphoma

Metastatic tumor

Tumors of male breast

Gynecomastia

Carcinoma:

In situ

Invasive

HISTOPATHOLOGICAL TYPE

Invasive breast carcinomas are a group of malignant tumors of the breast which have the potential to invade into adjacent tissue and has tendency to metastasize to distant sites.They are classified into various histological types with Invasive ductal carcinoma being the most common type.[20]

INFILTRATING DUCTAL CARCINOMA

Infiltrating ductal carcinoma comprises the largest group of primary breast carcinoma. These tumors vary in size from 1 cm to 10cm, firm to hard in consistency.[20] Microscopically tumor cells are arranged in cords, clusters and trabecular pattern. Nuclei may be regular, uniform or highly pleomorphic with prominent nucleoli. Cytoplasm is often abundant and eosinophilic, focal necrosis can be seen.[18,20] Prognosis is slightly worse with 35 – 50 % of 10 year survival, when compared to breast carcinoma as a whole with a 55 % 10 year survival.[21] [Figure 5]

INVASIVE LOBULAR CARCINOMA

Clinically presents as a bilateral or multifocal lesion, most commonly seen around 50 years of age group. Infiltrating lobular carcinoma is characterized by the presence of small and uniform tumor cells which are arranged in single linear cords (Indian file pattern) that invade the stroma. Neoplastic cells have round to notched ovoid nuclei with a thin rim of cytoplasm with occasional intracytoplasmic lumen.[20] Prognosis for classical type of lobular carcinoma has a favorable outcome and poor prognosis for pleomorphic lobular carcinoma.[20] [Figure 7]

TUBULAR CARCINOMA

These are special type of breast carcinoma with excellent prognosis, occurs in elderly patients more than 50 years. These tumors vary in size between 0.2 cm to 2 cm. They are composed of well differentiated tubular structures with open lumina lined by single layer of epithelial cells .These tubules are oval or rounded and angulated with cellular desmoplastic stroma.[18,20]

INVASIVE CRIBRIFORM CARCINOMA

Invasive cribriform is a rare form of breast malignancy, with excellent prognosis that grows in a cribriform pattern, where tumor cells are arranged as invasive islands often angulated, in which well defined spaces are formed by arches of cells. These cells are small and show mild pleomorphism.[18,20]

MEDULLARY CARCINOMA

These tumors are usually well circumscribed, composed of tumor cells arranged in syncytial pattern in 75 % of tumor masses, usually four or five cell thickness with no glandular or tubular structures. These tumor cells are usually round to pleomorphic with vesicular chromatin and prominent 1 to 2 nucleoli. These tumor cells are separated by lymphoplasmacytic stromal infiltrate with pushing margins.[18,20,22] It has better prognosis than infiltrative ductal carcinoma.[22] [Figure 9]

MUCINOUS (COLLOID) CARCINOMA

Mucinous carcinoma usually occurs in post-menopausal women, these tumors are well circumscribed with a glistening gelatinous appearance. They are characterized by proliferation of clusters of small uniform tumor cells floating in mucinous lakes with delicate fibrous septa divide the mucous lakes into compartments.[20] Pure mucinous carcinomas have a favorable prognosis.[18,20]

NEUROENDOCRINE TUMORS

Primary neuroendocrine tumors of breast exhibit morphological features which are similar to neuroendocrine tumors of gastrointestinal tract and lung. They express neuroendocrine markers in more than 50% of the tumors, and present as expansile or infiltrative lesion. These tumors are arranged in solid sheets or alveolar pattern separated by delicate fibrous septa, cells are small round cells with salt and pepper chromatin with scanty cytoplasm.[18,20]

INVASIVE PAPILLARY CARCINOMA

These tumors are clinically small and circumscribed in majority of the cases with relatively good prognosis. These tumors shows delicate blunt papillae and also shows focal solid areas, nuclei are intermediate grade with amphophilic cytoplasm with scant stroma.[20,23] [Figure 11]

METAPLASTIC CARCINOMA

They usually present as a well circumscribed palpable mass. It belongs to heterogeneous group of neoplasms characterized by admixture of adenocarcinoma with dominant areas of spindle cells along with squamous cell carcinoma or mesenchymal differentiation. It can be broadly classified into pure epithelial and mixed epithelial with mesenchymal components. They are more aggressive tumors than infiltrative ductal carcinoma.[18,20] [Figure 13]

APOCRINE CARCINOMA

Apocrine carcinoma is a rare form of breast malignancy predominantly composed of apocrine type of epithelium in more than 90% of the tumor cells. Tumor cells are large, having abundant acidophilic granular cytoplasm which contains golden brown granules that are strongly positive for PAS. Nuclei are vesicular with prominent nucleoli and glandular differentiation is usually found with apocrine snouts.[20, 24]

STAGING [20]

TNM Classification of carcinomas of the breast

TNM Clinical Classification

TX -Primary Tumor cannot be assessed

T0 - No evidence of primary Tumor

Tis - Carcinoma in situ

Tis (DCIS) Ductal carcinoma in situ

Tis (LCIS) Lobular carcinoma in situ

Tis (Paget) Paget disease of the nipple with no Tumor

T1 : Tumor 2 cm or less in greatest dimension

T1 mic : Microinvasion 0.1 cm or less in greatest dimension

T1a : More than 0.1 cm but not more than 0.5 cm in greatest dimension

T1b : More than 0.5 cm but not more than 1 cm in greatest dimension

T1c : More than 1 cm but not more than 2 cm in greatest dimension

T2 : Tumor more than 2 cm but not more than 5 cm in greatest dimension

T3 : Tumor more than 5 cm in greatest dimension

T4 : Tumor of any size with direct extension to chest wall or skin

Only as described in T4a to T4d

T4a : Extension to chest wall

T4b : Edema (including peau d'orange), ulceration of the skin of the breast or satellite skin nodules confined to the same breast

T4c - Both 4a and 4b, above

T4d - Inflammatory carcinoma

N - Regional Lymph Nodes

NX - Regional lymphnodes cannot be assessed

N0 - No regional lymph nodes metastasis

N1 - Metastasis in movable ipsilateral axillary lymphnode(s)

N2 - Metastasis in fixed ipsilateral axillary lymph node(s)or in clinically apparent ipsilateral internal mammary lymphnode (s) in the absence of clinically evident axillary lymphnode metastasis

N2a - Metastasis in axillary lymphnode(s) fixed to one another or to other structures

N2b - Metastasis only in clinically apparent internal mammary lymphnode (s) and in the absence of clinically evident axillary lymphnode metastasis

N3 - Metastasis in ipsilateral infraclavicular lymph node(s) with or without axillary lymphnode involvement or in clinically apparent ipsilateral internal mammary lymphnode (s) in the presence of clinically evident axillary lymphnode metastasis or metastasis in ipsilateral supraclavicular lymph node(s) with or without axillary or internal mammary lymphnode involvement

N3a - Metastasis in infraclavicular lymph node (s)

N3b - Metastasis in internal mammary and axillary lymph nodes

N3c - Metastasis in supraclavicular lymph node (s)

Metastasis – There are two metastatic classification values (M0 or M1)which depend on the presence or absence of breast cancer cells in locations other than the breast and lymphnodes (Distant metastasis eg :-Bone, Brain, Lung)but it includes supraclavicular lymphnode.

MX - Distant metastasis cannot be assessed

M0 - No distant metastasis

M1 - Distant metastasis

pTNM pathological classification [20]

pN – Regional Lymph Nodes

PNx - Regional lymph nodes cannot be assessed

pN0- No regional lymph node metastasis

pN1mi - Micrometastasis (larger than 0.2 mm, but none larger than 2 mm in greatest dimension)

pN1 - Metastasis in 1–3 ipsilateral axillary lymph node(s), and/or in internal mammary nodes with microscopic metastasis detected by sentinel lymph node dissection but not clinically apparent

pN1a -Metastasis in 1–3 axillary lymphnodes, including atleast one larger than 2 mm in greatest dimension

pN1b - Metastasis in internal mammary lymph nodes with microscopic metastasis detected by sentinel lymph node dissection but not clinically apparent

pN1c - Metastasis in 1–3 axillary lymph nodes and internal mammary lymph nodes with microscopic metastasis detected by sentinel lymph node dissection but not clinically apparent

pN2 - Metastasis in 4–9 ipsilateral axillary lymph nodes, or in clinically apparent ipsilateral internal mammary lymph node in the absence of axillary lymph node metastasis

pN2a - Metastasis in 4–9 axillary lymph nodes, including atleast one that is larger than 2 mm

pN2b - Metastasis in clinically apparent internal mammary lymph node(s), in the absence of axillary lymph node metastasis

pN3 - Metastasis in 10 or more ipsilateral axillary lymph nodes; or in infraclavicular lymph nodes; or in clinically apparent ipsilateral internal mammary lymph nodes in the presence of 1 or more positive axillary lymph nodes; or in more than 3 axillary lymph nodes with clinically negative, microscopic metastasis in internal mammary lymph nodes; or in ipsilateral supraclavicular lymph nodes

pN3a - Metastasis in 10 or more axillary lymph nodes (at least one larger than 2 mm) or metastasis in infraclavicular lymph nodes

pN3b - Metastasis in clinically apparent internal mammary lymph node in the presence of 1 or more positive axillary lymph node; or metastasis in more than 3 axillary lymph nodes and in internal mammary lymph nodes with microscopic metastasis detected by sentinel lymph node dissection but not clinically apparent.

pN3c - Metastasis in supraclavicular lymph node

HISTOLOGICAL GRADING

The tumor grade has been an important part of pathology report since the work of Broders and his predecessor's. Unlike stage which is based on the extent of disease, grade is based on the histopathological qualities of the primary tumor. This is an important determinant of prognosis that allows risk stratification within a given tumor stage. Tumor grade remained a statistically significant prognostic factor for disease free survival and overall survival. [25,26,]

Several histological grading systems were proposed. Some considered ductoglandular differentiation or tumor secretory state while some scored only nuclear and nucleolar characteristics and others use both duct formation and nuclear abnormalities**.** [27]

Greenough developed a histological grading system for breast carcinoma which was simplified by Patey and Scarf. Bloom and Richardson made it more acceptable by introducing a numerical scoring system to the method described by Patey and Scarf**.** [28]

Nottingham's modification of Bloom Richardson grading combines measurement of differentiation(Tubule formation) with details of cell morphology (Nuclear pleomorphism) and assessment of proliferation (Mitotic activity).[29]

PROGNOSTIC FACTORS

1. Age : Younger than 50 years has best or better prognosis.[16,18]
2. The risk of breast cancer increases with number of affected first degree relatives.[16,18]
3. Lymphnode metastasis:Axillary lymphnode status is the most important prognostic factor for invasive carcinoma in absence of distant metastasis. [16,18]
4. Tumor size: Tumor size is one of the most powerful predictors of tumor behavior in breast cancer. The risk of axillary lymphnode metastasis increases with the size of primary tumor. Both lymphnode metastasis and tumor size are independent prognostic factors.[16,18]
5. Histopathological type: Morphological variants of invasive ductal carcinoma with a more favorable prognosis are tubular, cribriform, medullary, pure mucinous, papillary and secretory carcinoma. A variant of lobular carcinoma associated with bad prognosis is signet ring carcinoma. Tumors which are aggressive than ordinary ductal carcinoma are squamous cell carcinoma and metaplastic carcinoma.[18,30]
6. Histological grade: Most commonly used grading system is Nottingham Histological score (Scarf Bloom Richardson). Survival for patients with well differentiated(Grade 1) carcinomas gradually declines to 70% at 24 years. Most deaths are seen in poorly differentiated (Grade 2) carcinomas which occur in first 10 years. Grade 2 (moderately differentiated) carcinomas have slightly better survival than Grade 3.[18,30]

7. Lymphovascular invasion:Strongly associated with presence of lymphnode metastasis is a poor prognostic factor for overall survival and risk factor for local recurrence.[16]
8. ER and PR receptors : 80% of carcinomas that are ER and PR positive respond to hormonal treatment.[18]
9. Her 2 neu overexpression is associated with poor survival.[16,18]
10. Microvessel Density : Attempts have been made to quantify the density of vessels and to correlate with various prognostic factors though only few showed impressive results. Others failed to show significant correlation. There have been several reports of a direct association between density of tumor microvessels and risk of metastasis.[18,30,31]

ANGIOGENESIS (NEOVASCULARIZATION)

In 1971, Judah Folkman, a visionary pioneer who first stated that tumor growing was directly dependent on the blood vessel network development (Angiogenesis).[32]

Angiogenesis is the formation of new blood vessels from endothelium of the existing vasculature. So these endothelial cells can switch from a resting state to rapid growth by release of various growth factors like VEGF (Vascular endothelial growth factor), PDGF (Platelet derived growth factor) and FGF (Fibroblast growth factor) from tumor cells and stromal cells. [33, 34]

Angiogenesis is the growth and remodeling of new blood vessels which has been recognized as one of the hall marks of cancer. It plays a crucial role in tumor

progression because tumor growth, invasion and metastasis rely on tumor angiogenesis.[35] Tumor growth is dependent on angiogenesis. Expansion of the tumor requires nutrition which is provided by induction of new blood vessels. These newly formed blood vessels are leaky which allow tumor cells to enter circulation and thus increase the metastatic potential and probably the outcome for the patient.[33, 36, 37]

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)

Vascular endothelial growth factor is secreted by mesenchymal and stromal cells. Of the various receptors, VEGFR -2 , a tyrosine kinase receptor is the most important in angiogenesis. [38]

VEGF induces the migration of endothelial precursor cells (EPC s) in the bone marrow and enhances the proliferation and differentiation of these cells at sites of angiogenesis. In angiogenesis originating from pre-existing local vessels, VEGF signaling stimulates the survival of endothelial cells, their proliferation and their motility initiating the sprouting of new capillaries.[38]

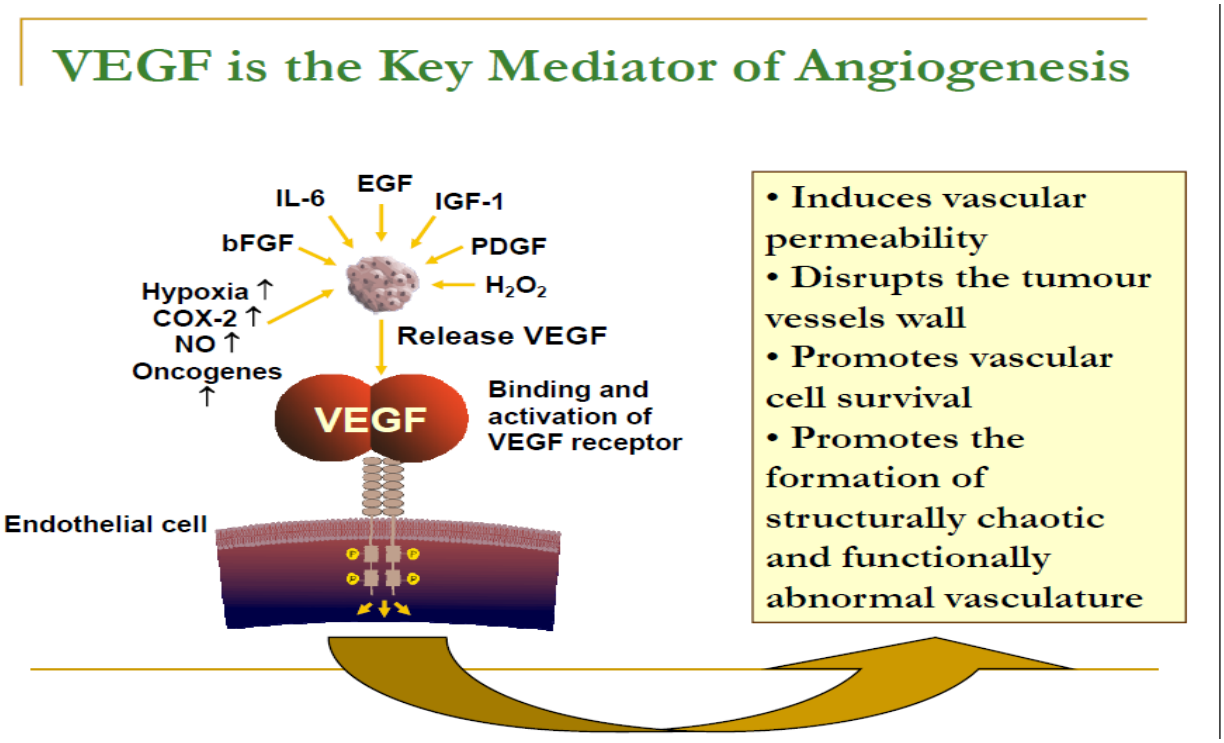


Figure 3: VEGF in Angiogenesis

The role of angiogenesis as an indicator of aggressiveness of the Tumor has been demonstrated in several kinds of neoplasms such as carcinoma breast, prostate, lung, larynx and bladder. [39]

Brem et al were among the first to suggest that the intensity of intratumoral angiogenesis may correlate with tumor grade and aggressiveness.[40]

Density of microvessels can be studied using various immunohistochemical stains like antibodies to CD 31, CD 34 and factor VIII. [41] Mast cells are studied using histochemical stains like Toluidine blue and Alcian blue .[42]

So far no endothelial marker developed has been trouble free. Anti-factor VIII/VWF remains the most specific endothelial marker, providing good contrast between micro vessels and other tissue components but doesn't highlight all intratumoral micro vessels.[43]

CD31 is sensitive but strongly cross reacts with plasma cells. These can obscure the micro vessels in those tumors with a prominent plasma cell inflammatory background. [44] CD34 is an acceptable alternative and the most reproducible endothelial cell highlighter in many laboratories but CD34 also highlight perivascular stromal cells and has been noted to stain a wide variety of stromal neoplasm.[45,46]

CD 34 molecule is a cluster differentiation molecule present on certain cells. It is a member of the family of single pass transmembrane sialomucin proteins. It is a highly glycosylated trans membrane cell surface glycoprotein expressed on the luminal cell membrane of endothelial cells of small blood vessels.[47,48] Cells expressing CD34 are normally found in umblical cord, bone marrow (hematopoietic cells), endothelial progenitor cells and endothelial cells of blood vessels . [47,48]

MICROVESSEL DENSITY (MVD)

Microvessel density assessment is the most commonly used technique to quantify intratumoral angiogenesis in breast cancer. Weidner et al first demonstrated angiogenesis by using panendothelial immunohistochemical staining of microvessels which correlated with distant metastasis in breast cancer patients.[49]

In breast carcinoma, intratumoral endothelial cells proliferate 45 times faster than the endothelial cells in adjacent benign stroma. Rate of progression is associated with increased intratumoral microvessel density, a morphological measure of tumor angiogenesis. [49,50]

Teo et al showed that higher the micro vessel densities in breast cancers, higher risk of subsequent in situ cancers and invasive recurrence of previous in situ cancers with poorer response to treatment .[51]

Increase in micro vessel density in breast carcinoma has been shown to correlate with malignant and metastatic potential and hence poor prognosis. Majority of published reports have shown a significant correlation between the density of intratumoral micro vessels in invasive breast carcinoma and the incidence of metastasis and or survival.[52]

The prognostic value of angiogenesis is controversial. Many studies proved that MVD in invasive breast carcinoma has a prognostic value. [49- 53] However some authors did not.[54,55]

The reason for these discrepancies may be variations in the methodologies used, antibodies used to identify microvessels," hotspots" counted, statistical analysis which have varied between the different studies.[56]

MAST CELLS

The presence of "Mast cell" in tumor tissue has been often reported since Ehrlich first described them in 1878. [57]

Mast cell(Mastocyte) is a resident cell of several types of tissue and contains many granules rich in histamine. Mast cell already known by their key role in IgE associated disorders also seem to play an important role in pro-inflammatory, immunoregulatory mechanisms and biological consequences such as mitogenesis, extracellular matrix degradation and spread of tumors by recruiting various growth factors and cytokines. [8, 9, 10, 11]

Mast cells are derived from specific bone marrow progenitor cell which migrate into tissue where they mature depending upon the micro environmental condition. They are present in the vicinity of blood vessel, skin, mouth and conjunctiva .[58]

Two types of mast cells:

- Mast cell TC type (Tryptase , Chymase) present in skin and intestinal sub mucosa
- Mast cell T type (Tryptase) present in alveolar mucosa

These two differ in the number and type of secretory granules they contain and responsiveness to stimuli [8]

ROLE OF MAST CELLS AND FUNCTION :

The role of mast cells in allergic disorders and inflammation is well established .[59,60]

Role of mast cells in tumor development is being investigated in various tumors. [12] Mast cell accumulations can be beneficial or detrimental for tumor growth, it could facilitate tumor angiogenesis through heparin like molecules and metastasis through its anti-clotting effect .[8, 12]

Moreover, mast cells secrete histamine and growth factors such as VEGF (Vascular endothelial growth factors), PDGF (Platelet derived growth factor), SCF (Stem cell factor) and NGF (Nerve growth factor) are also rich in metalloproteases that contribute to the majority of proteolytic components necessary for tumor invasiveness .[61]

Mast cells directly influence the growth of cancer cells. Histamine is the best known mast cell product which play role in tumor progression. Bowrey et al showed that histamine content correlates positively with the mast cell count in breast carcinoma.[62] Mast cells secrete histamine, interleukin 10 and tumor necrosis factor α which contribute to cancer growth by modulation of immune response by suppression of the cellular immunity.[63]

The most important factor by which mast cells influence cancer growth is stimulation of angiogenesis. Mast cells have also been shown to regulate proliferation of blood vessels and to participate in antigenic switch. Mast cell stimulates angiogenesis in the early phase of tumor development, while in the

later stages tumor cells become self-sufficient with regard to production of proangiogenic factors.[64] [Figure 4]

On the other hand, mast cells could also be detrimental to tumor growth by secreting several cytokines such as IL-4 and proteolytic enzymes participating in inducing apoptosis of malignant cells.[65]

Opposite effects of mast cells depend on its ability to degranulate or secrete various mediators in response to stimuli. Tryptase causes tumor cell disruption and chondroitin sulphate may inhibit tumor cell dissemination and metastasis formation.[66]

Mast cells release certain mediators like TNF α, IL- 1 and IL -6 which has inhibitory effect on tumor growth and angiogenesis. Mast cell recruit macrophages to the tumor site, which as inhibitory effect on tumor growth.[67][Table 1]

The Dual role of mast cells in inhibiting or promoting needs to be further investigated.[8]

In a study done by authors, showed that Perivascular mast cells in adenocarcinomas can secrete several cytokines and proteolytic enzymes that may be detrimental to the tumor cells, as well as compounds such as heparin, which act as both anticoagulant and angiogenic properties, mast cell tryptase can stimulate protease activated receptors (PAR 1 and PAR 2).[68,69]

Majority of the studies suggest that peritumoral mast cells are numerous than intratumoral and it was also observed that intratumoral mast cell contain less granules, it might indicate extensive secretion.[70]

Special stains to Identify Mast cells [71,72]

- Metachromatic staining of the granules with basic aniline dyes like Toluidine blue , Cresyl violet , Azure A , methylene blue and modified thionine acridine orange
- Histochemical demonstration of enzymes.
- Immunoperoxidase staining using monoclonal antibodies like anti Tryptase

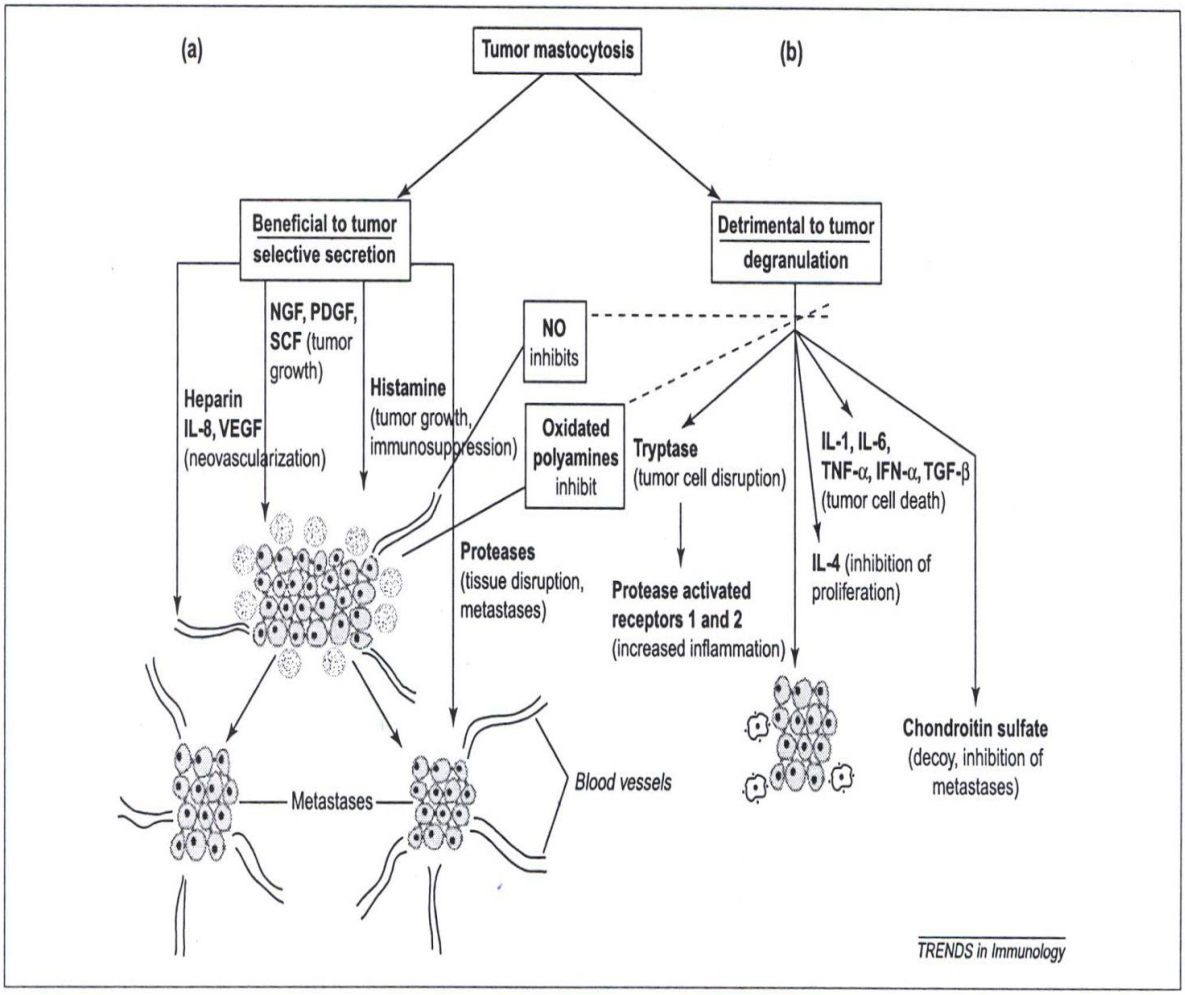


Figure 4 : Role of mast cells in tumor growth [8]

Mediators		Main pathophysiologic effects
Beneficial effects on cancer		
Mast cells	**Macrophages (M2)**	
Biogenic amines		
Histamine		Vasodilation, mitogenesis, immunosuppression
Chemokines		
MCP-1, RANTES	CCL17, 22, 24, 16, 18	Chemoattraction for mast cells
Cytokines		
IL-8	IL-1ra, IL-10, IL-13	Neovascularization
Enzymes		
Chymase		Tissue damage
Kinogenases		Synthesis of vasodilatory kinins
Tryptase		Tissue damage, metastases
Growth factors		
CSF, NGF, PDGF, SCF		Tumor growth
VEGF		Angiogenesis, neovascularization
Proteoglycans		
Heparin		Angiogenesis, NGF stabilization, patent vessels
Detrimental effects on cancer		
Mast cells	**Macrophages (M1)**	
Chemokines		
IL-8, MCP-3, MCP-4	CXCL8	Leukocyte chemoattraction
Cytokines		
IL-1, -2, -3, -5, -6, -9, -10, -13, -16	IL-1	Inflammation, leukocyte migration
IL-4	IL-6	Tumor cell apoptosis
IFN-γ	IL-12	Inflammation, leukocyte proliferation and activation
TNF-α	TNF-α	Inflammation, tumor cell death
Growth factors		
GM-CSF, TGF-β		Inflammatory cell proliferation
Proteoglycans		
Chondroitin sulfate		Acts as decoy and prevents metastases
Arachidonic acid products		
LTB_4		Leukocyte chemotaxis
PAF		Platelet activation and serotonin release
PGD_2		Vasoconstriction
LTC_4		Vasoconstriction

Abbreviations: CSF, colony stimulating factor; GM-CSF, granulcyte–monocyte-CSF; IFN-γ, interferon-γ; IL-8, interleukin-8; LTB_4, leukotriene B_4; MCP-1, macrophage inflammatory factor-1; NGF, nerve growth factor; PAF, platelet activating factor; PDGF, platelet-derived growth factor; PGD_2, prostaglandin D_2; SCF, stem cell factor; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial cell growth factor.

Table 1 : Role of mast cell mediators on cancer [8]

MAST CELL DENSITY (MCD)

Data from experimental tumor models suggest that mast cells accumulate near to tumor cells before the onset of angiogenesis and they are required for macroscopic expansion and metastatic spread of primary tumor cells. Evidence for a specific role for mast cells has been reported in mast cell tumors , head and neck , gastric and lung tumors, where mast cell density is highly correlated with extent of tumor angiogenesis .[73,74]

In the study done by Rajput et al, he concluded that stromal mast cell infiltration in invasive breast cancer is an independent good prognostic marker and has a critical role of local inflammatory responses in breast cancer progression.[75] Heidarpour M et al in his study stated that presence of mast cells in breast cancer is correlated with a much lower grade of the tumor.[76]

However there are limited literature published regarding correlations between mast cell and angiogenesis in primary breast cancer.[77]

RESEARCH METHODOLOGY

We have studied a total of 55 cases which included resected breast carcinoma specimens sent from department of surgery at R.L.Jalappa Hospital and Research Centre between January 2012 to July 2013 and available blocks of breast carcinoma cases between January 2008 to January 2012 in the department of pathology, Sri Devaraj Urs Medical College.

Study Duration:

The study was conducted from January 2012 to July 2013 for a period of 18 months.

Study Design:

Cross sectional study

Prior to the study ethical clearance was obtained from the institutional ethical board.

Inclusion Criteria:

All cases of resected breast carcinoma received at Department of Pathology during the period mentioned above

Exclusion Criteria:

1. Non –neoplastic lesions of breast

2. Patients on or receiving radiotherapy, chemotherapy or recurrence

DATA COLLECTION AND PROCESSING

DEMOGRAPHIC AND CLINICAL DETAILS

The following details of the patients were noted:

1. The age and sex of the patient
2. The location of the tumor, with respect to side (right / left)
3. The location of the tumor, with respect to quadrant (Outer , inner or central)
4. Whether the patient had received neoadjuvant chemotherapy or not

MACROSCOPIC (GROSS) EXAMINATION

1. The nature of the specimen received, measurement and description of the tumor were noted
2. The size of the tumor was noted

   The tumors were classified into three categories based on size

   a) Tumors less than 2 cm in greatest dimension

   b) Tumors with size between 2 – 5 cm in greatest dimension

   c) Tumors with size more than 5 cm in greatest dimension
3. Total number of lymphnodes received were recorded

MICROSCOPIC EXAMINATION

1. Surgical specimens were routinely processed and stained with H&E staining.
2. All H & E sections were examined and reviewed for histopathological type and pathological staging was done based on WHO classification of breast.
3. All sections were examined and reviewed for histopathological grading according to Modified Bloom Richardson system.
4. All H&E sections were reviewed for selection of the blocks, from which selected sections were taken for immunohistochemistry.

GRADING TECHNIQUE

Tubule formation

All sections with tumor are scanned and the percentage of the tumor displaying tubular structures is assessed . Only tubules with clear lumina should be considered and clefts formed due to shrinkage artifact are not taken into consideration. Tubule formation is scored 1 to 3 by taking 10% to 75% as cut off points. Score 1 is assigned when tubule formation is more than 75%, Score 2 when tubule formation is between 10 to 75% and Score 3 when tubule formation is less than 10%.

Nuclear pleomorphism

Assessment is done based on variation in nuclear size, chromatin pattern and nucleoli, score are assigned. When the nuclei are small with little increase in size, with regular outline and uniform chromatin, a score of 1 is assigned .When the cells appear larger than normal with vesicular nuclei and visible nucleoli, with moderate variability in size and shape, a score of 2 is given .A marked variation in size and shape, especially when there are large bizarre nuclei with presence of prominent nucleoli, a score of 3 is given.

Mitotic counts

This feature is best assessed where there is active growth .A minimum of ten fields is assessed. Hyper chromatic and pyknotic cells should be ignored as they represent apoptosis and not proliferation.

The grade is obtained by adding up the scores for tubule formation, nuclear pleomorphism and number of mitosis. The total score will range between 3 and 9 points which is translated into final grade as follows:

Grade I : 3-5 points

Grade II: 6-7 points

Grade III : 8-9 points

TABLE: 2 -NOTTINGHAM MODIFICATION OF BLOOM RICHARDSON GRADING

FEATURE	SCORE
Tubule formation	
>75 % of the tumor	1 point
10-75% of the tumor	2 points
<10% of the tumor	3 points
Nuclear pleomorphism	
Nuclei with minimal variation in size and shape	1 point
Nuclei with moderate variation in size and shape	2 points
Nuclei with marked variation in size and shape	3 points
Mitotic counts –per 10HPF (40x fields)	
0 – 5	1 point
6 – 10	2 points
>11	3 points

Representative blocks were selected and sections were prepared for IHC.Immunohistochemistry was done using a primary antibody CD34 for determination of microvessel density, followed by counterstaining with 0.1% toluidine blue for evaluation of mast cell count on the same section.

IMMUNOHISTOCHEMICAL TECHNIQUE (IHC)

The immunohistochemistry (IHC) was performed on 4-μm thick sections from 10% formalin-fixed paraffin-embedded tissues, using non - biotin polymer based HRP detection system.

Antigen	Clone	Species	Producer	Dilution	Control	Stain
CD34	QBEND/10	Mouse	Biogenex	Undiluted	Inbuilt	Endothelium

The IHC procedure includes following step :

1. Sections are cut 3-5mm thickness, floated onto organosialine coated slide and left on hot plate at 60^{o} over night
2. **Deparaffinization** using Xylene I and II for 15 min each
3. **Dexylinisation** using absolute alcohol I and II for1 min each
4. **Dealcoholisation** using 90% and 70% alcohol for 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 2 cycles.
7. Distilled water rinsing for 5 minutes. Transfer to TBS (Tris buffer solution pH- 7.6) - 5minutes x 2 times-wash.
8. **Peroxidase block**- 15-20minutes to block endogenous peroxidase enzyme. TBS buffer for 5 minutes washing for 3 times.

9. **Power block**- 15-20 minutes to block non- specific reaction with other tissue Antigen.

10. Cover sections with targeted antibody (primary) for 1hour.

11. TBS buffer- 5min x 3Times.

12. **Super Enhancer** - 45 minutes to enhance the reaction between primary and secondary antibodies.

13. TBS buffer- 5min x 3 times

14. Super sensitive poly- HRP (secondary antibody) for 30 min

15. TBS buffer- 5min x 3 times

16. **Color development** with working color development solution(DAB) for 5-8 min

    All the slides were examined for colour development

17. TBS wash- 5min x 3 times

18. Counter stain with 0.1% toulidene blue for 3 sec

19. Tap water wash for 5 minutes.

20. Dehydrate, clear and mount

21. Mount with DPX.

Micro vessel and Mast cell Quantitation (MVD and MCD)

Microvessels Density (MVD)

Vessels were counted according to standard technique described by weidner et al.[43]

Any brown staining endothelial cell or endothelial cell cluster that are clearly separate from adjacent microvessels, tumor cells and other connective tissue element is considered as single, countable microvessel. The presence of vessel lumen and RBC s was not required to classify a structure as a vessel.

Mast cell Density (MCD)

Mast cells are round to oval mononuclear cells with granular cytoplasm and single oval nucleus, measuring 4-20 μm in size. These mast cells are stained by basic aniline dyes like toluidine blue, methylene blue and cresyl violet, where they stain the granules to give purplish pink in color - metachromatic staining.

Counting (quantification)

The microvessel densities of IHC stained sections from the chosen paraffin blocks were assessed by light microscopy :

1. Sections were first examined at low magnification (10 X and 40 X magnification) using (Olympus CX 21i) microscope to identify areas of highest neovascularization (hotspot).
2. Three areas of hotspots which had high vessel density were selected, individual microvessel counts were carried out at 200 X field and then average of 3 areas was taken.
3. For MicroVessel Density (MVD), both intratumoral and peritumoral microvessels were counted separately. Intratumoral micro vessels were defined as vessels within the main tumor mass, surrounded by tumor cells, without muscle layer. Vessels more than one high power field (200 X) magnification away from the invasive tumor front were considered as peritumoral microvessels. This will give the number of microvessels per defined field.
4. Mast cells are stained by toluidine blue which are taken up by the metachromatic granules. For mast cell density (MCD) both intratumoral and peritumoral mast cells are counted at 200 X magnification , in the vicinity of the microvessels in 3 areas and averaged. This will give the number of mast cells per defined field.

Statistical analysis:

Data was analyzed using SPSS software version 6. Frequencies, mean and Standard deviation (SD) were used to describe the data. Further ANOVA was used to describe the association between Microvessel density (MVD) and Mast cell density (MCD) with histological grade and type. Pearson's co-relation co-efficient was employed to correlate between MVD (micro vessel density) and MCD (mast cell density) and a 'p' value less than 0.05 would be considered as significant.

RESULTS

The study period was from January 2012 to July 2013, for a period of 18 months. All cases which satisfied the inclusion criteria were included in the present study. A total of 55 cases were studied.

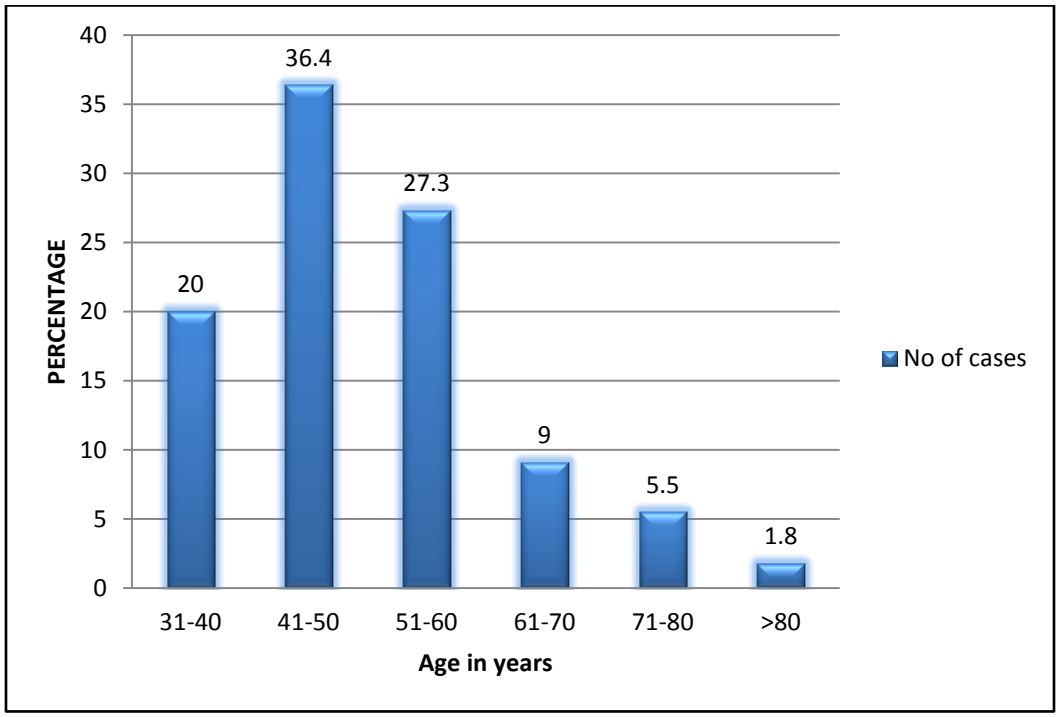
AGE DISTRIBUTION

In the present study, age group ranged from 35 to 85 years with mean age of 52.1±11.6 years. Majority of the patients belonged to 41- 50 years which constituted 20 cases(36.4%), followed by 15 cases (27.3 %) where patients belonged to age group 51-60yrs.

Table 3 : Age distribution of the cases

Age (in years)	No. of cases	Percentage (%)
31-40	11	20
41-50	20	36.4
51-60	15	27.3
61-70	5	9
71-80	3	5.5
>80	1	1.8
Total	**55**	**100**

Chart 1 : Distribution of cases according to age group



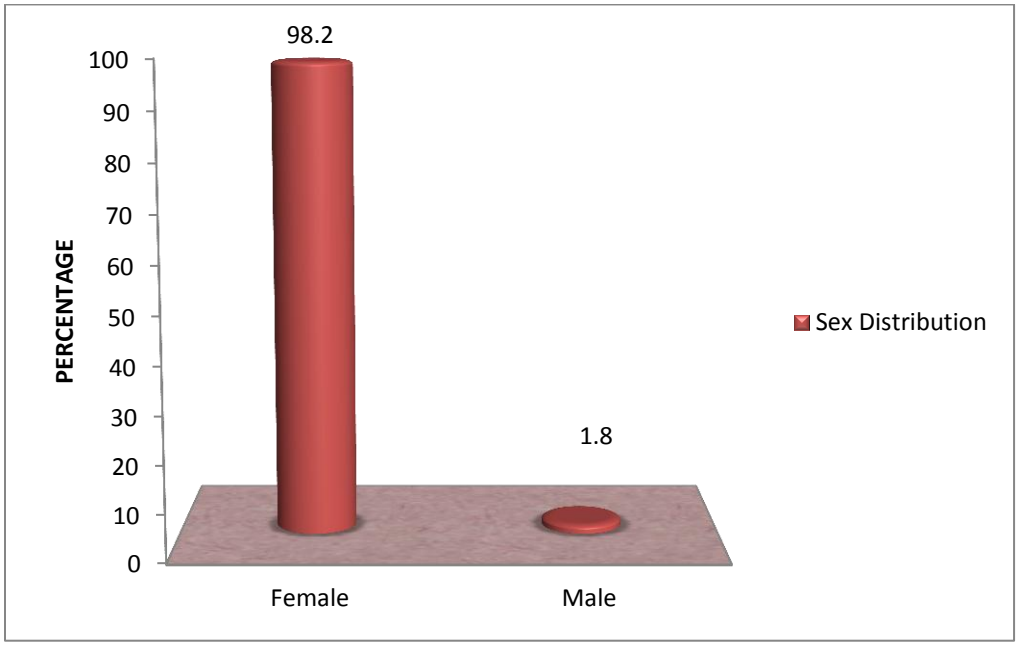
SEX DISTRIBUTION

In the present study, 54 (98.2%) were female and only 1(1.8%)case was male

Table 4 : Sex Distribution of the cases

Sex	No. of Cases	Percentage (%)
Female	54	98.2
Male	1	1.8
Total	**55**	**100**

Chart 2: Distribution of the cases by Sex



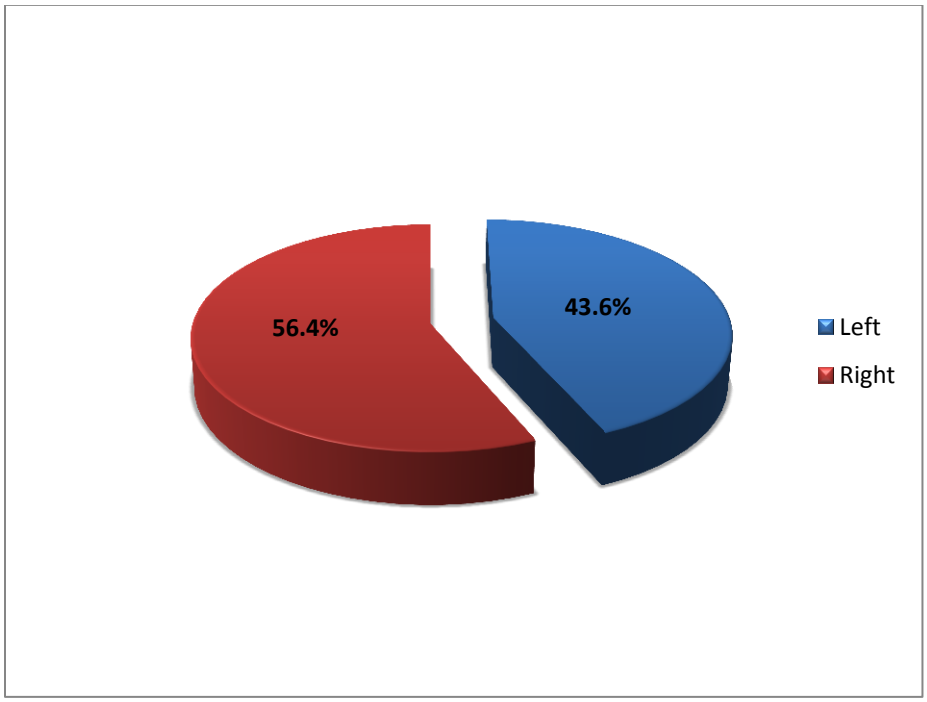
SIDE OF THE TUMOR

In the present study, breast carcinoma involving right breast was more common and comprised 31cases(56.4%) compared to left breast which comprised of 24 cases(43.6%).

Table 5: Side Distribution

Side	No. of cases	Percentage (%)
Left	24	43.6
Right	31	56.4
Total	**55**	**100**

Chart 3 : Distribution of the cases according to side of the breast involved



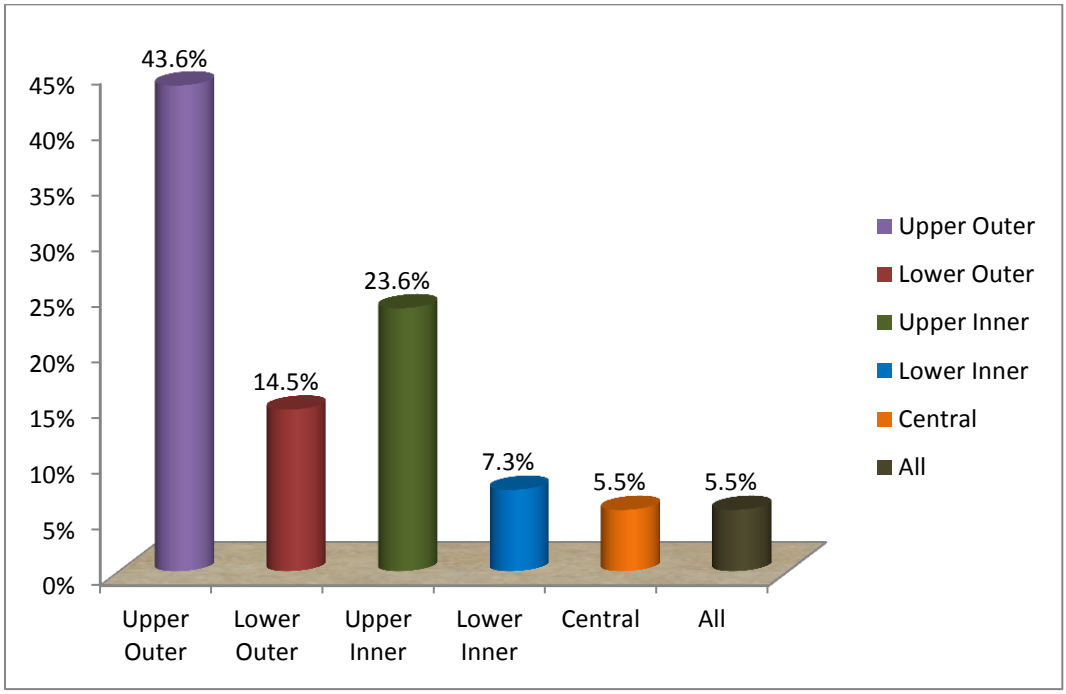
LOCATION

In the present study, the tumors were predominantly located in upper outer quadrant comprising of 24 (43.6%) cases, followed by upper inner quadrant in 13 (23.6%) cases.

Table 6 : Quandrant wise distribution

Quadrant	No of Cases	Percentage (%)
Upper Outer	24	43.6
Lower Outer	8	14.5
Upper Inner	13	23.6
Lower Inner	4	7.3
Central	3	5.5
All	3	5.5
Total	**55**	**100**

Chart 4 : Distribution of cases according to quadrant involved



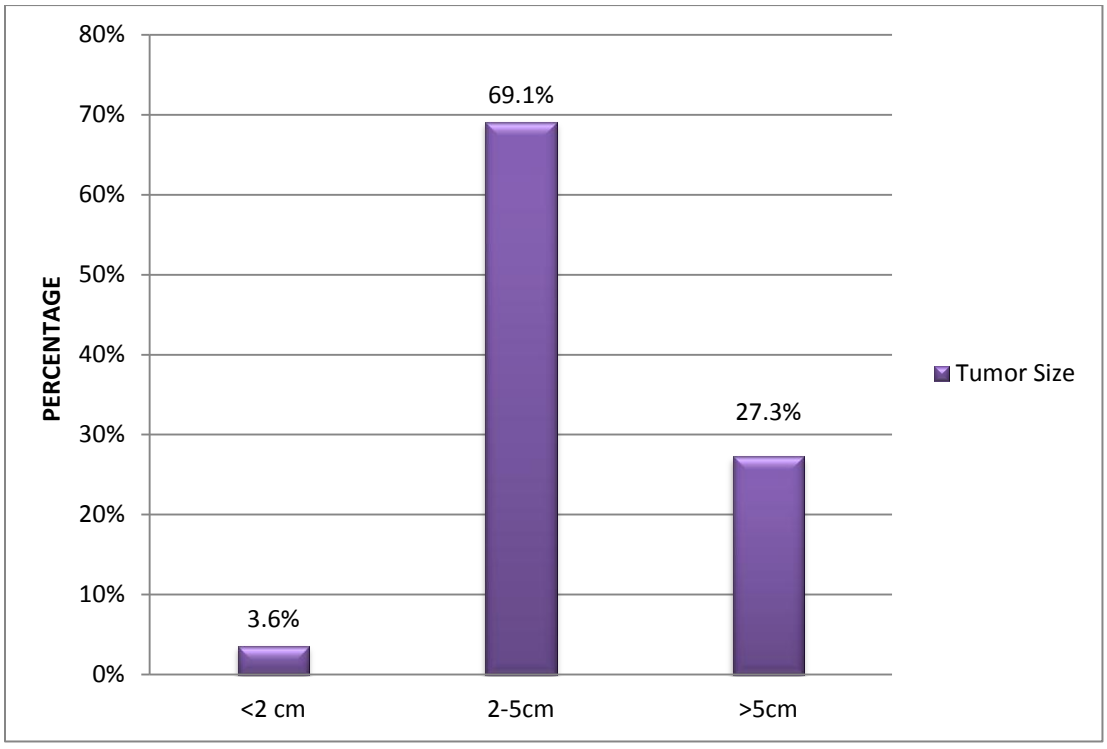
TUMOR SIZE

In the present study, majority of the cases of breast carcinoma presented with tumor size between 2- 5 cms (69.1%) followed by tumor size more than 5 cms (27.3%).

Table 7: Distribution of cases with regard to Tumor Size

Tumor size (in cm)	No. of cases	Percentage (%)
<2	2	3.6
2-5	38	69.1
>5	15	27.3
Total	**55**	**100**

Chart 5 : Distribution of cases according to Tumor size



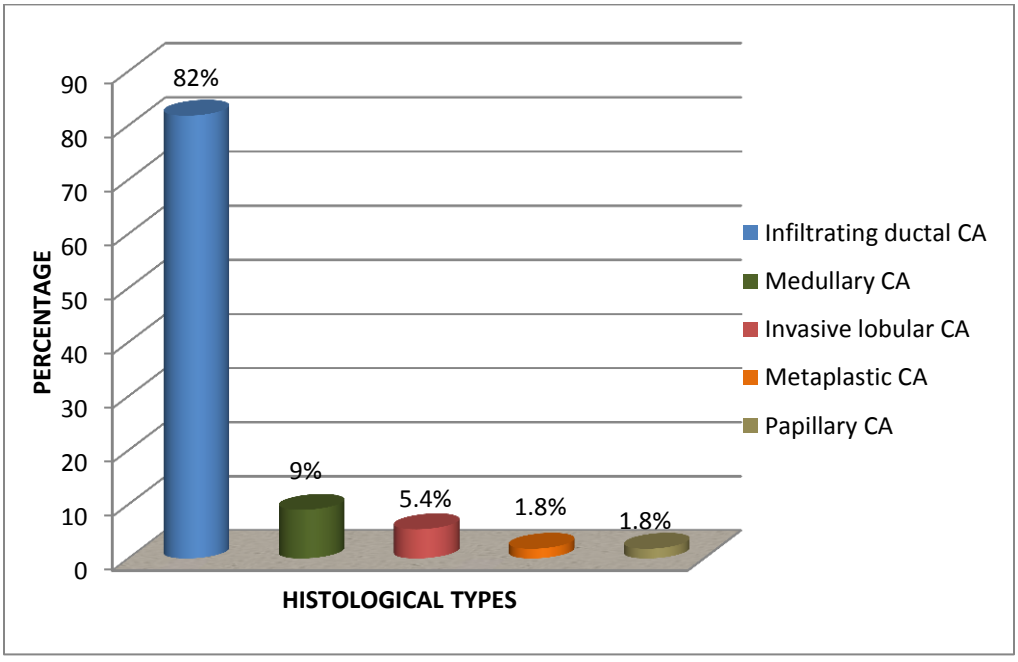
HISTOPATHOLOGICAL TYPE

In the present study, Infiltrating ductal carcinoma is the most common histological type of breast carcinoma seen in 45(82%) cases, followed by medullary carcinoma which was seen in 5(9%)cases. Other subtypes such as infiltrating lobular carcinoma, metaplastic and papillary carcinoma formed less than 10% of the total cases.

Table 8 : Distribution of cases according to Histological Type

Histopathology	No. of cases	Percentage (%)
Infiltrating ductal carcinoma(NOS)	45	82
Medullary Carcinoma breast	5	9
Invasive lobular carcinoma	3	5.4
Metaplastic carcinoma	1	1.8
Papillary carcinoma	1	1.8
Total	**55**	**100**

Chart 6 : Distribution of cases according to histopathological types



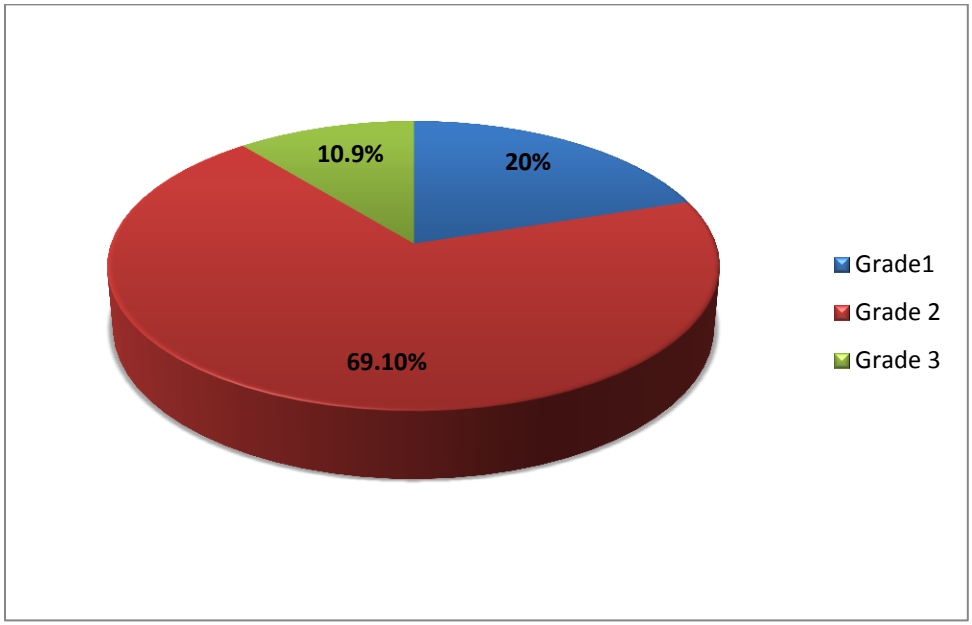
HISTOLOGICAL GRADE :

In the present study majority of the tumors presented as grade 2 tumors on histology comprising of 38 (69.1%) cases, followed by grade1 tumors which comprised of 11cases (20%) and remaining 6 cases (10.9%) were in grade 3 tumors.

Table 9: Distribution of cases with regard to histological grade

Grade	No. of cases	Percentage (%)
1	11	20
2	38	69.1
3	6	10.9
Total	**55**	**100**

Chart 7 : Distribution of cases according to histological grade



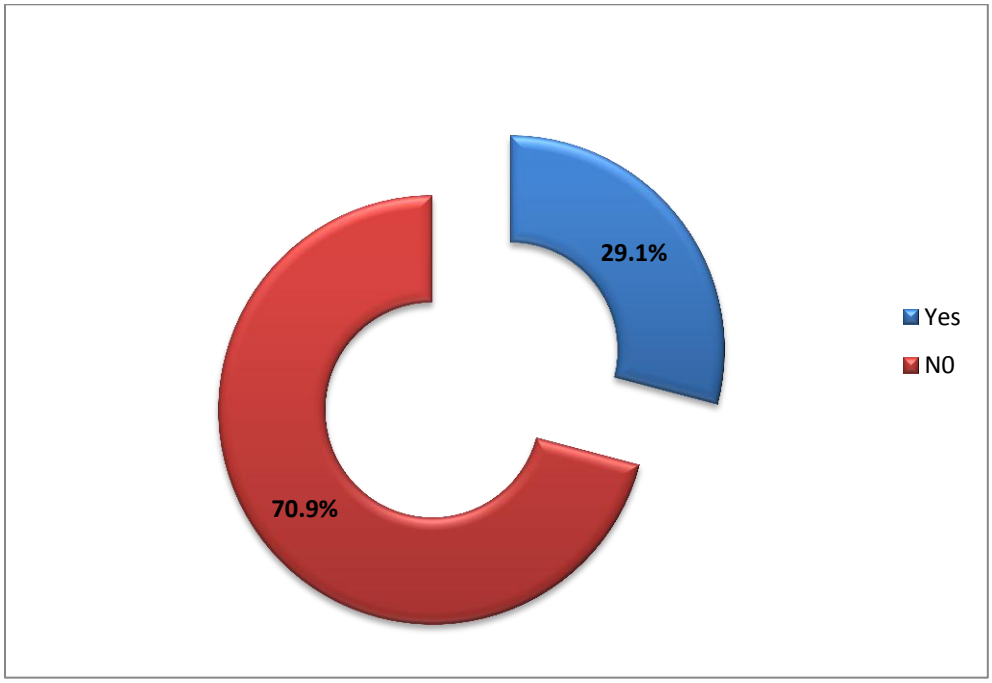
VASCULAR INVASION

In present study vascular invasion was seen in 16(29.1%) cases.

Table 10: Distribution of cases based on vascular invasion

Vascular invasion	No. of cases	Percentage (%)
Yes	16	29.1
No	39	70.9
Total	**55**	**100**

Chart 8 : Vascular Invasion



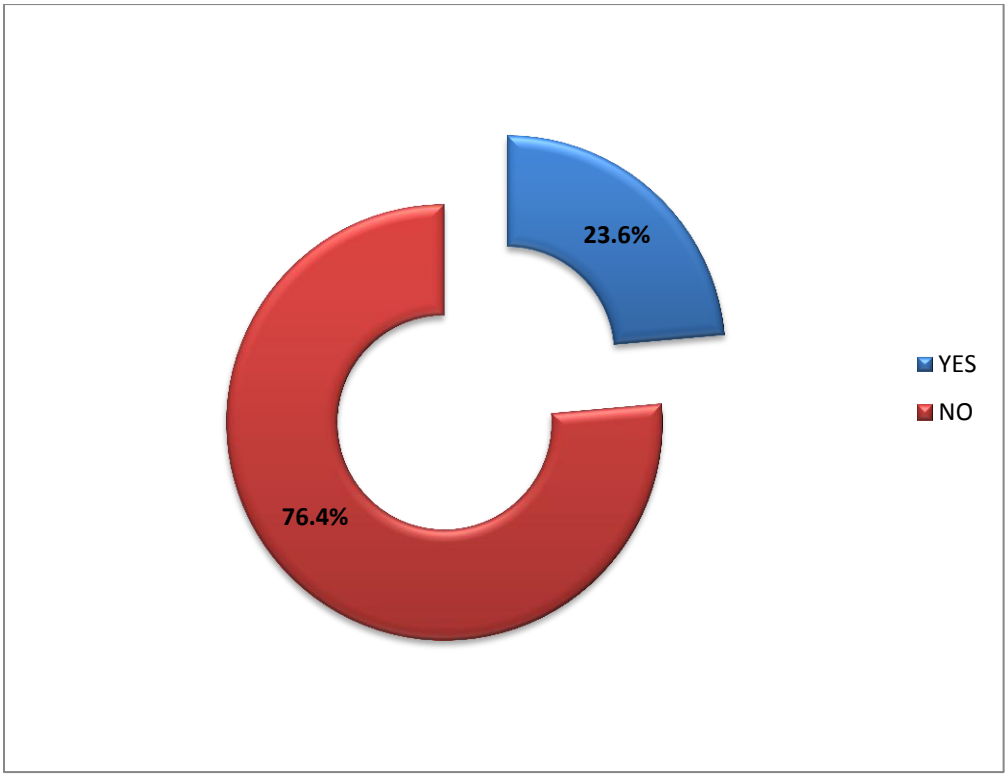
DCIS ASSOCIATION

In present study, only 23.6 %(13 cases) of the cases showed DCIS component with breast carcinoma.

Table 11 : Distribution of cases based on associated DCIS

DCIS association	No. of cases	Percentage (%)
Yes	13	23.6
No	42	76.4
Total	**55**	**100**

Chart 9 : DCIS Association



LYMPHNODE INVOLVEMENT

In the present study out of 55 cases, axillary clearance was done in only 45 cases, and hence only 45 cases were considered for lymphnode metastasis involvement. Out of 45 cases, lymphnode metastasis was seen in 64% (29 cases).Out of the 29 cases positive for lymphnode metastasis, majority of the cases ie 37.7%(17 cases) of the cases has lymphnode metastasis in 1-3 lymphnodes and remaining had lymphnode metastasis in >4.

Table 12: Distribution of cases based on lymphnode metastasis

Lymph node	No. of cases	Percentage (%)
0	16	35.7
1-3	17	37.7
≥4	12	26.6
Total	**45**	**100**

Chart 10 : Lymphnode Metastasis

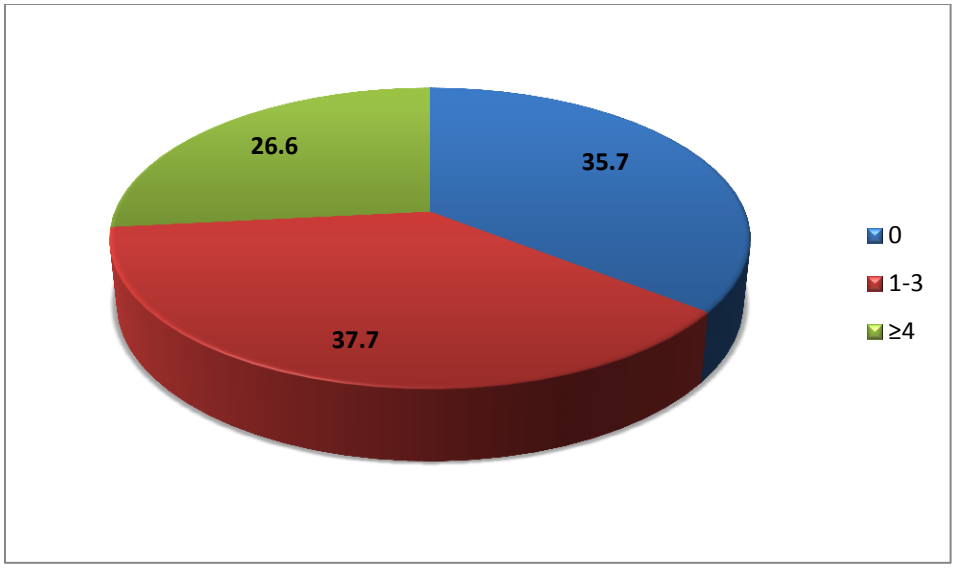


Table 13: Association between Intratumoral MCD and grade of the Tumor

Grade of the tumor	Mean MCD	SD	p value
1	3.27	2.00	0.68
2	2.82	2.24	
3	2.00	1.54	
Total	**2.82**	**2.12**	

In the present study, Mean MCD was highest (3.27±2) in Grade 1 tumors and the value decreased as the grade increased. However the p value was >0.05 implying lack of significance between intratumoral mean MCD and histological grade of tumor. The mean intratumoral MCD calculated was 2.8 ±2.1.

Chart 11 : Intratumoral MCD with grade

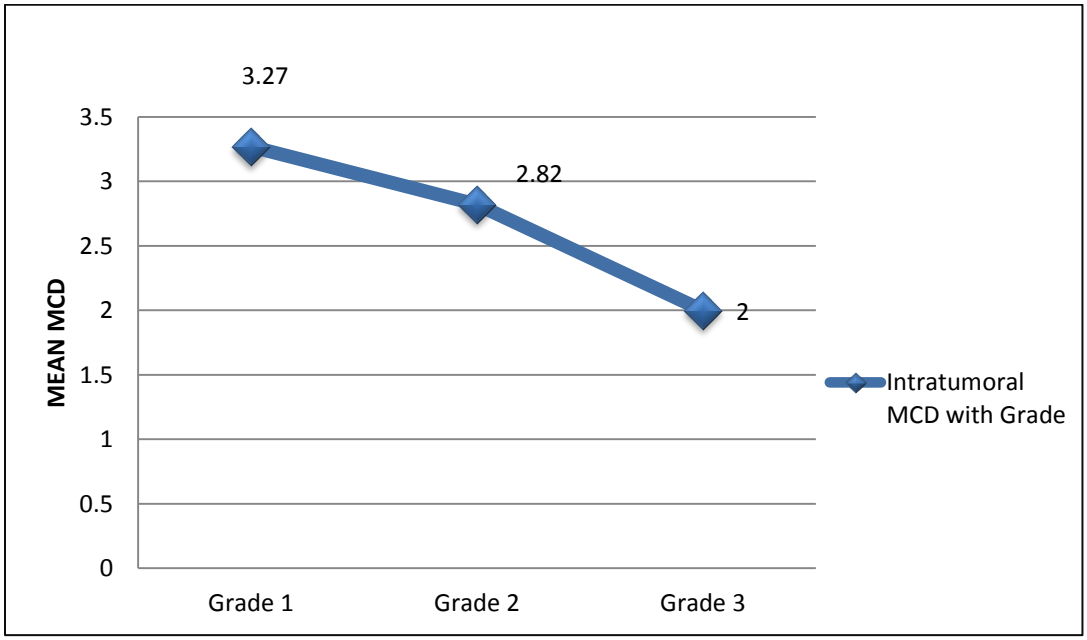


Table 14 : Association between Peritumoral MCD and grade of the Tumor

Grade of the Tumor	Mean MCD	SD	p value
1	16.64	11.12	0.70
2	15.24	9.66	
3	12.50	6.92	
Total	**15.60**	**9.75**	

In the present study, the peritumoral mean MCD was highest(16.64±11.12) in Grade1 tumors and the value decreased as the tumor grade increased. However p value was >0.05 implying lack of significance between peritumoral mean MCD and histological grade**.** Mean intratumoral MCD calculated was 15.6±9.7.

Chart 12 : Peritumoral MCD and grade

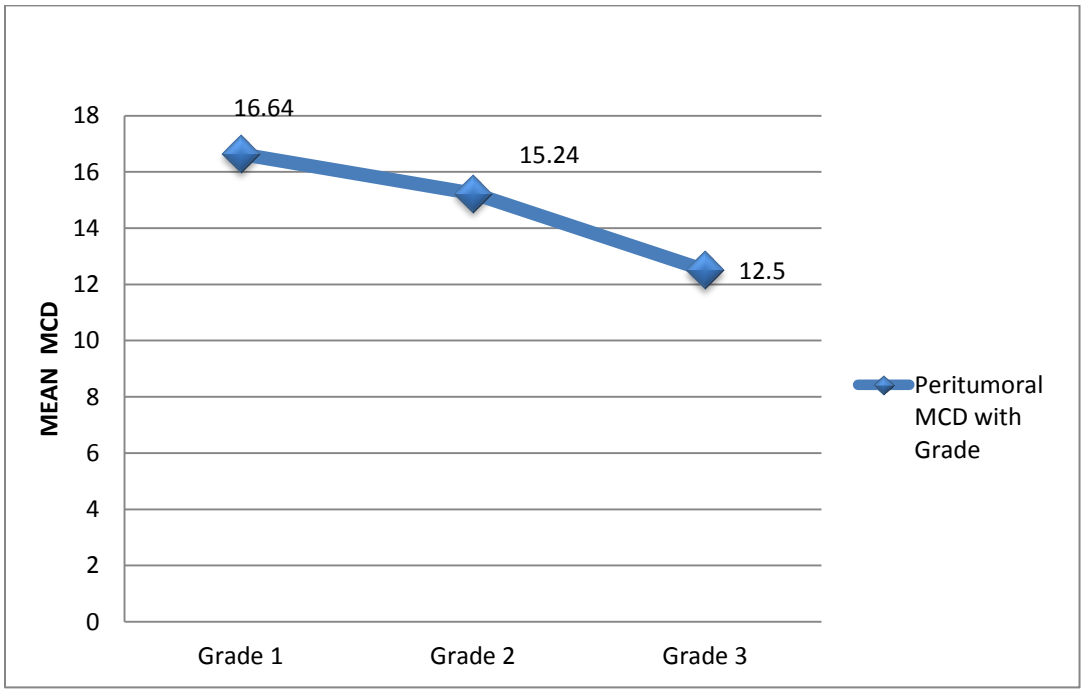


Table 15: Association between Intratumoral MVD and grade of the Tumor

Grade of the Tumor	Mean MVD	SD	p value
1	51.18	23.19	0.002
2	78.21	20.48	
3	132	18.77	
Total	**78.67**	**29.83**	

In the present study, the intratumoral mean MVD was highest (132 ±18.7) in Grade 3 tumors and the value increased as the tumor grade increased. p value was <0.05 implying a strong significance between intratumoral mean MVD and histological grade**.** Mean MVD calculated was 78.6 ± 29.8

Chart 13 : Intratumoral MVD with grade

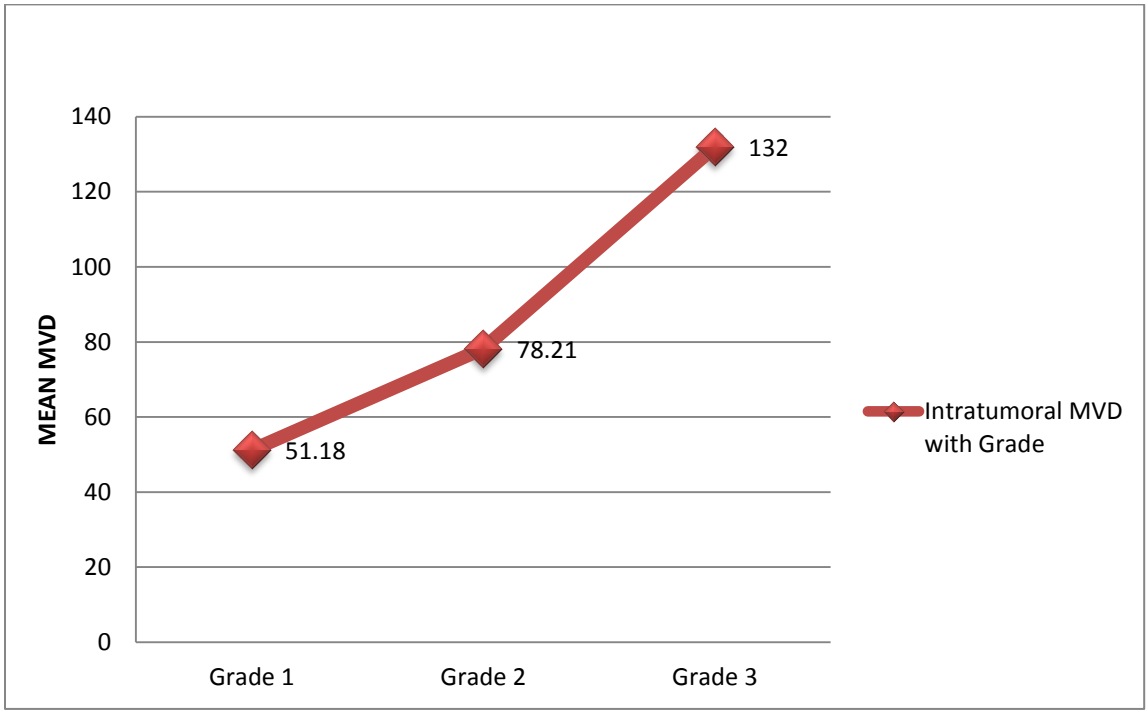
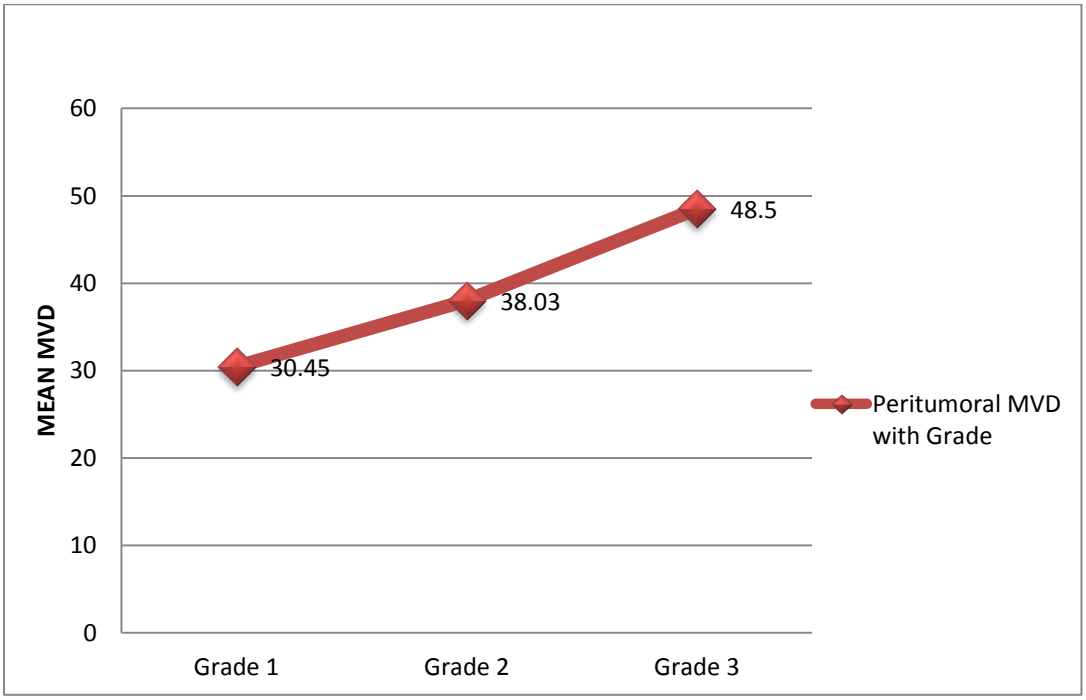


Table 16 : Association between Peritumoral MVD and grade of the Tumor

Grade of the Tumor	Mean MVD	SD	p value
1	30.45	10.79	0.01
2	38.03	10.91	
3	48.50	14.95	
Total	**37.65**	**12.14**	

In the present study, the peritumoral mean MVD was highest(48.50 ±14.95) in Grade 3 tumors and the value increased as the tumor grade increased. P value was <0.01 and showed a strong significance between peritumoral mean MVD and histological grade**.** Mean MVD calculated was 37.6±37.6.

Chart 14 :Peritumoral MVD with grade



For statistical purpose, histopathological type was categorized into 3 groups :

1) Infiltrating ductal carcinoma
2) Invasive lobular carcinoma
3) Others (Medullary , Metaplastic , papillary carcinoma)

Table 17 : Association between histopathological type and intratumoral MCD

Histological Type	**No of cases**	**Mean MCD**	**SD**	P
Infiltrating Ductal CA	45	3.13	2.191	0.05
Lobular CA	3	2.00	1.732	
Others	7	1.14	0.378	
Total	**55**	**2.82**	**2.126**	

In the present study, the intratumoral mean MCD was highest (3.13±2.91) in Infiltrating ductal Carcinoma compared with other histological types of breast carcinoma. p value calculated was 0.05 implying no significance between histological type and intratumoral MCD.

Table 18: Association between histopathological type and peritumoral MCD

Histological Type	No of cases	Mean MCD	SD	p value
Infiltrating Ductal CA	45	16.62	10.15	0.16
Lobular CA	3	6.67	3.21	
Others	7	12.86	6.30	
Total	**55**	**15.60**	**9.75**	

In the present study, the peritumoral mean MCD was highest (16.62±10.15) in Infiltrating Ductal Carcinoma**.** p value calculated showed no significance between histological type and peritumoral MCD.

Table 19 : Association between histopathological type and intratumoral MVD

Histological Type	No of cases	Mean MVD	SD	p value
Infiltrating Ductal CA	45	78.89	28.0	0.15
Lobular CA	3	49.67	28.3	
Others	7	89.71	37.6	
Total	**55**	**78.67**	**29.830**	

In the present study, the intratumoral mean MVD was highest (89.71±37.6) in Other tumors(Medullary, Papillary and metaplastic Carcinoma)**.** p value calculated showed no significance between histological type and intratumoral MVD.

Table 20 : Association between histopathological type and peritumoral MVD

Histological Type	No of cases	Mean	SD	p value
Infiltrating Ductal CA	45	38.00	11.44	0.51
Lobular CA	3	30.67	9.29	
Others	7	38.43	17.70	
Total	**55**	**37.65**	**12.148**	

In the present study, the intratumoral mean MVD was highest (38.43±17.7) in Other tumors(Medullary, Papillary and metaplastic Carcinoma)**.** p value calculated showed no significance between histological type and peritumoral MVD.

Table 21 : Correlation between IntraTumoral MCD vs IntraTumoral MVD (Pearson correlation)

	No of cases	Mean	SD	Pearson correlation	p (2 tailed)
Intratumoral MCD	55	2.82	2.12	-0.217	0.11
Intratumoral MVD	55	78.67	29.830		

Pearson Correlation test done showed a negative correlation between intratumoral MCD and intratumoral MVD suggesting that the MCD decreases as MVD increases. However p value (>0.05) showed no significance between the two parameters.

Table 22 : Correlation between peritumoral MCD vs Peritumoral MVD (Pearson correlation)

	No of cases	Mean	SD	Pearson correlation	p (2 tailed)
Peritumoral MCD	55	15.60	9.75	0.39	0.003
Peritumoral MVD	55	37.65	12.148		

Pearson correlation test done shows a positive correlation between peritumoral MCD and peritumoral MVD suggesting that MCD increases as MVD increases. However p value (<0.05) showed strong significance between the two parameters.

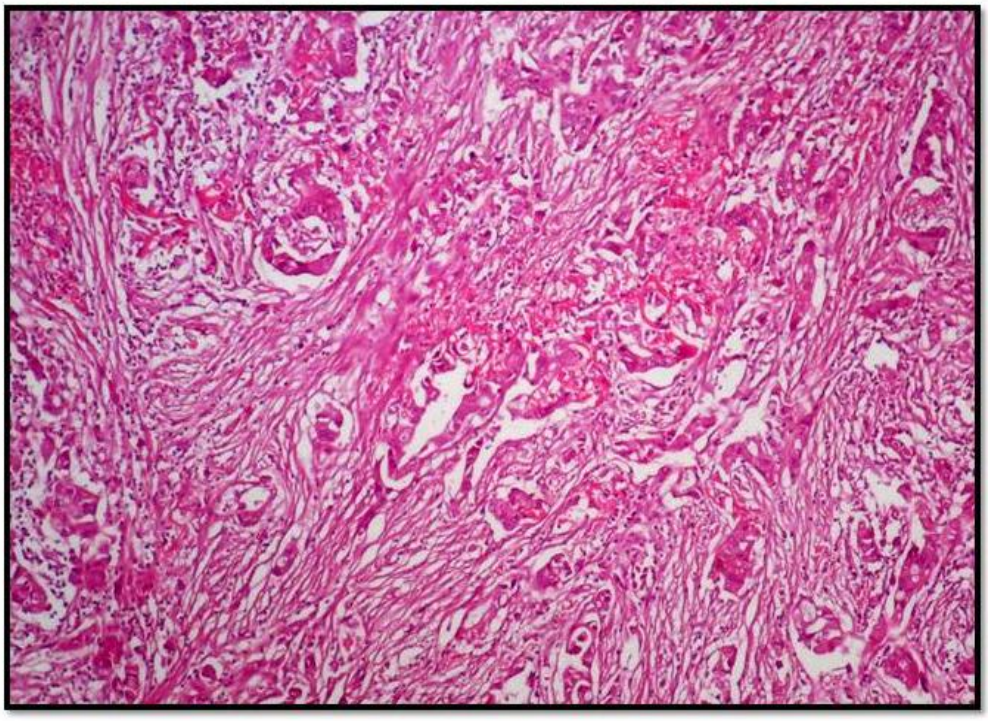


Figure 5: Photomicrograph showing Infiltrating ductal carcinoma

(H&E, 200X)

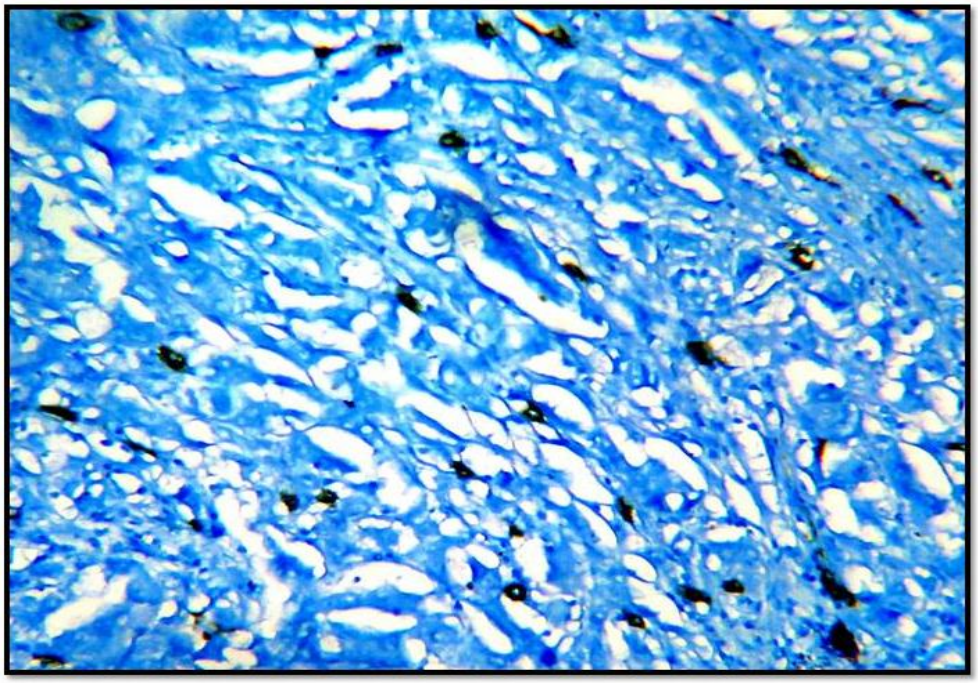


Figure 6 : CD 34 immuno stained microvessels in IDC (IHC, 200X)

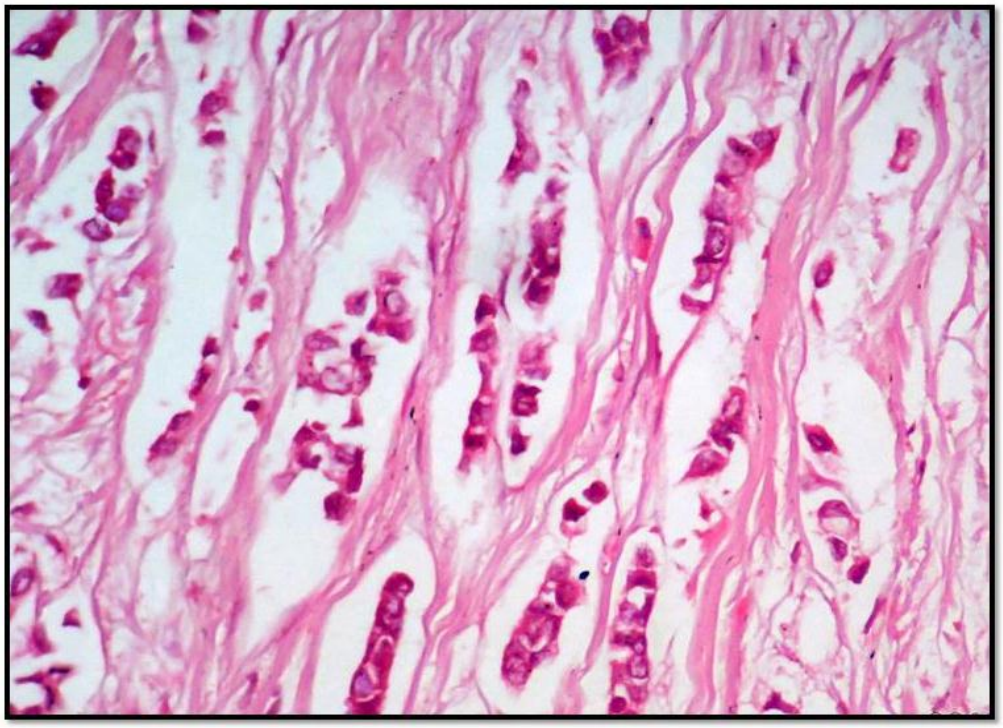


Figure 7 : Photomicrograph showing Invasive lobular carcinoma

(H&E, 200X)

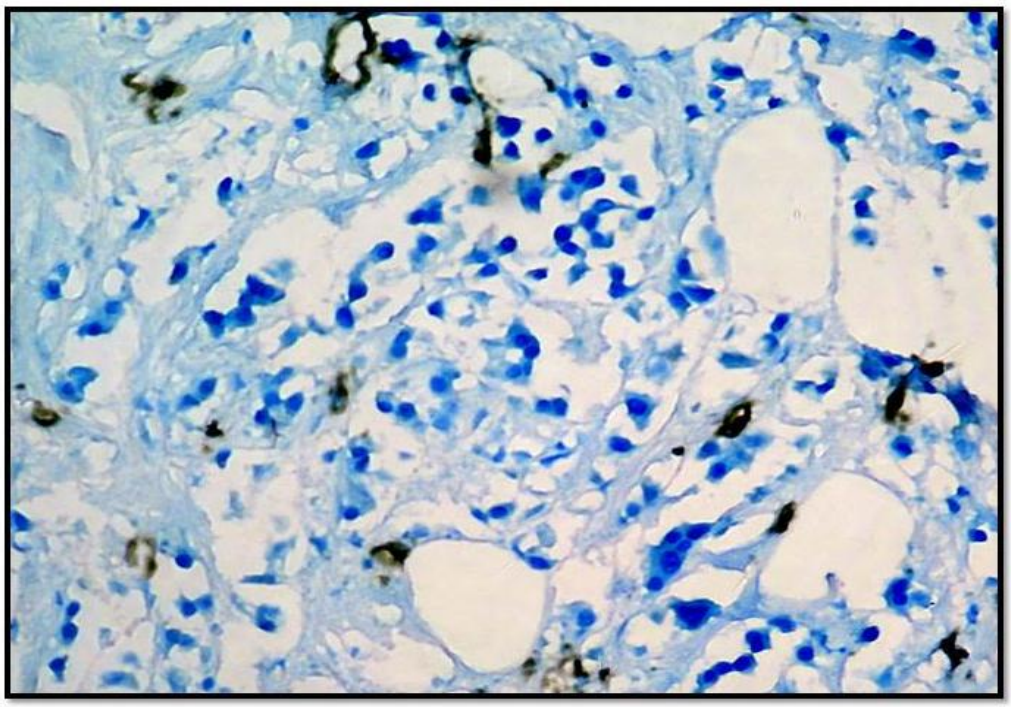


Figure 8 : CD 34 immuno stained microvessels in ILC (IHC, 200X)

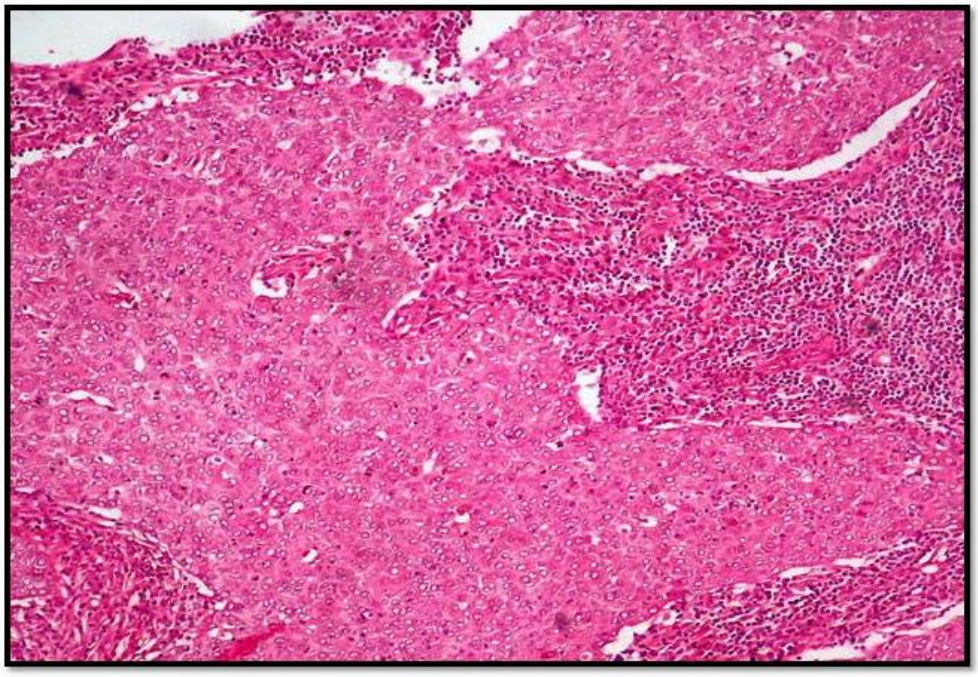


Figure 9 : Photomicrogragh showing Medullary carcinoma (H&E, 200X)

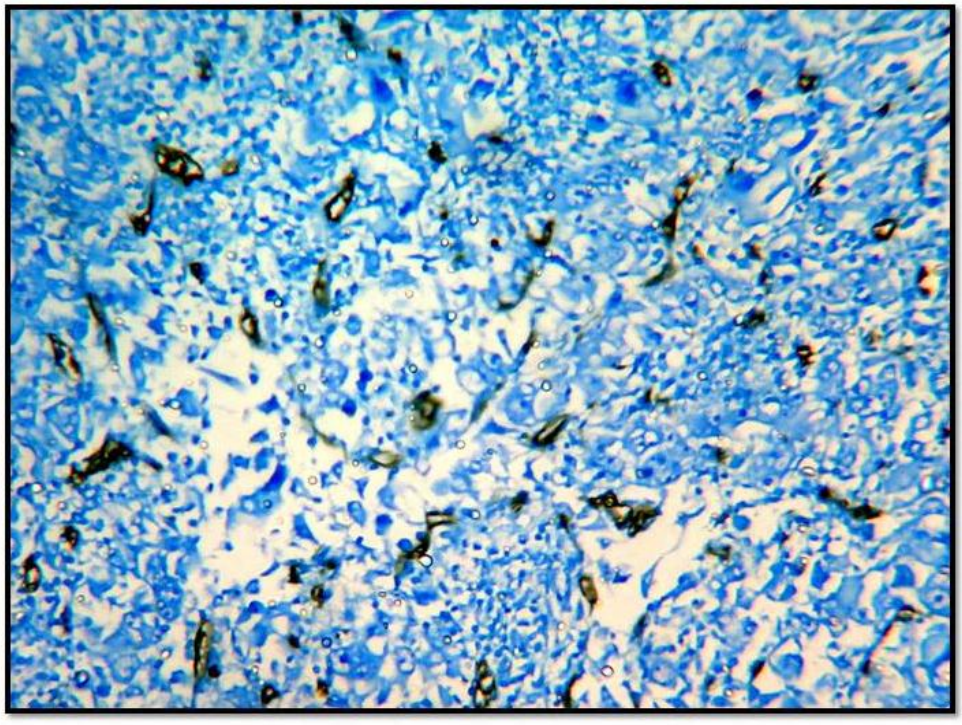


Figure 10 : CD 34 immuno stained microvessels in medullary Carcinoma (IHC, 200X)

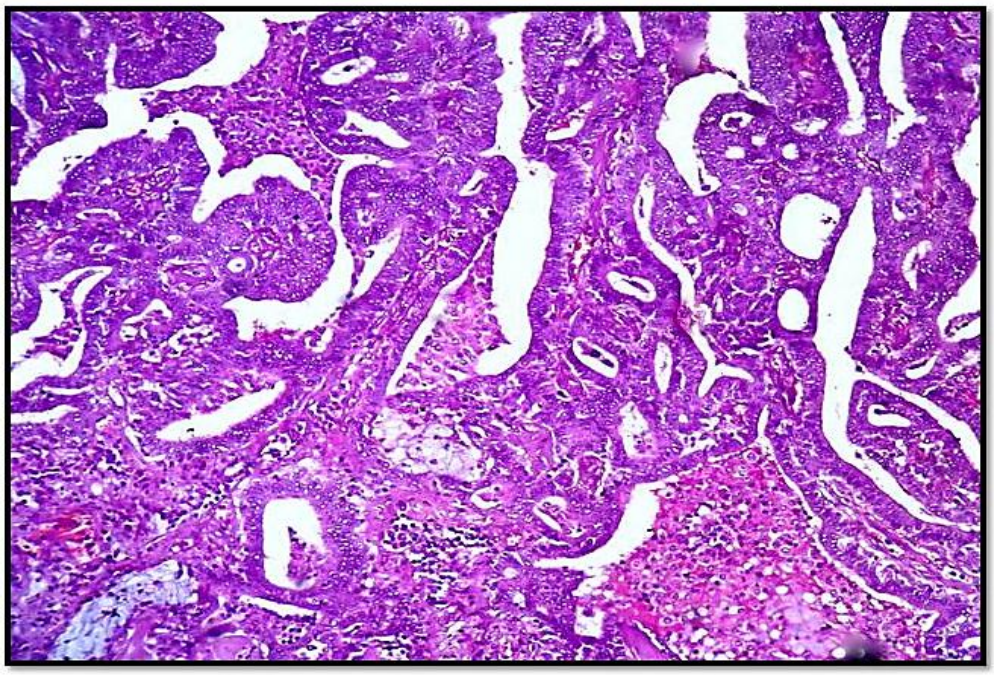


Figure 11 : Photomicrogragh showing Papillary carcinoma (H&E, 200X)

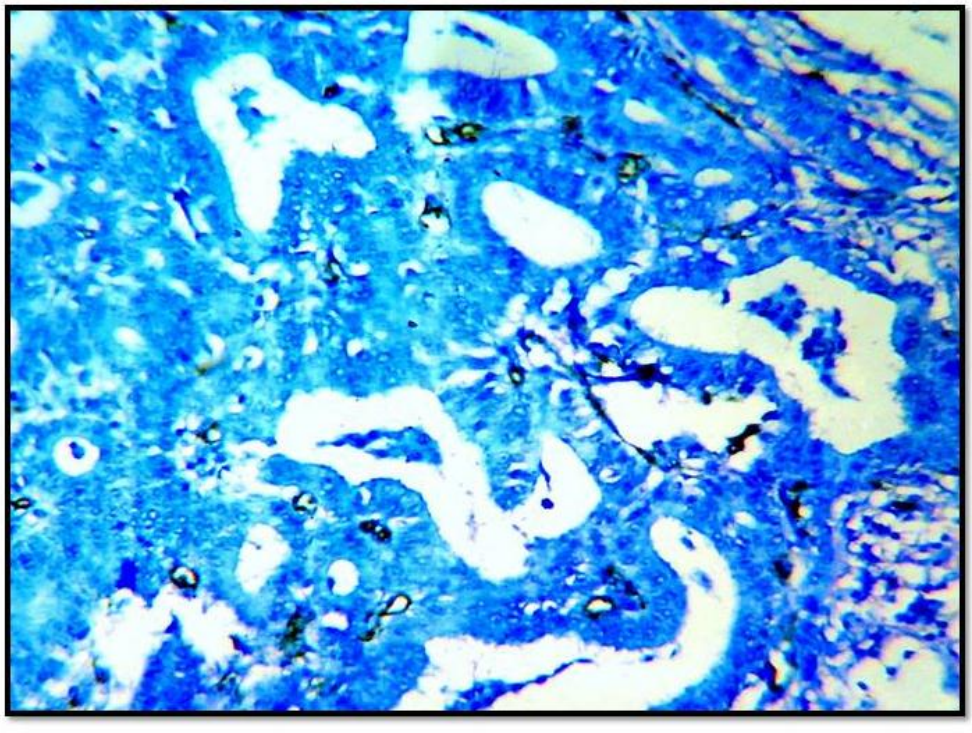


Figure 12 : CD 34 immuno stained microvessels in Papillary carcinoma (IHC, 200X)

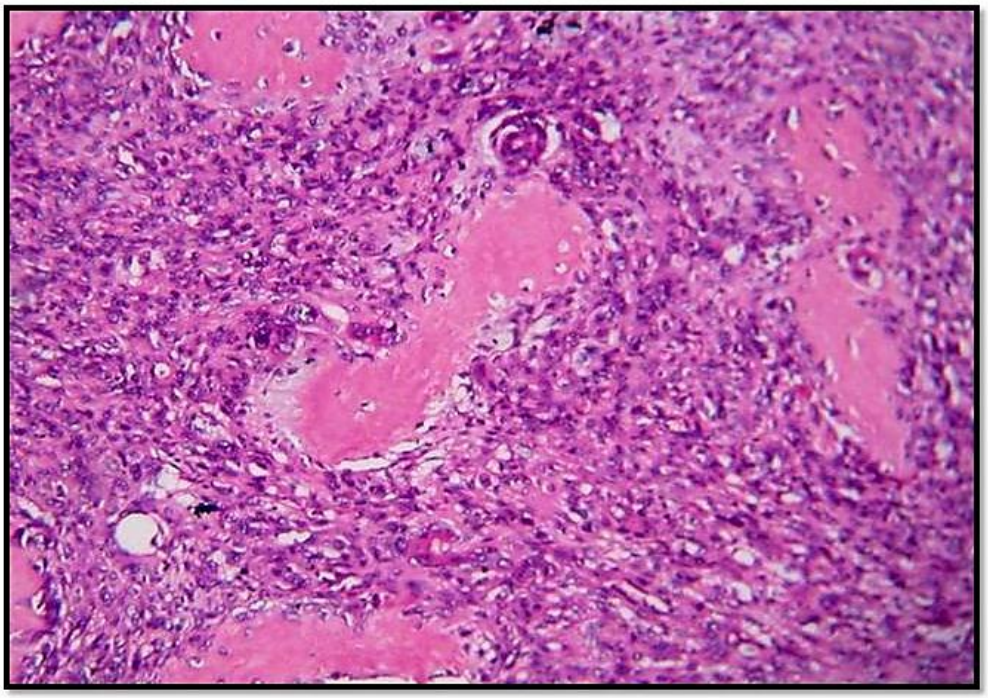


Figure 13 : Photomicrogragh showing Metaplastic Carcinoma (H&E,200X)

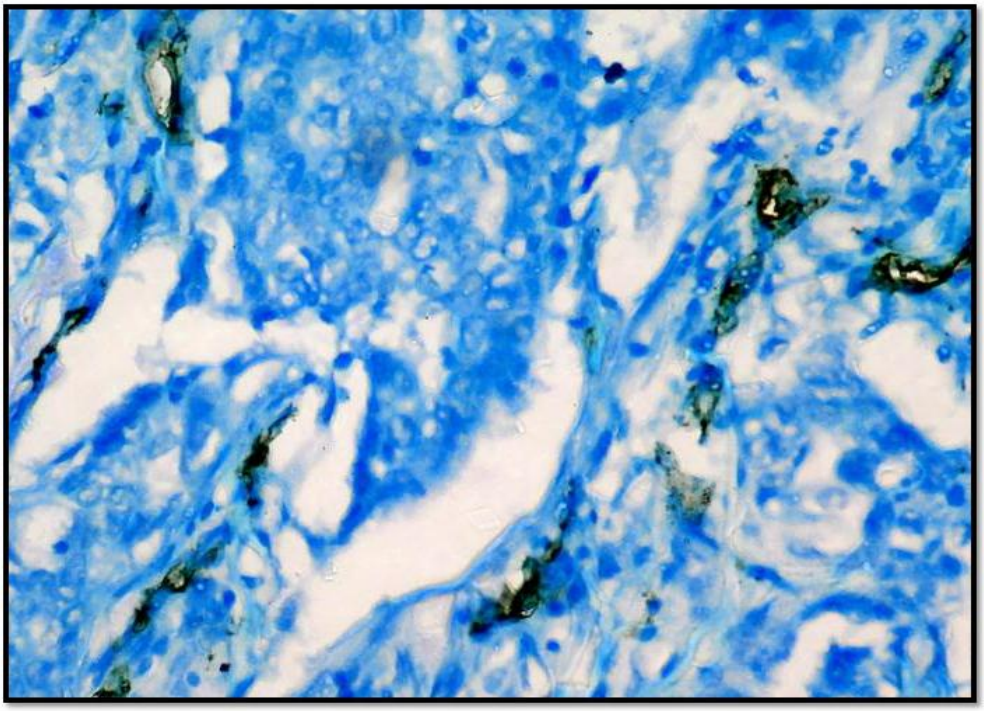


Figure 14 : CD 34 immuno stained microvessels in Metaplastic carcinoma (IHC, 200X)

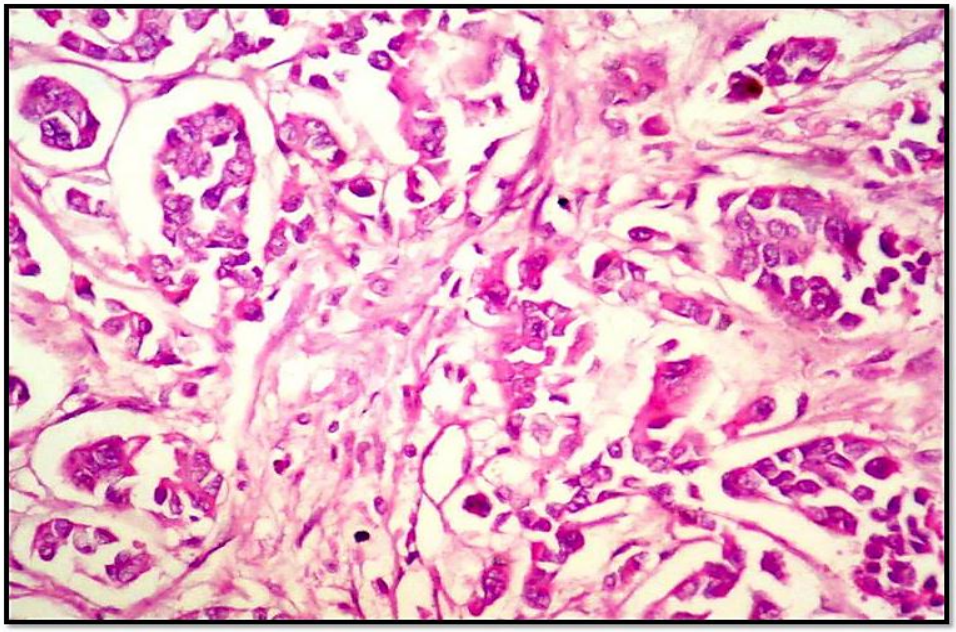


Figure 15 : Histological grade 1, showing tubule formation in > 75% of the tumor, mild nuclear pleomorphism and low mitotic count (H&E , 400X)

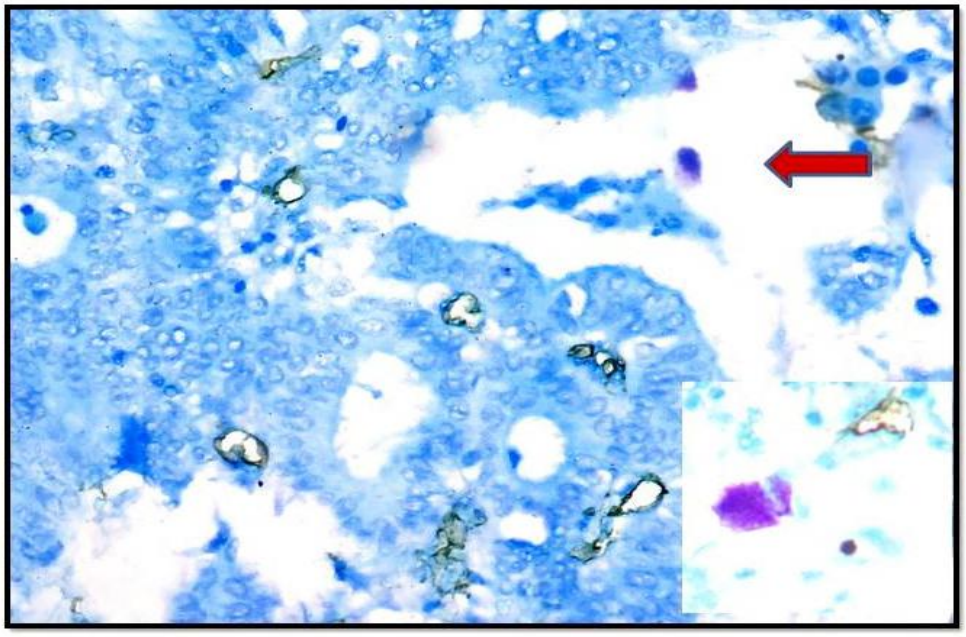


Figure 16 : CD 34 IHC slide counterstained with toluidine blue showing microvessels and mast cells (Arrow) in Grade 1 tumors(400X) with inset showing mast cells(1000 X)

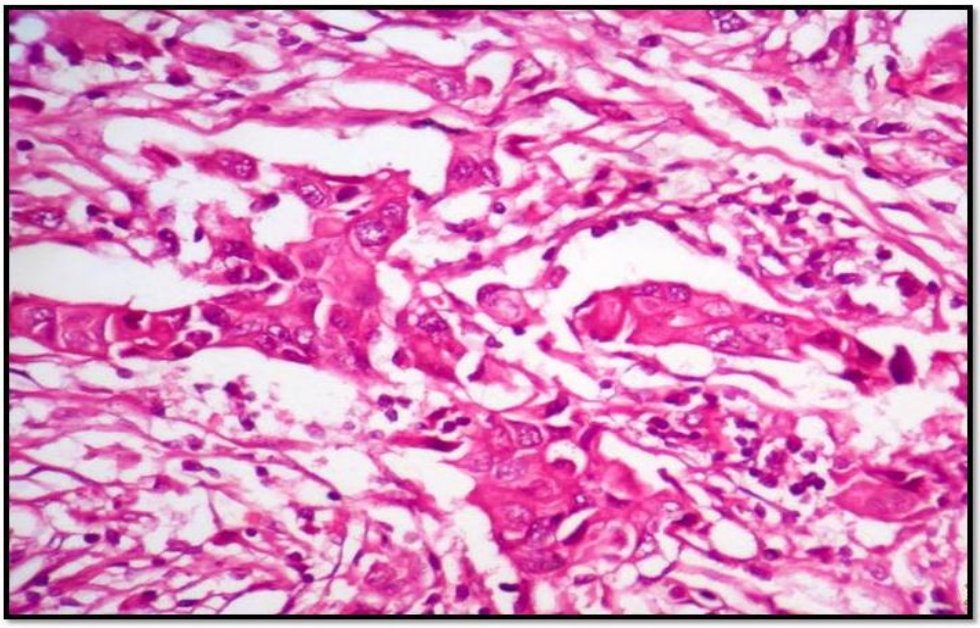


Figure 17: Histological grade 2 showing tubule formation in 10-75% of the tumor, moderate nuclear pleomorphism and few mitotic figures(H&E,400X)

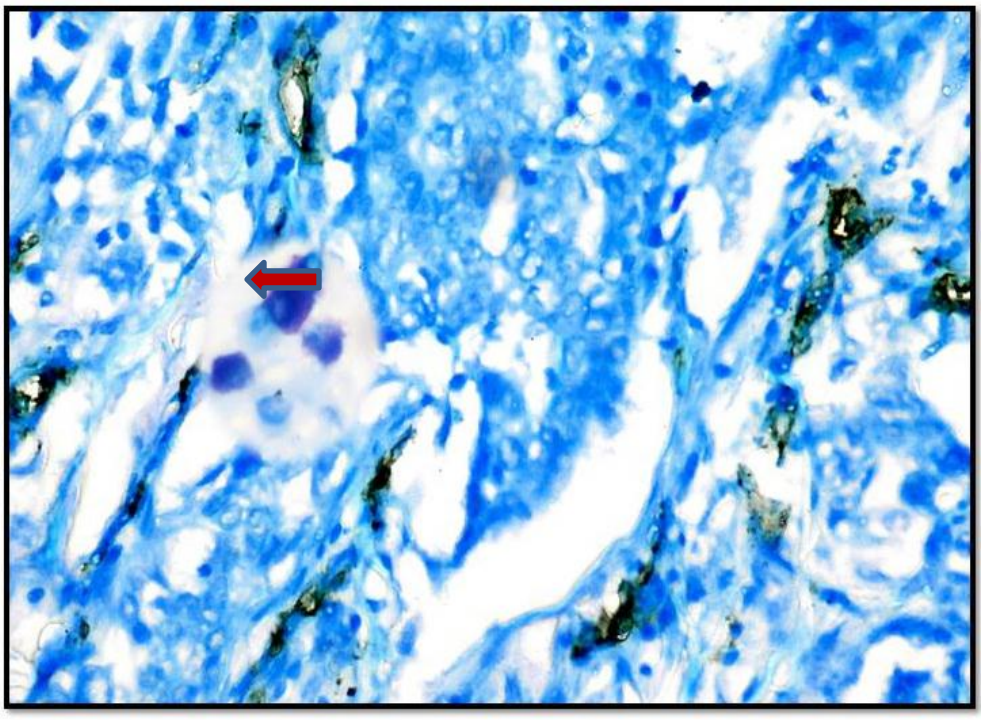


Figure 18: CD 34 IHC slide counterstained with toluidine blue showing microvessels and mast cells (Arrow) in grade 2 tumors(IHC, 400X)

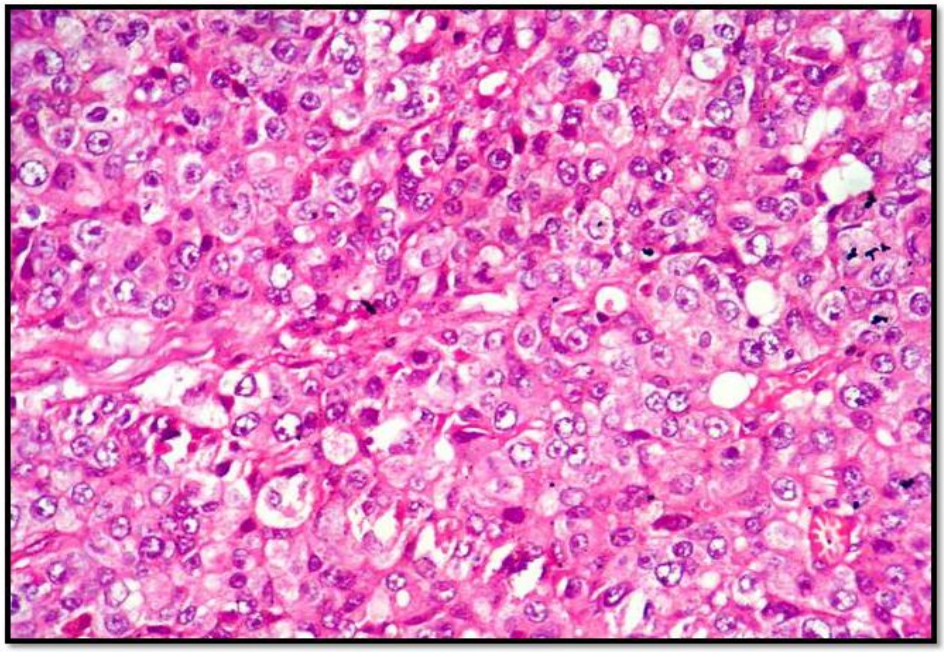


Figure 19: Histological grade 3, showing tumor cells arranged in sheets with marked nuclear pleomorphism and abundant mitotic activity (H&E,400X)

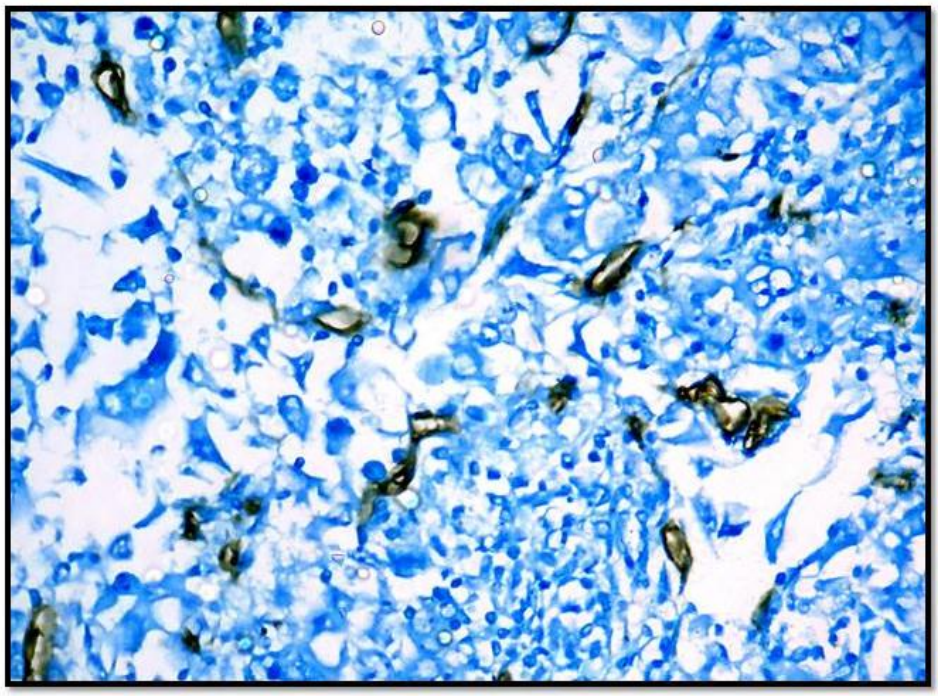


Figure 20 : CD 34 IHC showing microvessels grade 3 tumor (IHC, 400X)

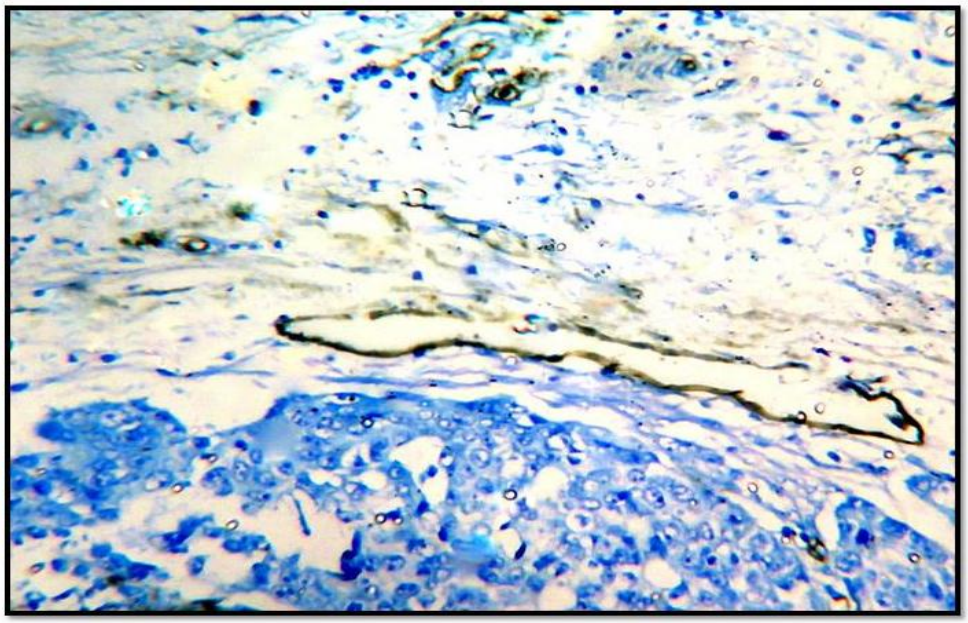


Figure 21 : CD 34 IHC slide showing both intratumoral and peritumoral areas (IHC, 200 X)

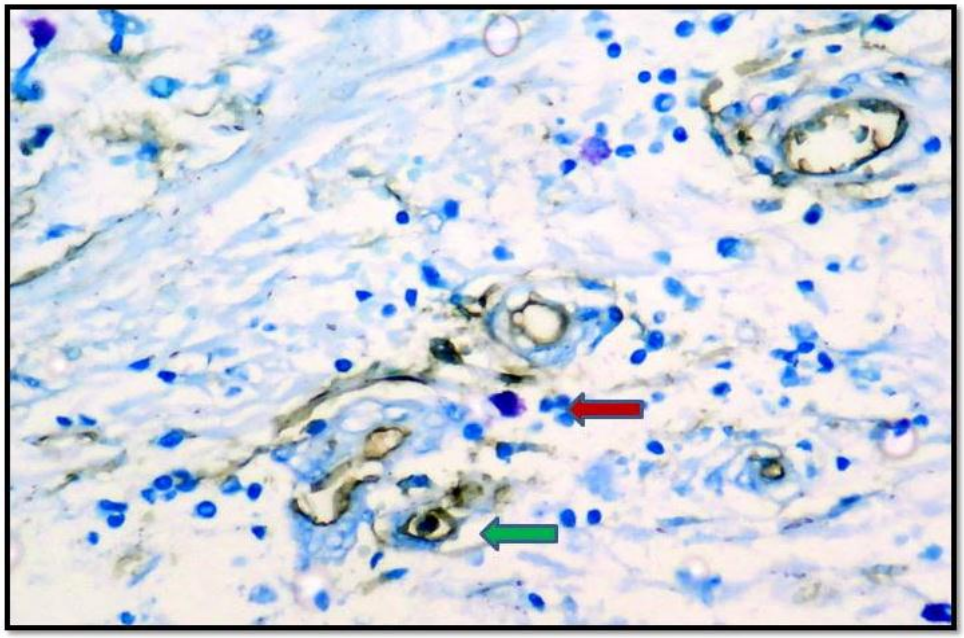


Figure 22 : CD 34 IHC slide counterstained with toluidine blue showing microvessels (green arrow) and mast cells (red arrow) in peritumoral area (IHC,400X)

DISCUSSION

Breast cancer is the leading cause of cancer deaths among women. Results from experimental studies suggest that tumor progression and metastasis are angiogenesis dependent.[78]

Angiogenesis is a highly regulated process balanced by inhibitors and stimulators of endothelial cell proliferation and endothelial cell migration. Methods to quantify the degree of tumor angiogenesis have shown to provide important prognostic information for the patients. This involves assessing the angiogenesis by light microscopic estimation of vascular density or micro vessel density on tissue sections probed for endothelial markers by immunohistochemistry (IHC).[79]

Experimental evidence suggest that the growth of tumor beyond a certain size requires angiogenesis for further expansion of tumor.[80] So the concept of antiangiogenic therapy as an alternative adjuvant to traditional anti-cancer therapies has attracted enormous attention for the past 3 decades.[81]

The present study was conducted from January 2012 to July 2013 in department of pathology, Sri Devraj Urs Medical College, Kolar. The total number of cases done during this period was 55 .

AGE DISTRIBUTION

Table 23 : Comparison of age distribution in different studies

	Bolat et al (2006)	Ching ESet al (2012)	Present study
Age range (years)	25-81	28-89	35 – 85
Mean Age	52.38±12.7	52.5±11.1	52.1±11.6

In the present study, age group ranged from 35 to 85 years, with mean age of 52.1±11.6 years, which was similar to the observations made by Ching ES et al [82] with age ranged 28-29 years and mean age being 52.5±11.1 years and Bolat et al [83] with age ranged 25- 81 years with mean age of 52.38±12.7 .Due to lack of screening programmes and awareness in our rural set up, patients usually present at the later age.

SEX DISTRIBUTION

Table 24 : Comparison of sex distribution with Different studies

Authors	**Sex distribution**			
	Female		**Male**	
	No of cases	**Percentage (%)**	**No of cases**	**Percentage (%)**
ChingES (2012) etal	93	98.9	1	1.1
Present study	54	98.2	1	1.8

In the present study 98.2 % were female and 1.8 % were male, similar observation was made by Ching SE et al [82] with 98.9% female and 1.2 % male cases.

LOCATION

Table 25 : Comparison of quadrant distribution with other studies

Quadrant	Meena (2005)et al		Lee (2005) etal		Present study	
	No of cases	Percentage (%)	No of cases	Percentage (%)	No of cases	Percentage (%)
Upper outer	54	54	217	62	24	43.6
Lower outer	12	12	30	9	8	14.5
Upper inner	5	5	61	18	13	23.6
Lower inner	12	12	18	5	4	7.3
Central	7	7	21	6	3	5.5
All	10	10	-	-	3	5.5
Total	100	100	347	100	55	100

In the present study, majority of the cases 43.6% presented with lump in the upper and outer quadrant. Similar observation was made in a studies done by Meena et al[84] and lee et al [85] with majority of the cases presenting with lump in the upper outer quadrant (54 % and 62 % resepectively).

TUMOR SIZE

Table 26 : Comparison of size of the tumor in different studies

	Ogaway (1995)etal		Kwon GY(2005)etal		Present study	
	No of cases	**Percentage (%)**	**No of cases**	**Percentage (%)**	**No of cases**	**Percentage (%)**
<2cm	65	42	11	24.5	2	3.6
2-5cm	74	47.7	25	55.5	38	69.1
>5cm	16	10.3	9	20	15	27.3
Total	**155**	**100**	**45**	**100**	**55**	**100**

In the present study majority of the cases (69.1%) had tumor size between 2 and 5 cm, followed by tumor size greater than 5 cm (27.3%).Similar findings were observed in studies by Ogaway et al [36] and Kwon GY et al [86] with majority of their cases also having tumor size between 2 to 5cm (47 and 55% respectively).Due to lack of awareness of self-breast examination and breast cancer screening programmes, majority of our cases presented with tumor size more than 2 cms.

HISTOPATHOLOGICAL TYPE

Table 27 : Comparison of histopathological types with other studies

Author	Medri (2000)et al		Erdogan N(2009) etal		Present study	
	No of cases	Percentage %	No of cases	Percentage %	No of cases	percentage %
IDC	301	82.2	27	89	45	82
Lobular	30	8.2	4	12.9	3	5.4
Others	35	9.6	-	-	7	12.72
Total	366	100	31	100	55	100

In the present study, infiltrating ductal carcinoma (IDC) was seen in majority of our cases comprising of 82 %. In a study done by Medri et al [87] similar observations were seen with majority of the cases (82.2 %) being Infiltrating ductal carcinoma. Another study by Erdogan N et al [88] showed similar observations with majority of the cases (89%) being IDC.

HISTOPATHOLOGICAL GRADING

Table 28 : Comparison of histological grading with other studies

Histological Grade	Ching SE (2012) et al		Verma MS (2013)etal		Present study	
	No of cases	percentage %	No of cases	percentage %	No of cases	percentage %
Grade 1	10	10.6	30	31.2	11	20
Grade 2	35	37.2	24	25	38	69.1
Grade 3	49	52.1	42	43.7	6	10.9
Total	**94**	**100**	**96**	**100**	**55**	**100**

In the present study, Nottingham modification of Bloom Richardson grading system was used. Out of 55 cases, 20% were Grade 1 tumors and 69.1% were in Grade 2 tumors and 10.9% were Grade 3 with majority of the cases being Grade 2. However in studies done by Varma MS et al[89] and Ching SE et al [82] showed majority of their cases (43 and 52% respectively) being Grade 3.

Microvessel Density(Intratumoral MVD) with Grade of tumor

Table 29 : Comparison of MVD with grade of tumor in different studies

Authors	Grade 1 Mean MVD±SD	Grade 2 Mean MVD±SD	Grade 3 Mean MVD±SD
Kwon GY(2005) et al	63.5 ± 57.01	101.09 ± 41.64	104.36 ± 53.15
Bolat (2006)et al	14.7 ± 3.2	67.2 ± 17.4	85.3 ± 21.3
Safwat MD(2009) et al	56.3±7.63	88.9± 7.53	96.12 ±7.18
Present study	**51.18 ±23.1**	**78.21 ±20.4**	**132 ±18.7**

In present study mean intratumoral MVD was found to be highest in Grade 3 tumors (132 ±18.7) followed by Grade 2 (78.21 ±20.4) and Grade 1 (51.18 ±23.1) respectively. Similar findings were noted in studies by Safwat et al [78] (96.12 ±7.18), Bolat et al [83] (85.3 ± 21.3) and Kwon et al [86](104.36 ± 53.15) showing mean MVD highest in Grade 3 tumors. All studies above showed mean MVD increasing as the grade increases.

This can be explained by the hypothesis put forward by Noel Weidner who stated association between intratumoral microvessel density and tumor aggressiveness. He suggested that a high grade tumor is usually a solid tumor with two interdependent components i.e. malignant cells and stroma and measuring microvessel density represents the capacity of the particular tumor in enhancing the tumor angiogenesis by converting stromal cells into endothelial cells.[34]

Brem et al were among the first to suggest that the intensity of intratumoral angiogenesis may correlate with tumor grade and aggressiveness.[40]

In breast carcinoma, intratumoral endothelial cells proliferate 45 times faster than the endothelial cells in adjacent benign stroma. Rate of progression is associated with increased intratumoral micro vessel density, a morphological measure of tumor angiogenesis. [49, 50]

Microvessel Density(Peritumoral MVD) with Grade of tumor

In the present study, the peritumoral mean MVD was highest (48.5 ±14.9) in Grade3 tumors, followed by (38.0±10.9) in Grade 2 and 30.4±10.7 in Grade 1 tumors. The value decreased as the tumor grade decreased. p value (0.01) showed a high significance between peritumoral MVD and histological grade. However Peritumoral MVD has not been documented in breast carcinoma cases occurring in Indian population. As explained previously intratumoral MVD had a positive association with grade. Similarly it can be hypothesized that peritumoral MVD showed positive association with grade.

Mast cell Density (Intratumoral MCD) with grade of the tumor

In the present study, Mean MCD was highest (3.27±2) in Grade 1 tumors and the value decreased as the grade increased. The role of intratumoral mast cells in breast carcinoma has limited literature.

In a study done by Amini et al stated that the presence of mast cells was associated with low grade tumors and correlated with favorable prognosis in breast cancer.[58]

Mast cells are attracted to the tumor by tumor derived chemo attractants where they degranulate to release potential tumor cytotoxic compounds depending on local tumor conditions.[75] They also release certain mediators like TNF α, IL- 1 and IL -6 which has inhibitory effect on tumor growth and angiogenesis.[67]

These findings might indicate a protective effect of mast cells, possibly exerting a cytotoxic effect on the tumor cells. However, the studies are still few and further investigations are needed in order elucidate the precise role of mast cells in the tumorigenesis.[58]

Mast Cell Density(Peritumoral MCD) with Grade of tumor

Table 30 : Comparison of MCD with grade of tumor in other studies

	Kwon GY (2005)et al **Mean MCD ±SD**	**Present study** **Mean MCD ±SD**
Grade 1	25.13 ± 6.75	16.6±11.12
Grade 2	39.43 ± 21.03	15.2±9.66
Grade 3	43.07 ± 25.66	12.5±6.92

In the present study, the peritumoral mean MCD was highest (16.6±11.12) in Grade1 tumors, followed by Grade 2 (15.2±9.66) and Grade 3 (12.5±6.92). The value decreased as the tumor grade increased. However in a study done by Kwon GY et al[86], the peritumoral mean MCD was highest in Grade 3 (43.07 ± 25.66) tumors and the value increased as the tumor grade increased.

Regarding the presence of mast cells in the peritumoral stroma of invasive breast carcinomas, few studies have been done and there was evidence indicating that mast cells accumulate around tumors and they could inhibit tumor growth depending on the local stromal conditions. [61,75]

Dabiri et al observed that the presence of mast cells in the stroma correlated with good prognosis in breast carcinoma.[41]

The result in the present study is supported by the theory put forward by Heidarpour M et al suggesting presence of mast cells was associated with low tumor grade which is associated with favorable prognostic factor. [76]

The controversy in results between different studies might be attributed to those mast cells accumulation around tumors, could either promote or inhibit tumor growth depending on the local stromal conditions.

The role of stromal mast cells for prognostic significance in breast cancer warrants further studies

Microvessel Density(Intratumoral MVD) with Histological type of tumor

Table 31 : Comparison of MVD with histological type in other studies

Histological Type	Bolat (2006) et al Mean MVD ±SD	Present study Mean MVD ±SD
Infiltrating ductal CA	60 .7 ± 29.9	78.89±28.0
Lobular CA	41.2 ± 12.9	49.67±28.3
Others	-	89.71±37.6

In the present study, intratumoral mean MVD (89.71±37.6) was highest in other tumors (Medullary, Papillary and metaplastic Carcinoma). However when only IDC and ILC were compared similar findings were noted in studies by Bolat et al[83] showing mean MVD to be higher in IDC than ILC.

Correlation between peritumoral MCD vs Peritumoral MVD

Pearson Correlation test done shows a positive correlation between peritumoral MCD and peritumoral MVD suggesting that the MCD increases as MVD increases. One of the possible explanation could be: Peritumoral area has increased mast cell density. These mast cells secrete proangiogenic factors which supports angiogenesis aiding tumor growth. Hence raised MVD is seen in peritumoral areas. However, the studies are still few for comparison and further investigations are needed .

Correlation between IntraTumoral MCD vs IntraTumoral MVD

Pearson Correlation test done showed a negative correlation between intratumoral MCD and intratumoral MVD suggesting that the MCD decreases as MVD increases, which can be explained by the angiogenic factor synthesis by tumor cells which act by autocrine pathway and hence leading to increase MVD in intratumoral site, which is independent of the proangiogenic factors produced by mast cells.

SUMMARY

- The present study was conducted in Department of Pathology, Sri Devraj Urs Medical College, Kolar from January 2012 to July 2013.
- A total of 55 cases were studied of which 54 were females and one was male patient.
- Majority of patients belonged to 41 - 50 years which constituted 36%.
- Majority of the lumps 31(56.4%) were seen in right breast and 24 (43.6%) were in left breast.
- Out of 55 cases, majority 24 (43.6%) were located in upper outer quadrant of the breast
- Majority of the cases 38(69.1%), had tumor size between 2-5cm
- Infiltrating ductal carcinoma was seen in majority 45 (82%) cases, followed by medullary carcinoma 5(9%), invasive lobular in 3(5.4%) and metaplastic and papillary seen in 1(1.8%) case each.
- Majority of the tumors were in histological grade 2
- Vascular invasion was seen in 16(29.1%) cases
- DCIS component was seen in 13(23.6%) cases
- Out of 45 cases, lymphnode metastasis was seen in 29 cases
- Intratumoral and peritumoral mean MCD was highest (3.27±2 and 16.64±11.12) respectively in Grade 1 tumors and the value decreased as the tumor grade increased. However p value(>0.05) showed no significance between intratumoral and peritumoral MCD with grade of tumor.

- Intratumoral and peritumoral mean MVD was highest (132 ±18.7 and 48.50 ±14.95) in Grade 3 tumors respectively and the value decreased as the tumor grade decreased. P (< 0.05) value showed a strong significance between MVD and histological grade of tumor**.**
- Intratumoral and peritumoral mean MCD was highest(3.13±2.91and 16.62±10.15) in Infiltrating ductal Carcinoma respectively. P (>0.05) value calculated showed no significance between histological type and MCD.
- Intratumoral and peritumoral mean MVD was highest (89.71±37.6 and 38.43±17.7) respectively in other tumors (Medullary, Papillary and metaplastic Carcinoma). P value (>0.05) calculated showed no significance between histological type and MVD.
- Pearson Correlation test done shows a negative correlation between intratumoral MCD and intratumoral MVD. P value(>0.05) showed no significance between the two parameters.
- Pearson Correlation test done shows a positive correlation between peritumoral MCD and peritumoral MVD. p value(<0.05) showed strong significance between the two parameters.

CONCLUSION

The study has been undertaken to know the role of mast cells and microvessels in breast carcinoma and also to see whether there is correlation between Mast cell density and Micro vessel density with histological type and grading in breast carcinoma.

In the present study, intratumoral and peritumoral mean MVD was highest in grade 3 tumors suggesting that intensity of tumor angiogenesis correlates with tumor grade.

In the present study, intratumoral and peritumoral mean MCD was highest in grade 1 (low grade) tumors suggesting the protective effect of mast cells which exerts a cytotoxic effect on the tumor cells

In the present study intratumoral MVD was seen to be higher in infiltrating ductal carcinoma than infiltrating lobular carcinoma.

There was a positive correlation between peritumoral MCD and peritumoral MVD in our study suggesting an increase in angiogenesis for tumor growth and progression in response to angiogenic factors produced by mast cells and other stromal cells.However a negative correlation between intratumoral MCD and intratumoral MVD was seen in our study which can be explained by angiogenesis (MVD) via proangiogeneic factors synthesized by tumor cells, which is independent of the proangiogenic factors produced by mast cells.

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ANNEXURE 1

PROFORMA

"Significance of mast cell density and Micro vessel density in breast carcinoma"

Case No:

Name:

Age: Sex: IP/OP no: Biopsy no:

Presenting complaints:

General Physical examination:

Pallor - Icterus -

Local examination of breast:

Side: Site:

No of lesions:

Gross: polypoidal /Ulcerative / infiltrative /fungating:

Size:

Consistency:

Skin changes:

Lymphadenopathy:

Group: Level:

No of LN involved:

Unilateral /Bilateral:

Size: consistency:
mobility:

History of Neoadjuvant chemotherapy received :

Nature of specimen: Biopsy / mastectomy

Side:

Gross features:

Measurements:

Whole -

Skin -

Cut surface:

Description of tumor:

Size:

Type:

Color and consistency:

Margins:

Histopathological Diagnosis:

Histological Type:

Histological Grading:

Vascular invasion:

Associated with DCIS: Yes /No

Axillary lymph node:

Total number examined:

No of are positive:

PTNM staging

Nottingham prognostic index: (0.2 x size of tumor) + LN s grade +grade =

(Nodes graded as no of nodes positive: - 0=1, 1-3=2 ,>3 =3)

ER/PR Status:

Immunohistochemistry (IHC)

Mast cell Density: Toluidine blue

No of mast cells /200X field :

Intratumoral -

Peritumoral -

Micro vessel Density: CD 34

No of micro vessels /200 Xfield:

Intratumoral -

Peritumoral -

Signature of Guide : Signature of candidate:

ANNEXURE 2

TOLUIDINE BLUE SOLUTION PREPARATION

Preparation of 0.1 percent Toluidine stain.

- **Solution A:**

  Toluidine blue-1gm.

  70% alcohol -100 ml.

- **Solution B:**

  Sodium chloride– 0.5 gm.

  Distilled water – 50 ml.

Mix the Solutions A (5 ml) and B (45 ml).

Adjust pH to 2.0~2.5 using glacial acetic acid or HCl

Solution is prepared freshly

Case number	Hospital number	Biopsy number	Age	Sex	Lump					Lymphnode	Nature of specimen Received	Size of the tumor	Histopathological Type	Histopathological grading			Final grade	Vascular invasion	Association with DCIS	Total no of lymph nodes	Number of lymph nodes positive	Pathological(TNM) staging	Immunohistochemistry(IHC)			
					Side	Quadrant	Size(cm)	Consistency	Skin over lump					Tubule formation	Nuclear Atypia	Mitosis/10 HPF							Mast cell density(MCD)/200 X field(3 areas)		Microvessel density (MVD)/ 200 X field (3 areas)	
																							Intratumoral	Peritumoral	Intratumoral	Peritumoral
1	401920	B/169/8	55	F	R	UO	3X2	firm	Normal	Axillary	Modified Radical Mastectomy	2 to 5	Medullary Carcinoma breast	3	2	2	2	No	No	2	0	pT2N0Mx	1	7	71	55
2	423709	B/577/8	40	F	L	Central	7X4	firm	Normal	Axillary	Modified Radical Mastectomy	>5	Infiltrative ductal carcinoma(NOS)	2	3	2	2	No	No	4	0	pT3N0Mx	1	12	80	31
3	426831	B/624/8	62	F	L	UO	4X4	firm	Normal	Axillary	Modified Radical Mastectomy	2 to 5	Infiltrative ductal carcinoma(NOS)	2	1	1	1	No	No	4	0	pT1cN0Mx	6	4	77	25
4	486180	B/633/8	35	F	R	UO	3X2	firm	Normal	Axillary	Modified Radical Mastectomy	2 to 5	Infiltrating ductal carcinoma(NOS)	3	3	3	3	No	No	10	0	pT2N0Mx	5	22	128	61
5	428052	B/679/8	60	F	R	UI	4X4	firm	Ulcerated	Axillary	Modified Radical Mastectomy	2 to 5	Infiltrating ductal carcinoma (NOS)	2	3	2	2	yes	No	4	3	pT3N1cMx	3	14	72	38
6	235811	B/763/8	50	F	L	UI	3X2	firm	Normal	Axillary	Modified Radical Mastectomy	2 to 5	Infiltrative ductal carcinoma(NOS)	2	2	2	2	yes	No	11	4	pT2N2aMx	3	19	67	30
7	435996	B/820/8	47	F	R	All	8X2	soft - firm	Normal	Axillary	Modified Radical Mastectomy	>5	Infiltrative ductal carcinoma(NOS)	3	2	2	2	yes	yes	14	2	pT3N1cMx	1	7	77	30
8	450944	B/1235/8	50	F	R	UO	2X1	soft - firm	Normal	No	Mastectomy	2 to 5	Infiltrative ductal carcinoma(NOS)	1	2	1	1	No	yes	Nil	Nil	pT2 No Mx	3	32	59	45
9	476818	B/60/9	57	F	R	LO	3X2	soft	Normal	No	Mastectomy	2 to 5	Infiltrating ductal carcinoma(NOS)	2	2	1	1	No	No	Nil	Nil	pT2NxMx	3	10	48	30
10	480183	B/162/9	50	F	L	UO	6x4	firm	Normal	Axillary	Modified Radical Mastectomy	>5	Infiltrating ductal Carcinoma(NOS)	2	2	2	2	yes	No	5	1	pT4b N1a Mx	5	12	82	28
11	495033	B/674/9	53	F	R	LO	5X3	Soft	Normal	Axillary	Modified Radical Mastectomy	>5	Medullary Carcinoma breast	3	3	2	3	No	No	12	0	pT3 N0 Mx	2	18	120	68
12	532827	B/1924/9	43	F	L	UI	3X2	soft - firm	Normal	Axillary	Modified Radical Mastectomy	2 to 5	Infiltrating ductal carcinoma(NOS)	2	3	2	2	No	yes	9	2	pT3N1Mx	5	27	64	42
13	528743	B/2037/9	40	F	R	All	8X6	firm	Normal	Axillary	Modified Radical Mastectomy	>5	Infiltrating ductal carcinoma(NOS)	2	3	2	2	No	No	2	2	pT4c N1 Mx	3	26	63	49
14	544388	B/2219/9	51	F	L	UO	5X3	firm	Normal	Axillary	Modified Radical Mastectomy	>5	Infiltrating ductal Carcinoma(NOS)	2	2	2	2	yes	No	6	6	pT4b N2a Mx	2	16	69	31
15	506115	B/2350/9	45	F	L	UI	3X2	firm	Normal	Axillary	Radical Mastectomy	2 to 5	Infiltrating ductal Carcinoma(NOS)	2	2	2	2	No	No	6	1	pT2 N1 Mx	2	38	111	82
16	567207	B/64/10	60	F	L	UO	3X2	firm	Normal	Axillary	Modified Radical Mastectomy	2 to 5	Infiltrating ductal Carcinoma(NOS)	2	2	2	2	NO	NO	6	1	pT2N1aMx	5	19	53	39
17	555767	B/69/10	48	F	R	LO	5X4	firm	fungating	No	Mastectomy	>5	Infiltrating ductal Carcinoma(NOS)	3	2	2	2	No	yes	Nil	Nil	pT3NxMx	2	49	70	42
18	566573	B/80/10	85	F	R	UO	6x4	firm - hard	ulcerated	No	Mastectomy	2 to 5	Infiltrating ductal Carcinoma(NOS)	1	2	1	1	No	No	Nil	Nil	pT3NxMx	3	16	53	30
19	566847	B/241/10	70	F	L	LO	5X4	firm	Nodule	Axillary	Modified Radical Mastectomy	>5	Infiltrating ductal Carcinoma(NOS)	2	2	2	2	No	No	5	0	pT3 NoMx	1	27	101	65
20	576341	B/411/10	50	F	R	UO	2X2	firm	Normal	Axillary	Modified Radical Mastectomy	2 to 5	Infiltrating ductal Carcinoma(NOS)	3	2	2	2	No	No	4	0	pT2N0Mx	1	8	55	27
21	599241	B/1290/10	36	F	R	UI	3X2	firm	Normal	Axillary	Modified Radical Mastectomy	2 to 5	Infiltrating ductal Carcinoma(NOS)	2	2	2	2	No	yes	4	1	pT2 N1Mx	2	25	73	53
22	637467	B/2373/10	47	F	R	UO	3X2	firm	Normal	Axillary	Modified Radical Mastectomy	2 to 5	Infiltrating ductal Carcinoma(NOS)	2	2	2	2	No	No	4	1	pT2 N1a Mx	8	29	79	42
23	649523	B/2553/10	60	F	R	LO	3X2	firm	fungating	Axillary	Modified Radical Mastectomy	2 to 5	Medullary Carcinoma breast	3	2	2	2	No	No	Nil	Nil	pT1c NxMx	1	21	67	31
24	660835	B/2748/10	50	F	L	LI	4X3	firm	Normal	Axillary	Modified Radical Mastectomy	>5	Infiltrating ductal carcinoma(NOS)	2	2	2	2	No	No	5	0	pT3N0Mx	5	17	83	39
25	659784	B/57/11	60	F	L	UO	4X2	firm	Normal	Axillary	Modified Radical Mastectomy	>5	Infiltrating ductal carcinoma(NOS)	2	2	1	1	No	yes	2	2	pT4bN1Mx	2	33	29	25
26	689026	B/525/11	50	F	R	UO	6x4	firm	Normal	Axillary	Modified Radical Mastectomy	>5	Infiltrating ductal carcinoma(NOS)	3	2	2	2	yes	No	9	7	pT3N2aMx	8	21	56	40
27	686600	B/545/11	37	F	R	UO	3X2	Hard	Normal	Axillary	Modified Radical Mastectomy	<2	Metaplastic carcinoma	2	3	2	2	No	No	1	1	pT1c N1Mx	1	16	84	39
28	690510	B/684/11	45	F	R	UI	6x4	Hard	Normal	Axillary	Modified Radical Mastectomy	>5	Infiltrating ductal Carcinoma(NOS)	3	3	2	3	yes	No	7	3	pT3N1Mx	2	11	103	35
29	716752	B/1504/11	55	F	R	UO	4X3	firm	Normal	Axillary	Modified Radical Mastectomy	2 to 5	Infiltrating ductal carcinoma(NOS)	2	3	1	2	No	No	6	1	pT2N1aMx	3	17	49	36
30	722385	B/1601/11	75	F	R	LO	5X7	firm	Normal	Axillary	Modified Radical Mastectomy	2 to 5	Medullary Carcinoma	2	2	2	2	No	No	5	0	PT2N0mx	1	3	95	26
31	738156	B/2112/11	55	F	R	UO	10x7	Hard	Retraction	Axillary	Modified Radical Mastectomy	2 to 5	Infiltrating ductal carcinoma	2	2	1	1	No	yes	10	2	pT2N1Mx	1	14	99	37
32	771600	B/436/12	51	F	L	UI	3X2.5	firm	Normal	Axillary	Modified Radical Mastectomy	2 to 5	Infiltrating ductal carcinoma(NOS)	2	2	2	2	yes	No	10	10	pT2 N2b Mx	5	13	99	37
33	781356	B/457/12	57	F	R	Central	4X4	firm-hard	Ulceration	Axillary	Modified Radical Mastectomy	2 to 5	Infiltrating ductal carcinoma(NOS)	2	3	1	2	yes	No	6	5	pT2N2aMx	1	3	63	30
34	784592	B/486/12	48	F	R	LI	5X4	firm	Normal	Axillary	Modified Radical Mastectomy	2 to 5	Infiltrative ductal carcinoma(NOS)	2	2	1	1	No	yes	4	0	pT2N0Mx	4	6	54	35
35	795926	B/843/12	37	F	L	UI	8X6	firm-hard	Normal	Axillary	Medial quadrentectomy	2 to 5	Infiltrtaive ductal carcinoma(NOS)	2	2	2	2	No	No	2	0	pT1c N0Mx	1	9	135	36
36	808270	B/1138/12	80	M	L	LO	4x3	Soft	Normal	NO	Mastectomy	2 to 5	Papillary carcinoma	2	2	1	1	No	No	Nil	Nil	pT2NxMx	1	11	38	16
37	816016	B/1297/12	45	F	R	UO	5X4	firm - hard	Normal	Axillary	Modified Radical Mastectomy	>5	Infiltrating ductal Carcinoma	2	3	2	2	No	yes	6	0	pT2N0Mx	3	6	82	32
38	815307	B/1363/12	50	F	L	UO	5X4	firm	Normal	Axillary	Modified Radical Mastectomy	2 to 5	Infiltrtaive ductal carcinoma(NOS)	2	3	2	2	yes	No	11	9	pT2 N1Mx	2	17	61	31
39	819116	B/1461/12	40	F	R	UI	4X4	firm	Normal	NO	Toilet Mastectomy	2 to 5	Medullary carcinoma	3	3	2	3	No	No	Nil	Nil	pT4bNxMx	1	14	153	34
40	815918	B/1489/12	37	F	L	LO	4X3	flrm- Hard	Normal	Axillary	Modified Radical Mastectomy	2 to 5	Invasive lobular carcinoma	2	2	2	2	yes	No	14	14	pT2N2aMx	1	3	67	35
41	827149	B/1552/12	71	F	L	UO	4X4	flrm	Normal	Axillary	Modified Radical Mastectomy	2 to 5	Invasive lobular carcinoma	2	2	2	2	No	No	4	0	pT2 N0Mx	4	9	65	37
42	827513	B/1573/12	60	F	R	UI	4X3	Hard	Normal	NO	Mastectomy	2 to 5	Infiltrating ductal carcinoma (NOS)	1	2	2	1	No	No	Nil	Nil	pT3 Nx MX	6	15	59	51
43	830889	B/1644/12	50	F	L	UO	6X5	firm	Normal	Axillary	Modified Radical Mastectomy	2 to 5	Infiltrating ductal carcinoma(NOS)	2	2	3	2	No	No	4	0	pT2 N0Mx	1	2	72	29
44	843983	B/1908/12	45	F	L	UI	4X2	firm	Normal	Axillary	Modified Radical Mastectomy	2 to 5	Infiltrating ductal carcinoma(NOS)	2	3	2	2	No	yes	7	1	pT2 N1 Mx	10	11	65	40
45	844930	B/1988/12	65	F	R	UI	2x1	firm	Puckering	Axillary	Modified Radical Mastectomy	2 to 5	Infiltrating ductal carcinoma(NOS)	2	2	1	1	No	No	6	0	pT2 N0Mx	6	34	30	21
46	851459	B/2027/12	50	F	L	All	8X5	Hard	Normal	NO	Mastectomy	>5	Infiltrating ductal carcinoma	3	3	2	3	yes	No	6	5	pT3 N2a MX	1	4	148	37
47	851410	B/2205/12	47	F	R	UO	6X3	firm	Normal	Axillary	Modified Radical Mastectomy	>5	Infiltrating ductal carcinoma(NOS)	3	3	2	3	yes	yes	12	5	pT3 N2a Mx	1	6	140	56
48	868865	B/84/13	70	F	L	UO	4X3	flrm	Normal	Axillary	Modified Radical Mastectomy	2 to 5	Infiltrating ductal carcinoma(NOS)	2	2	2	2	No	No	3	0	pT2 N0Mx	1	18	50	40
49	874942	B/142/13	60	F	R	Central	3X2	firm	Normal	NO	Mastectomy	2 to 5	Infiltrating ductal carcinoma(NOS)	2	3	1	2	No	No	Nil	Nil	pT2NxMx	3	11	116	34
50	872784	B/147/13	68	F	R	LI	4X2	firm	Normal	Axillary	Modified Radical Mastectomy	2 to 5	Infiltrating ductal carcinoma(NOS)	2	2	2	2	Yes	No	37	27	pT2N3aMx	1	9	90	32
51	877207	B/156/13	35	F	L	UO	2X1	firm	Normal	NO	Mastectomy	<2	Infiltrating ductal carcinoma(NOS)	3	2	2	2	No	No	Nil	Nil	pT1c NxMx	2	9	121	32
52	877207	B/255/13	35	F	R	UO	4X3	firm	Normal	NO	Mastectomy	2 to 5	Invasive lobular carcinoma	2	2	1	1	NO	NO	4	1	pT2N1aMx	1	8	17	20
53	879988	B/269/13	60	F	L	UI	4X3	flrm	Ulceration	Axillary	Modified Radical Mastectomy	2 to 5	Infiltrating ductal carcinoma(NOS)	2	3	1	2	No	yes	9	7	pT2 N1 Mx	4	13	87	32
54	888120	B/462/13	45	F	L	LI	4X2	firm	Normal	Axillary	Modified Radical Mastectomy	2 to 5	Infiltrating ductal carcinoma(NOS)	2	3	1	2	Yes	No	7	4	pT2N2Mx	3	20	68	40
55	921434	B/1206/13	40	F	R	UO	3X3	firm	Normal	Axillary	Modified Radical Mastectomy	2 to 5	Infiltrating ductal carcinoma(NOS)	2	3	1	2	Yes	Yes	6	3	pT2N2Mx	1	17	110	33