"EVALUATION OF IMMUNOHISTOCHEMICAL EXPRESSION OF COX-2 IN BREAST CARCINOMA PATIENTS-A HOSPITAL BASED STUDY AT KLES DR. PRABHAKAR KORE HOSPITAL& MRC,BELAGAVI."

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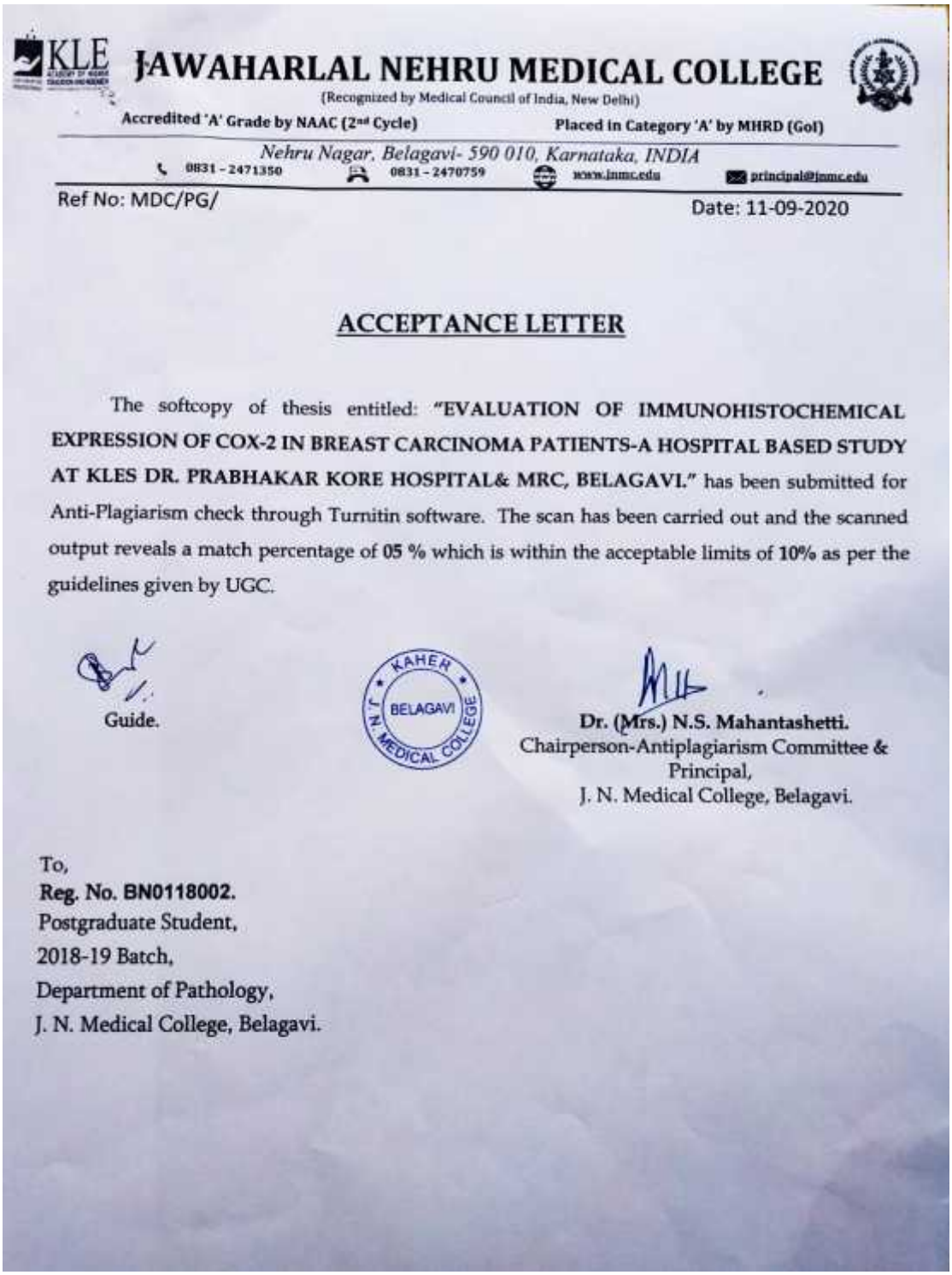

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LIST OF ABBREVIATIONS USED

BC	-	Breast carcinoma
DCIS	-	Ductal Carcinoma in-situ.
H&E	-	Haematoxylin and eosin
IDC	-	Infiltrating ductal carcinoma
IHC	-	Immunohistochemistry
LVI	-	Lympho vascular invasion
LN	-	Lymph node
LNM	-	Lymph node metastasis.
MEC	-	Myoepithelial cell.
NPI	-	Nottingham Prognostic Index
RBC	-	Red blood cell
SBR	-	Modified Scarff-Bloom-Richardson
TDLU	-	Terminal duct lobular unit
TNBC	-	Triple negativebreast cancer
WHO	-	World Health Organisation

ABSTRACT

EVALUATION OF IMMUNOHISTOCHEMICAL EXPRESSION OF COX-2 IN BREAST CARCINOMA PATIENTS-A HOSPITAL BASED STUDY AT KLES DR. PRABHAKAR KORE HOSPITAL& MRC,BELAGAVI

Background & Objectives: Breast cancer is the most common female cancer worldwide representing nearly a quarter of all cancers. Along with China and United States, India accounts for almost one third of global burden of breast cancer. COX-2 derived prostanoids have been shown to induce invasion, increase metastasis and promote angiogenesis. This study is to evaluate the expression of COX-2 in histologically diagnosed cases of breast carcinoma and its with clinical parameters and histological features of breast carcinoma.

Materials and Methods: A total of 40 cases of breast carcinoma were included from January 2018 to December 2020. Clinical details and gross findings were obtained from medical records and grossing notes using a structured proforma. Paraffin embedded blocks were archived and histological findings & IHC expression of COX-2 were analysed. Statistical analysis was done using chi square test and p value of less than 0.05 was considered significant.

Results: Majority of the patients in the study were in sixth decade of their life and 95% were females. Among them, 70% were postmenopausal. The expression of COX-2 was observed in 32.5% of cases. 32.5% females, including 7.5% premenopausal and 25% postmenopausal were positive for COX-2 expression.80% of total cases belonged to IDC type and 30% of IDC showed COX-2 positivity. Tumors

ranging from 2-5cm were 60% and among them COX-2 positive cases were 25% . Histological grade II and III were 72.5% and 25% respectively with COX-2 positivity of 17.5% in grade II and 15% in grade III. 70% cases showed lymph node metastases with COX-2 positivity of 22.5% and 75% possessed lympho-vascular invasion with 25% positivity for COX-2.

Conclusion:COX-2 expression did not show statistically significant association with the evaluated clinico-pathological parameters.

Keywords: Breast cancer, COX-2, immunohistochemical

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INTRODUCTION

Breast cancers are among the leading causes 0f morbidity and deaths among females. Cancer of the breast is considered as the most widely diagnosed cancer of women with median incidence of 1.38 million new cases annually [(1).] In Indian population also, it is the most common cause of cancer. [(2)]

As of 2012, the annual age standardized incidence rate of breast carcinoma was 43.1 per 1,00,000 women worldwide whereas in India it was 25.8 per 1,00,000 women. [(3)] In the state of Karnataka incidence is 36.6 per 1,00,000 women which is phenomenally a high value. [(4)]

The incidence and detection rate of breast cancer in India has increased in the past few years. The major reason behind which is the introduction of breast cancer screening programs.However, other factors such as lifestyle changes also play a significant role. [(5)]

Immunohistochemistry plays a prominent role in breast disease pathology and also in many tumors of both benign & malignant etiology. Most common markers that are used in prognosis and for therapeutic purposes in breast cancers include: ‘estrogen receptor’, ‘progesterone receptor’, ‘Ki-67’,‘human epidermal growth factor receptor-2’ & ‘p53’. In addition to these, there are markers of angiogenesis & apoptosis that play an important role [(6).]

“Cyclooxygenase (COX)” are the group of enzymes that are essential in the conversion to prostaglandings from precursor molecule- archidonic acid. “Cyclooxygenase-1 (COX-1)” is seen to be expressed constitutively at a near constant

rate all throughout cell cycle in majority of the organs & tissues. The other form, is the 'inducible isoform' known as "Cyclooxygenase-2 (COX-2)".This form is seen to be highly expressed in many cancers especially of the breast.

COX-2 acts as a critical target in chemoprevention of breast cancer. Rodent models and numerous studies on transgenic mice have suggested that COX-2 may be involved specifically in mammary carcinogenesis as selective inhibitors of COX-2 enzyme suppress development of tumor in rats [6,7].

The metabolites derived from COX-2 are found to assist in upregulation of premalignant proliferation, viability of tumor tissue, growth & transformation of tumor and invasive and metastatic properties. COX-2 is thereby implicated in many human malignancies and malignant tumors.[2,6,7] This study aims to investigate the immunohistochemical expression of COX-2 marker with respect to various clinico-pathological entities that are considered as risk factors for development of breast cancers and also as essential markers of prognosis.

As the role of COX-2 in breast cancer seems to be pivotal and millions of women all over the world are suffering immensely due to cancers of breast and their related morbidity, the current study aims to investigate and evaluate the expression of COX-2 marker by use of immunohistochemistry with respect to various clinico-pathological entities that are considered as risk factors for development of breast cancers and also as essential markers of prognosis.

AIMS AND OBJECTIVES

1. To study the expression of COX-2 in histologically diagnosed cases of breast carcinoma.
2. To study COX-2 expression in relation with clinical parameters and histological features of breast carcinoma.

REVIEW OF LITERATURE

Mammary glands, commonly referred to as breasts, are secondary sexual characters and are well developed in females serving as a source of nutrition for newborns. The breasts develop from the mammary ridge which is also known as the milk line. They appear as a thickening of epidermis on the ventral surface of the body of the fetus which extends from axilla to the upper medial aspect of thighs bilaterally in the 5^{th} week of gestation. In humans, the ridge eventually disappears with development except in the anterior pectoral region. On day 56, nipple formation begins. Mammary sprouts, which form the primitive ducts start development on day 84 and get canalized at around day 150. Myoepithelial cells appear around theseducts between 23 and 28 weeks of gestation. (1)

Thelarche is the term given for mature breast development. It begins with cyclical estrogen and progesterone secretion. The mammary ducts undergo branching under the influence of estrogen. Accumulation of adipose tissue occurs in the parenchyma which leads to enlargement of the breast. (1)

GROSS ANATOMY

Mature breast is a bilateral accessory organ located on the anterior chest wall. It has an eccentric configuration with the long axis running diagonally along the pectoralis major muscle and extends into the axilla as the axillary tail of Spence. The breast lies within the superficialfascia which is continuous superiorly as the cervical fascia and inferiorly as the superficialabdominal fascia. The suspensory ligament of axilla is formed by the pectoralis major muscle fascia. The retromammary space or

the sub mammary space lies deeper to the breast tissue which contains loose areolar connective tissue.

The morphological and functional unit of the breasts are the lobes which are comprised of glands, ducts, lobules intermingled in a stroma comprising of connective tissue. stroma. The term terminal duct lubular unit or TDLU is given for the same. The TDLU is the most commonsite for a primary malignancy of the breast. It is hormone responsive and act as functional lactational component of breast.

Nipple Areola Complex: The terminal secretory ducts from each of the lobules converge at the base of the nipple and form the lactiferous sinus. The skin surrounding the nipple is called as the areola which contains numerous sweat glands, opening into the skin surface. These glands can become very evident near term pregnancy or in lactating mothers as Montgomery's tubercles, the circumferentially arranged elevations.

Arterial supply & venous drainage: Breast derives its arterial supply from branches of internal thoracic, axillary & intercostal arteries. The major artery supplying the breast in most individuals is the internal mammary, that is a branch of internal thoracic.

Lymphatic drainage: Lymph from the breasts drain into the axillary group of lymph nodes via the lymphatics running along the external thoracic vein and into the internal mammary lymph nodes via the lymphatics running along the internal thoracic vein. A plexus of lymphatic vessels is seen in the subareolar regionwhich is called as the subareolar plexus of Sappey. There are three important routes to drainage of lymph in breast. Approximately 75% of the lymphatic flow is into the axillary group

of lymph nodes, around 25% is via the Internal mammary lymph nodes. Posterior intercostal lymph nodes are the third draining sites for the breast.

The lymphatic route has been acknowledged as an important route for dissemination of breast cancer. Lymphatics from the right breast drain into the right subclavian vein and those via left drain into thoracic duct finally terminating into subclavian vein on left side.

Axillary lymph nodes are divided into anterior, posterior, lateral and apical group of lymph nodes which range from 20-40 in number. These lymph nodes are described in three levels surgically, based on their relation to pectoralis minor muscle.

Level 1 nodes are those which lie lateral to the lateral border of pectoralis minor, level 2 nodes lie posterior to the muscle and level 3 lymph nodes lie medial to the medial border of pectoralis minor. Rotter's nodes which are interpectoral group of lymph nodes usually 1-2 in number may be present in some instances. A sentinal lymph node is the one which is first draining lymph node of a malignant lesion.

Identifying the sentinel lymph node is an important step for breast cancer-early staging. It is removed surgically and further histopathological analysis is mandated to detect presence of matastasis. In cases where sentinel lymph node comes out to be negative, radical surgery can be avoided. [(94)]

Nerve supply: Ant. & Lat. branches of 4-6 intercostal nerves innervate the breast. Lateral cutaneous branch of T4 supply the nipple areolar complex.

Microstructure: Each mammary gland comprises of 15-20 lobes of tubuloalveolar glands separated from each other by dense collagenous and adipose

tissue. Each lobe is a separate gland in itself havingits own lactiferous duct, opening individually into the nipple.

Each duct branches to form multiple terminal ducts within the lobes which ends in a lobule forming a Terminal duct-lobular unit (TDLU).

The lining of these ducts and acini is formed by double layer of cells; the inner layer formed by epithelial cells and the outer layer formed by myoepithelial cells.

The luminal epithelial layer is formed by cuboidal cells in smaller ducts and acini and by tall columnar cells in the larger ducts . Surrounding these cells is a layer of stellate shaped myoepithelial cells. During the reproductive ages, the ductal epithelium undergoes mild cyclical changes under the influence of ovarian hormones.

BREAST CARCINOMA

Epidemiology:

Breast carcinoma is the 5th most common cause of cancer deaths in the world.

Approximately 1.7 million new cases of breast carcinoma are diagnosed every year. [8] In India, breast carcinoma accounts for the most common malignancy in women. [2] In India as of 2012, the annual age standardized incidence rate of breast cancer was 25.8 per 100000 women. [3] However, in Karnataka alone the rates were as high as 36.6 per 100000 women. [4]

On the basis of histology, breast carcinomas are divided into carcinoma in situ and invasive carcinoma. Carcinoma in situ can be further classified as ductal and lobular, which are differentiated from each other based on cytological features and pattern of the growth. Ductal carcinoma in situ(DCIS) is more common than Lobular

carcinoma in situ(LCIS) which is further classified on the basis of morphology as Papillary, Micropapillary, Cribriform, Solid and Comedo carcinoma.

In "Atypical ductal hyperplasia" and "Carcinoma in situ", cellular proliferation is till lumen of ductal system and there is no invasion of myoepithelial cell layer or the basement membrane. [8,9]

Among invasive types, invasive ductal carcinoma is the most common variant, accounting for 47-80%. On gross examination, these tumors range from 0.5 to 10 cm in size, are firm to hard in consistency having ill-defined margins. Microscopically, the neoplastic cells are arranged in sheets, cords or often diffusely infiltrative. These cells have hyperchromatic, pleomorphic nucleus with prominent nucleoli and abundant eosinophilic cytoplasm. Atypical mitotic figures are a common occurence.

The surrounding stroma might be cellular or may show desmoplastic reaction. At times a focus of DCIS can also be seen along with the invasive carcinoma. [10]

Risk Factors

- Age: The risk of breast carcinoma increases with age. [11] Reproductive factors which may act as risk factors are "early menarche" (12 years), "late first birth" (30 years) & "late menopause" [12]

- Family history: The risk is nearly double in women whose mother or sister have had a history of breast carcinoma. [13]

- Genetic factors: The most common cause of hereditary breast cancer is the BRCA1 which is located on chromosome 17q and BRCA2 located on 13q. Mutation in the TP53tumour suppressor protein gene (Li-Fraumeni syndrome)

leads to incerased risk of development of breast mailgnancy along with sarcomas, bone tumours and adrenal tumours, brain tumours, leukemia. An inherited abnormality, Ataxia Telangiectasia and Mantle cell lymphoma or ATM gene codes for a DNA repair protein ,causing cancer of the breast.

Other genetic abnormalities that are associated withbreast cancer are "Phosphatase and tensin homologue", "tumour suppressor gene, CHEK2 (Checkpoint kinase 2), CDH1 (E-Cadherin) tumour suppressor gene and "Partner And Localizer of BRCA2". [(12)]

Weight: Obesity and weight gain is associated with increased risk of development of breast cancer especially during the perimenopausal age. In the postmenopausal age, marked weight gain is associated with nearly double risk of development of breast cancer. Gain of weight after surgery can increase chances of relapse.[(12)]

- Exercise: Physical fitness may act as protective factor by lowering various hormone levels. Some studies detail that an active lifestyle after treatment of cancer reduces the overall risk of mortality and chances of relapse.

- Diet: The effect of individual dietary components as a risk factor for breast cancer is little fruitful. There are studies suggesting risk of ER negative tumours, reduced by low fat intake and high vegetable intake. Alcohol and tobacco play no important role in the etiology of breast cancer. However, low calorie diet, increased exercise and reduced environmental exposure to disturbances of normal circadian rhythm can lower chances of breast malignancy by 1/3rd .[(14)]

- Breast feeding: Prolonged breast feeding reduces risk of malignancy of breast. Independent of age of first child birth there is 30% less chance of development of cancer. among females who start breastfeeding at 20-24 years of age as compared to women who have never breastfed their child [15]

- Parity: Low parity and late pregnancy are significant risk factors contributing to breast cancer. Nulliparous women have a 30% higher risk ofmalignancy as compared to parous women. Women who have their first child birth at an age greater than 35 years are at a 40% increased risk of malignancy ~40% as compared to ones that give birth before the age of 20 years. [16]

- Hormone replacement therapy (HRT): The risk of breast cancer is higher in women taking combined estrogen and progesterone therapy. However, current evidence suggests that HRT does not increase breast cancer mortality. [17] Prolonged use (5 years) of postmenopauasal HRT is an important risk factor. [12]

- Estrogen exposure: Use of Selective Estrogen Receptor Modulator (SERM) have shown that the risk is reduced by 38% from the start of five year treatment plan up to10 years. [14]

- Radiation: Radiation exposure in young women constitutes a higher risk of development of a neoplasm especially <30 years of age and this risk is seen to increase with radiation dose. [12]

- Precancerous conditions of the breast: The risk of development of breast cancer is four times in patients with atypical ductal hyperplasia or lobular hyperplasia. A benign breast disease in the past is associated with an increased

risk. Patients having both biopsy findings of high breast density & benign diseases of breast are at higher risk of developing breast cancer in near future. (12,18).

E-cadherin - Negative expression of E-cadherin is associated with higher grade and development of distant metastasis.(41)\

Carcinoma of contralateral breast or endometrium : Study done by Monika K. Graeser et al reported that contralateral breast cancer risk depends on age at first breast cancer and on the affected BRCA gene. Younger age at first breast cancer is associated with a significantly higher risk of breast cancer on contralateral side in patients with mutation of BRCA 1.

After 25 years, 62.9% of the patients with BRCA1 mutation who were seen to younger than 40 years of age at development of first breast cancer, developed contralateral breast cancer. Whereas only 19.6% of those who were older than 50 years of age at first breast cancer developed contralateral breast cancer. (39)

Geographic influence - White women are slightly more likely to develop breast cancer than African-American women. The women of African-American descent are more likely to die of breast cancer. Breast cancer is more common in African-American women who are less than 45 years old. Asian, Native American ,and Hispanic females are seen to have less chances of development of breast malignancy & dying from the same.(32,40) Smoking: Increased risk is seen with increased exposure of tobacco smoke. (32)

2019 WHO classification of tumors of the breast

(Annexure VI)

The classification of breast carcinoma mentioned above has a prognostic value, but it fails to predict the response of the newer targeted therapy. Thus,the molecular classification was then introduced to predict the response.

MOLECULAR CLASSSIFICATION

This classification was first proposed by Peroi & Sorlie in 2000 to highlighten differences among gene expression. [19] Malignancies of breast were divided into small groups, "Luminal" which is further subdivided into 3 types reflecting estrogn receptor."HER-2neu positive" reflecting its overexpression & "Basal". Few places, a "Normal like" subgroup has also been discussed but its importance is not that clear.[20]

The existence of five main molecular subtypes, namely basal-like, HER2/neu, luminal A, luminal B, and normal breast-like, but luminal C and interferon-regulated subtypes have also been described [2,3,11,12,19,21,22] These five main subtypes have been reported to have distinct clinical presentations [23] ,sites of relapse [13], histological features [14], responses to chemotherapy [19,17] and outcomes.[15] There are five intrinsic or molecular subtypes of breast cancer that are based on the genes a cancer expresses: "Luminal A" They are hormone-receptor positive that is, estrogen receptor and/or progesterone-receptor positive, HER2/neu negative, with low levels of the protein Ki-67. Luminal A cancers are low-grade, and are known to have best prognosis.

"Luminal B" Generally seen to have fast growth than cancers of Luminal A type and prognosis is poor. This type is seen mostly in women with mutation of BRCAl gene.

"HER2-enriched"

These cancers are faster growing than luminal cancers and have the worst prognosis among all. However, treatment modalities such as Herceptin (chemical name: Trastuzumab), Tykerb (chemical name: Lapatinib),Perjeta (chemical name: Pertuzumab) have introduced successfull treatment in such cases.

"Normal-like"

These are similar to luminal A disease: hormone receptor positive, i.e. estrogen-receptor and/or progesterone-receptorpositive,HER2/neu negative, and with low Ki-67 protein levels.

Normal like breast cancer has a good prognosis, its prognosis is slightly worse than luminal A cancer's prognosis. [(16)]

Recently another molecular subtype, 'claudin –low' has been identified. (24) Microarray-based gene expression analysis was done to identify these molecular subtypes. The molecular classification predicts the overall survival (OS) and the disease free survival (DFS). The basal like or the TNBC was shown to have the shortest survival. Molecular classification stratifies ER positive population into several subtypes, each of which has a difference in patient survival. This is important because although ER, PR, HER2 status is used for clinical assessment, it doesnot separate the two distinct ER positive subtypes (i.e., Luminal A and Luminal B) which have different clinical outcomes.[(25)]

MORPHOLOGY OF BREAST CARCINOMA

Breast cancers can be classified into biological and clinical subgroups according to histological grade (Elston and Ellis, 1991) [18] and histological type (Ellis et al., 1992) [26]

Grade assesses the degree of differentiation (i.e. tubule formation and nuclear pleomorphism) and proliferative activity (i.e. mitotic index) of a tumor, and tells about its aggressiveness (Elston and Ellis, 1991). [18]

Histological type shows the pattern of growth of tumors.

"Ductal Carcinoma In Situ or DCIS" : It is a intraductal lesion that is neoplastic in nature known by its epithelial proliferation, cellular atypia which can be subtle or marked & a tendency to progress into invasive type.[27]

The term ductal is used as it involves the lobules and the expanded acini appear like small ducts. The Myoepithelial layer of cells is preserved in the involved ducts. DCIS has two major architectural subtypes – comedo and non comedo.

Comedo DCIS usually presents as a vague mass. Tumor cells are pleomorphic with high grade nuclei, with areas of central necrosis.

Non comedo DCIS lacks high grade nuclei as well as central necrosis. Cribriform DCIS has round spaces within the ducts or has a solid DCIS pattern. The micropapillary DCIS produces bullous protrusions which lack a fibrovascular core. Focal calcifications, along with focal necrosis and intraluminal secretions maybe seen in non comedo DCIS. [28]

Low grade DCIS is composed of small, monomorphic cells, forming micropapillae, cribriform or solid patterns. The cells have uniform sized nuclei with regular chromatin pattern and inconspicuous nucleoli. High grade DCIS is composed of highly atypical cells patterns such as cribriform, solid & micropappilary. [29]

Lobular carcinoma in situ (LCIS) : defined as a clonal proliferation of cells within ducts and lobules that grow in a discohesive fashion, usually due to an acquired loss of tumor suppressive adhesion protein E-cadherin. It is almost always an incidental biopsy finding without any calcification or stromal reactions. Around 20-40% of LCIS cases are bilateral.

The cells of affected lobules comprise of uniform population of cells which have round to oval nuclei and small nucleoli. Signet ring cells are commonly encountered. The cells do not form cribriform spaces or papillae. Pagetoid spread is common. [28] "Invasive lobular carcinoma or ILC" represents ,<= 15% of invasive malignancies of breast.

Most women present with a palpable mass. They usually present as irregular and poorly delimited tumors, and have diffuse growth pattern of the cell infiltrate.

Microscopically, there is proliferation of small cells, invading the stroma that is arranged in linear cords of single file.

"Invasive ductal carcinoma not otherwise specified or NOS" : This type is most common type of invasive breast malignancies upto 75%. It is also the most common diagnosed subtype. [30] These tumours have no characteristic macroscopic findings. Size shows a marked variation from 0.1 to 1.0cm. They can have irregular, nodular or stellate outline. There is no regularity of shape or structure. [29]

Medullary carcinoma (MC) : Medullary carcinoma represents 1 to 7% of all breast carcinomas. The mean age of women diagnosed with MC ranges from 45 to 52 years. The tumor is well aligned and soft to touch. Well circumscribed mammographic features can lead to confusion with benign lesion. On gross, well defined, well defined,rounded margins and soft texture. Fleshy, gray to tan appearance,hemorrhage & necrosis are commonly seen.There are five morphological traits characterize MC are known.1. Syncytial architecture. Cells of tumor tissue are seen in 4-5cm thick sheets,divided by connective tissue that is scanty. 2. Glandular or tubular structures are absent 3. Stromal infiltration by diffuse lymphoplasmacytic infiltrate is hallmark feature. 4.

Tumor cells are round with plenty cytoplasm & vesicular nuclei with one or few nucleoli.

There is nuclear pleomorphism which can be marked 5. Under low power, complete histological circumscription of tumour . [29]

Mucin producing carcinomas : This type is characterized by proliferating clusters of uniform cells that are small & seen floating in abundant extracellular mucus usually visible by naked eye. It has shiny glistening appearance with pushing margins and soft on palpation.

Tumors size ranges from less than 1 cm to over 20 cm. Microscopically, Mucinous carcinomas are comprised of clusters of uniform, round cells with scant amount of eosinophilic cytoplasm, floating in lakes of mucus. [29]

Neuroendocrine tumours (NE) : This type represents 2-5% of total carcinoma breast The consistency is soft and gelatinous in mucin producing tumors. Most of

these carcinomas form solid sheets m or alveolar pattern and have a affection towards production of peripheral palisading.

Invasive papillary carcinoma : Papillary intraductal carcinoma on invading, assumes the pattern of infilltration lacking papillary architecture. Grossly, they are usually not distinguished from No special type of invasive cancer of breast. In about two-third of the cases, that invasive papillary carcinoma is grossly circumscribed (Fisher et al.).

Microscopically circumscribed papillae that are blunt, focally solid areasare seen. The cytolplasm in these cells are amphophillic with apocrine character.Invasive Micropapillary Carcinoma “Pure micropapillary carcinoma“ has lobulated appearance because of its expansile growth pattern.

Tubular carcinoma Pure tubular carcinoma accounts for less than 2% of invasive breast cancer. There is no characterstic gross finding to distinguish it from other types only size of tumor which is usually small. The most important featutre is presence of tubules composed of epithelial cells in single layer enclosing a clear lumen within themselves.

“Invasive cribriform carcinoma or ICC” this type is seen in <=3.5% of breast malignancies and usually in sixth decade. Few instances, tumor can be seen as a mass but usually it is occult clinically. Mammographic findings show spiculated mass with microcalcifications.

More than 90% of the tumor comprises of an invasive cribriform pattern. The tumour cells are arranged as a sieve-like or in cribriform pattern. [(29)]

PAGET'S DISEASE OF NIPPLE

Paget's disease of the nipple is a rare manifestation of breast carcinoma which is seen in 1-4% of the cases. It presents as a unilateral erythematous eruption with a scale of crust. The lesion mimics eczema. Pruritis is a common feature. The malignant cells extend into the nipple from the DCIS within the ducts, disrupting the epithelial barrier. This allows extracellular fluid to seep onto the nipple surface. In 50-60% of the cases, patient presents with a palpable mass, are poorly differentiated, ER-negative and overexpress HER2. [(28)]

PROGNOSTIC FACTORS

The routine clinical management of breast cancer relies on the prognostic factors including nodal status, tumor histological grade, and primary tumor size. in addition to these traditional factors, estrogen receptor, progesterone receptor, and HER2 are also evaluated nowadays.

Evaluation of these biomarkers is valuable to predict therapeutic response to the targeted therapy.[(41)]

Small tumors confined to the breast, without micro-metastasis have high chances of cure. Big tumors which spread to axillary lymph nodes are associated with subclinical systemic spread and strongly predict advanced disease [(42)]. A study conducted including 13,464 node negative patients, showed patients with tumors 90%. In contrast, node-negative patients with high uPA/PAI-1 have a higher risk for relapse.

HISTOLOGIC GRADING

Histologic grading is done on the basis of Nottingham histological score, also referred to as Scarf Bloom Richardson scoring system. The histologic grading for breast carcinoma was introduced by Greenhough assessing eight morphological factors. Scarf and his colleagues followed Greenhough's method but emphasized more on the amount of tubule formation, inequality in the size of nuclei and hyperchromatism. Bloom followed Scarff's histological grading and added mitotic figures as additional criteria for grading. Bloom along with

Richardson gave the numerical scoring system. The Patey and scarff method, modified by Bloom Richardson, showed correlation between grade and prognosis, which was subsequently adopted by WHO as the preferred grading system.[43] Elston and Ellis published the Nottingham modification of the Bloom Richardson method which includes the percentage of tubule formation, degree of nuclear pleomorphism and mitotic count, using a defined field area.[44]

Table 1: Histologic Grading Using Nottingham Modification of Scarff Bloom Richardson System [44]

CRITERIA	SCORE 1	SCORE 2	SCORE 3
Tubule formation	>75% of tumor	10-75% of tumor	<10% of tumor
Nuclear pleomorphism	Minimal variation in shape and size of nuclei	Moderate variation in shape and size of nuclei	Marked variation in shape and size of nuclei
Mitotic count per 10hpf	0-5	6-10	>11

Table 2: The Scores of the Histological Grading

SCORES	GRADES
3-5	Grade 1
6-7	Grade 2
8-9	Grade 3

TNM STAGING

The American Joint Committee for Cancer (AJCC) introduced the TNM (primary tumor [T], regional lymph nodes [N], distant metastases [M]) staging in 1959. [45] With the advancement in imaging techniques, treatment and prognosis, periodic revisions are made.

Currently, the TNM staging includes size of the tumor, lymph nodal status & presence of metastatic disease. In the eighth edition, which is effective as of January 2018, incorporates biologic biomarker which would improve the prognostic discrimination. [46] TNM staging is the most widely accepted staging system which is used by clinicians to obtain& provide patient information. It is periodically updated according to the advancement in the field of oncology. [47]

Pathologic TNM staging of breast carcinoma, AJCC 8th edition

(Annexure VII)

OTHER STAGING SYSTEMS:

There are two other staging systems – "Summary Stage system" – used by cancer registries of state. It broadly classifies cancers as a. localized b. regional c. distant. Information pertaining to incidence & extent of cancer can be obtained. The other is the "Extent of Disease system or the EOD system". It was developed in the 1970s and used frequently for "National Cancer Institute Surveillance Epidemiology and End Results Program" also known as NCISEER". [47]

HORMONAL STATUS IN BREAST CARCINOMA

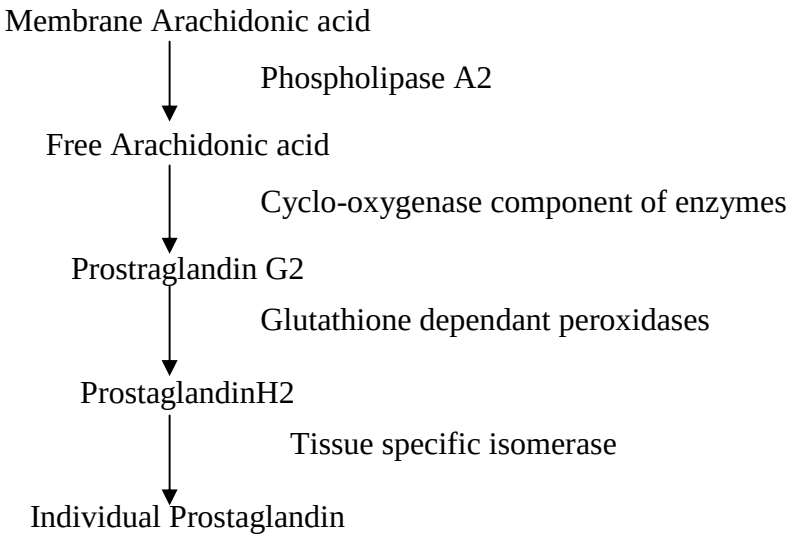
Breast cancer is a heterogeneous disease and gene expression profiling has identified four different malignant subtypes: basal-like, human epidermal growth factor receptor 2 (HER2)-enriched, luminal A and luminal B cancers.[20] This type of classification is dependant on expression of estrogen receptor, prrogesterone and HER-2neu receptor.Their expression is seen to predict prognoses and clinical response. [13]

ROLE OF COX 2 IN BREAST CANCER

The Cyclo-oxygenase group of enzymes:

Cyclooxygnase or the COX are group of enzymes that are implied in rate limiting step of oxidation during prostaglandin synthesis by conversion of arachidonic acid using PGG2 which acts as an important substrate for other PG synthases

Flowchart- Role of cyclo-oxygenase (COX) in prostaglandin synthesis



COX enzymes have two main isoforms, COX-1 and COX-2 [48]. A recently discovered isoenzyme has been introduced by the name COX-3 [49,50]

COX-1 is also called as a “housekeeping enzyme” in its constitutive form in humans. It is implied in homeostasis maintaenance by acting out in internal body functions like platelet aggregation, protection of stomach mucosa, functioning of “renal blood flow” & “glomerular filtration rate regulation”, etc. COX-2 is usually not detected in healthy tissues or organs. In Fetal tissue COX-2 is seen in heart,kidneys, lungs, and skin. However in adults, it is found only in the central nervous system, kidneys, vesicles, and placenta. [49,50]

COX-2 is induced rapidly in response to growth factors, tumour promoters, hormones, bacterial endotoxin, cytokines and shear stress (51), and has a number of inducible enhancer elements.

COX-2 is also called as "inducible isoenzyme" [52]. The expression of COX-2 is caused by alarge variety of oncogenes and growth factors [53] including "Tyrosine

kinase type 1, Human HER2 / neu, NFB, NFIL6" and various other pathways to inflammation. COX-2 over-expression has been observed in numerous~ 70% of invasive carcinoma and in situ carcinomas of breast.(54)

Both COX-1 and COX-2 are seen to exist as integral, membrane-bound proteins, located primarily on the luminal side of the endoplasmic reticulum (ER) and the nuclear envelope.

COX2 is studied to be more concentrated in the nuclear envelope. (55)

The COX-3 isoform is the one which is yet to be fully understood. Few studies have shown that it occurs in humans as COX-1 splice type, in the cerebral cortex and in the heart (55)

COX-2 and Cancer:

COX2 has much to do with carcinogenesis through several mechanisms, direct and in direct.

Prostaglandins boost mitogenesis directlyby impacting fibroblasts, osteoblasts along with mammary cells. COX-2 has an indirect effect on angiogenesis, genesis

of mutations and enhanced cell migration or even apoptosis. (56)

A study done by Liu and Rose in 1996 concluded that COX-2 is ubiquitously expressed in breast malignancy cell lines such as the metastases line "MDA-MB-231" and other tumors. (57)

Parrett et al in 1997 studied COX-2 and revealed findings that The expression of COX-2 was detected by PCR in 13 human tumor cells of breast although no expression was identified in normal breast tissue. (58)

However, many studies were done on a small population resulting in conflicting results in the past. In the year 2002, Ristimaki et al went on an intrusive immunohistochemical analysis in 1576 carcinomas to the breast. They not iced that COX2 expression was considerably high in 37 per cent samples.This finding was repeated further in other studies with massive numbers of patients and should be considered conclusive and positive evidence of connection of COX-2 in breast cancer in humans.(59)

Recent immunochemical analysis of breast cancers in humans has revealed a significant COX-2 expression in different types of breast carcinomas . The degree of expression shown by COX-2 has been positively correlated with poor prognosis of tumor (60-62).

One study also noted that elevated level of COX-2 mRNA are seen in the tissue adjacent to cancerous lesions and thus implicating its role in malignancies of breast. (63)

Expression of COX-2 has been closely linked to prognostic factors which indicate poor chance of survival. Important prognostic markers include tumour size, axillary lymph node metastases, ductal histology, tumour grade, HER-2 amplification and receptor negative disease (59)

A study done by Ranger et al, 2004 also revealed that The increased expression of COX-2 was shown to demonstrates close association with metastases in breast cancer.[64]

The correlations between COX-2 expressivity and clinicopathological factors have been done by various studies. However, due to different sample size and other factors there is variability in findings. [59,65-70]

COX-2 acting as a potential target for treatment & prevention:

Experiments on animals via translation have shown to connect C0X-2 to development of malignancies of breast. "Transgenic mice" were inserted A COX-2 gene regulated by the "mammary tumor virus promoter" has shown that mammary tumours grow after several pregnancy and lactation cycles and who were not meddled with, remained tumour free. This indicates that over-expressed COX2 in itself is sufficient to cause tumorigenesis.[57]

Another study where transgenic mice were inserted with a C0X-2 inhibitor, production of mammary tumors was slowed down showing potentially greater clinical evidence of its associated with breast cancer.[71]

Inhibitors of COX, commonly known as COX inhibitors, commonly used "nonsteroidal anti- inflammatory drugs or NSAIDs" cause pain relieving, anti-inflammatory & anti-pyretic displays.

A number of epidemiological studies have investigated if long-term NSAID reduces breast cancer risk.

Our study aims to study the immunohistochemical expression of C0X-2 in breast carcinoma cases. Correlation 0f COX2 positivity with various clinico-pathological parameters influencing prognosis and outcome of patients is studied to provide a better insight of the functioning of this novel marker.

METHODOLOGY

The present study has been carried out at the Pathology Department of KAHER's Jawaharlal Nehru Medical College, Dr. Prabhakar Kore Hospital and Research Centre, Belagavi.

Study design : This is a descriptive observational study

Inclusion criteria : Well fixed surgically resected and biopsy specimens diagnosed as carcinoma breast.

Exclusion criteria : Specimens which had not been fixed optimally.

Sample Size Calculation: The sample size was calculated using the formula 4pq/d2

P- expected prevalence , q- 100-p , d- Sample error [10]

Substituting the values in the above formula a sample size of 40 was obtained.

Ethical clearance: The present study was approved by Jawaharlal Nehru Medical College's Institutional Ethics Committee on Human subjects Research.(Ref.: MDC/DOME/37) (Annexure III)

Sampling Procedure: All surgically resected specimens of carcinoma breast during the period of January 2019 to December 2020 were included in the study.

Case Selection : Forty surgically resected specimens of breast carcinoma were collected from KLES Dr.Prabhakar Kore hospital and research centre during the period of 2019 to 2020 and were studied in the Pathology Department of KAHER's

Jawaharlal Nehru Medical College, Dr. Prabhakar Kore Hospital and Research Centre, Belagavi.

The clinicopathological parameters, including age, tumor size, axillary lymph node status, surgery type were obtained from the patient's outpatient & inpatient records and requisition forms as per proforma given in (Annexure II).

The specimens were adequately fixed in 10% neutral buffered formalin. Four to five micron thick sections were cut from paraffin embedded blocks. One section from each block was taken for Haematoxylin and eosin(H and E) staining as in (Annexure IV)

The Hematoxylin and Eosin stained slides were evaluated for

- Histological type
- Tumor grade
- Lympho-vascular invasion
- Lymph node metastases

Immunohistochemistry :

All cases were studied for the expression of COX-2.

For immunohistochemical analysis, 4 micron thick serial tissue sections were prepared from formalin fixed, paraffin embedded blocks were used on coated slides. Slides were air dried for 2 hours at 58 C. Slides were deparaffinised, dehydrated and rehydrated. The rehydrated slides were subjected to heat induced Antigen retrieval in a microwave. Slides were incubated for 15mins on high heat. After 15mins,

microwave was opened and slides were immediately transferred to room temperature. Slides were then washed with IHC wash buffer. The sections were then stained according to the IHC procedure. The polymer based IHC kit of Dako Corp. "Cox-2-specific antihuman mouse monoclonal antibody" (1g/ml; 1:50dilution; Dako - Hamburg, Germany) was used. Slides were incubated with respective optimized primary antibody for 60min. A brown precipitate is produced on addition of substrate chromogen. Slides were removed and stained with hematoxylin. Appropriate controls were run with each batch of slides. (Annexure II)

The tumours were typed according to the WHO classification system and appropriate Bloom Richardson grade were assigned. [72] All forty cases were then studied for expression of COX-2 . Digital images were obtained.

Assessment of expression of COX-2:

The expression of COX-2 was assessed semiquantitatively by evaluating intensity at a score of 0-3 & estimation of percentage of positive tumor cells in each intensity. The assessment is done in 10 high power fields. Scoring criteria was formulated before the beginning of analysis and is as follows:

0	"No staining"
1	"Weak staining" < 20% of cells
2	"Strong granular cytoplasmic staining" 20-100% of cancer cells

Thus, a score of >= 1 was considered positive, and a score of 0 was negative. [59]

Statstical Analysis:

Descriptive analysis has been performed in the present study and the analysis of data was done using the statistical software stata 14.2 .Microsoft word and Excel have been employed to generate graphs, tables etc. Descriptive statistics such as mean & percentages were calculated. The relationship between expression of COX-2 marker and other clinico-pathological variables was analysed using chi-square test. Probability (P) value: < 0.05 was considered significant.

RESULTS

A descriptive observational study was carried out in the Pathology Department of KAHER's Jawaharlal Nehru Medical College, Dr. Prabhakar Kore Hospital and Research Centre, Belagavi to study the expression COX-2 in breast carcinoma cases.

A total of 40 cases were evaluated.

The data obtained from the study was compiled, tabulated and subjected to statistical analysis. The results are presented here under the headings of the various parameters considered for the study.

The H & E stained microphotographs elaborated the histological type and grade of tumor tissue. H & E study of slides also detailed the grade of tumor. On the basis of Modified Scarff Bloom Richardson Grading all 40 cases were given histological Grades of I, II and III. Lympho-vascular invasion and tumor emboli are also seen in few of the cases .For COX-2 immunoreactivity, we studied it into three groups viz. negative for COX-2 expression, weak positive i.e. only few tumor cells show positivity or if tumor cells are positive diffusely but the intensity of their staining is less and lastly, strong positive.The label of strong positive was assigned for cases that showed diffuse cytoplasmic positivity for COX-2, that was distributed throughout majority of the slide, with high intensity of staining as well. In few cases, only the endothelial cells of blood vessels showed reactivity to COX-2 but the tumor cells failed to show any reactivity. Such cases are considered as negative for COX-2 expression in our study.

Out of 40 cases, 13 cases were positive for COX-2 immunohistochemical expression, including both weak and strong positive cases. Two cases among 13 positive ones showed strong diffuse positivity. So, in our study 32.5% cases showed COX-2 positivity among which 27.5% were weak positive cases and 5% were strong positive.

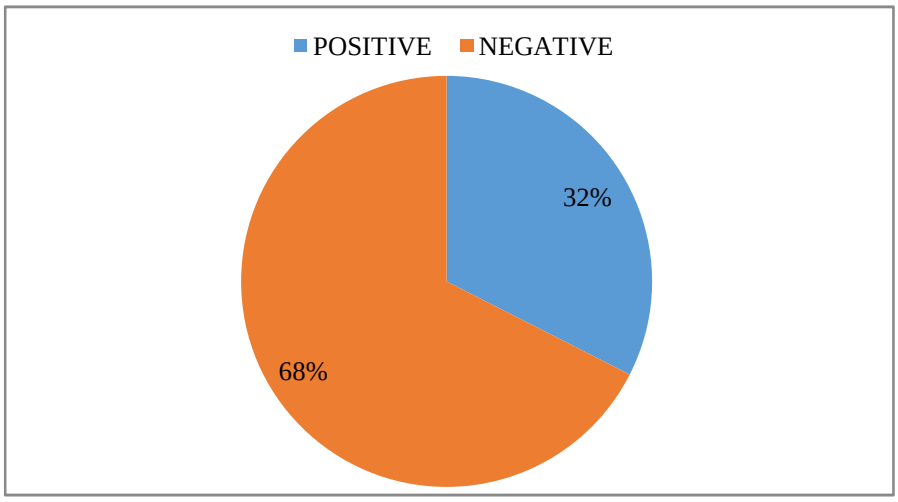
Table 3: COX-2 expression of sample

COX-2 Positivity	Number	Percentage
Positive	13	32.5
Negative	27	67.5
TOTAL	40	100

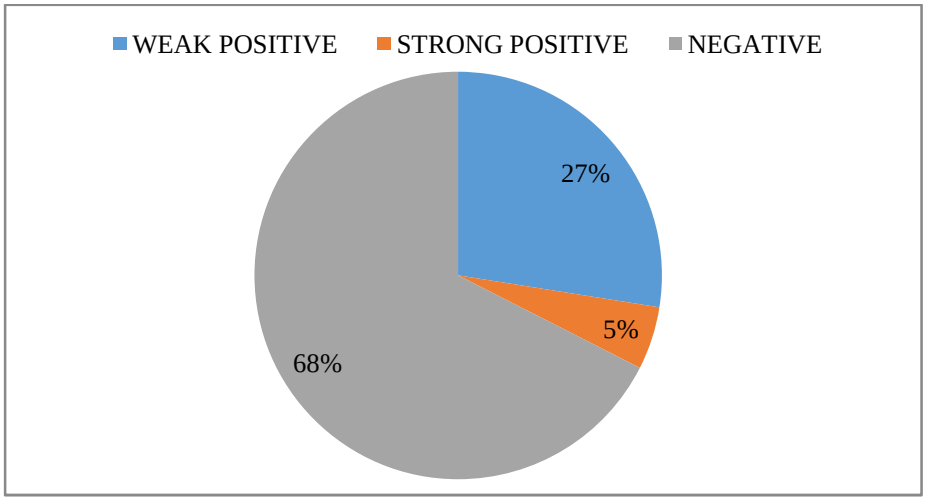
Table 4: Strong v/s weak v/s negative COX-2 expression

COX-2 Positivity	Number	Percentage
Weak Positive	11	27.5
Strong positive	2	5
Negative	27	67.5
TOTAL	40	100

Graph 1- COX-2 Positive v/s COX-2 negative sample distribution



Graph 2- Weak v/s Strong v/s Negative expression of COX-2



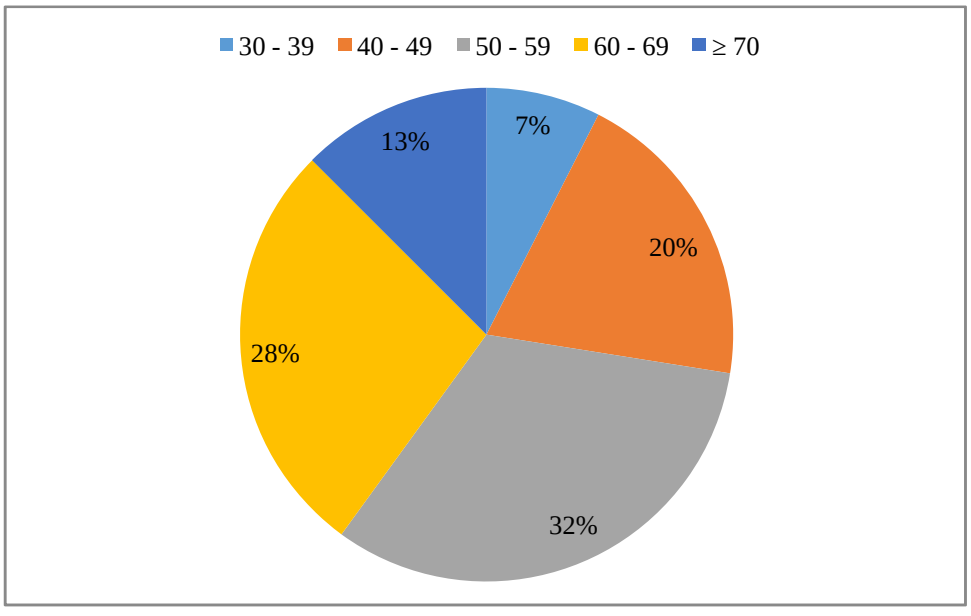
AGE:

All the patients irrespective of gender were divided into various groups according to their age. The age ranged from 30-87 years. The mean age of the sample +/- 2SD was 57 years +/-13.The age groups were divided in the difference of 10 years starting from the age of 30 up till 70 years and thereafter all cases above 70years of age were taken into one group [96]. Majority of the sample i.e. 32.5% of the patients belonged to the age group of 50-59 years.

Table 5- Age wise sample distribution

Age (in years)	Number	Percentage
30 – 39	3	7.5
40 – 49	8	20
50 – 59	13	32.5
60 – 69	11	27.5
≥ 70	5	12.5
TOTAL	40	100

Graph 3- Age wise sample distribution (in years)



Immunohistochemical expression of COX-2 was evaluated with respect to age.

Table 6- Age wise COX-2 expression

Age(in years)	COX-2 Positive	COX-2 Negative	TOTAL
30 - 39	1 (2.5%)	2 (5%)	3 (7.5%)
40 - 49	2 (5%)	6 (15%)	8 (20%)
50 - 59	6 (15%)	7 (17.5%)	13 (32.5%)
60 - 69	2 (5%)	7 (17.5%)	9 (22.5%)
≥ 70	2 (5%)	5 (12.5%)	7 (17.5%)
TOTAL	13 (32.5%)	27 (67.5%)	40 (100%)

On performance of chi square test, the p-value is 0.87. The result is not statistically significant .

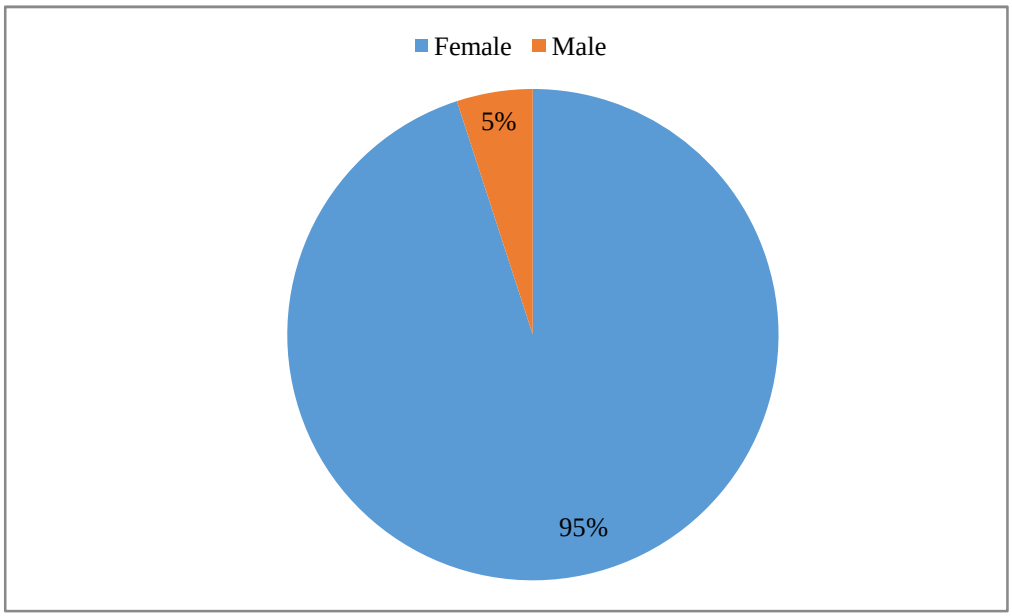
GENDER-

Table 7- Gender wise sample distribution

Among the total of 40 cases, 95% cases were of females and 5% cases were males.

Gender	Number	Percentage
Female	38	95
Male	2	5
Total	40	100

Graph 4- Gender wise sample distribution



While evaluating for COX-2 expression, all 13 cases which were positive for COX-2 staining belonged to females. There were only two males in the study and both were negative for the expression of COX-2. 32.5% females were positive for COX-2 expression.

Table 8- Gender wise COX-2 expression

Gender	COX-2 Positive	COX-2 Negative	TOTAL
Female	13 (32.5%)	25 (62.5%)	38 (95%)
Male	0 (0)	2 (5%)	2 (5%)
TOTAL	13 (32.5%)	27 (67.5%)	40 (100%)

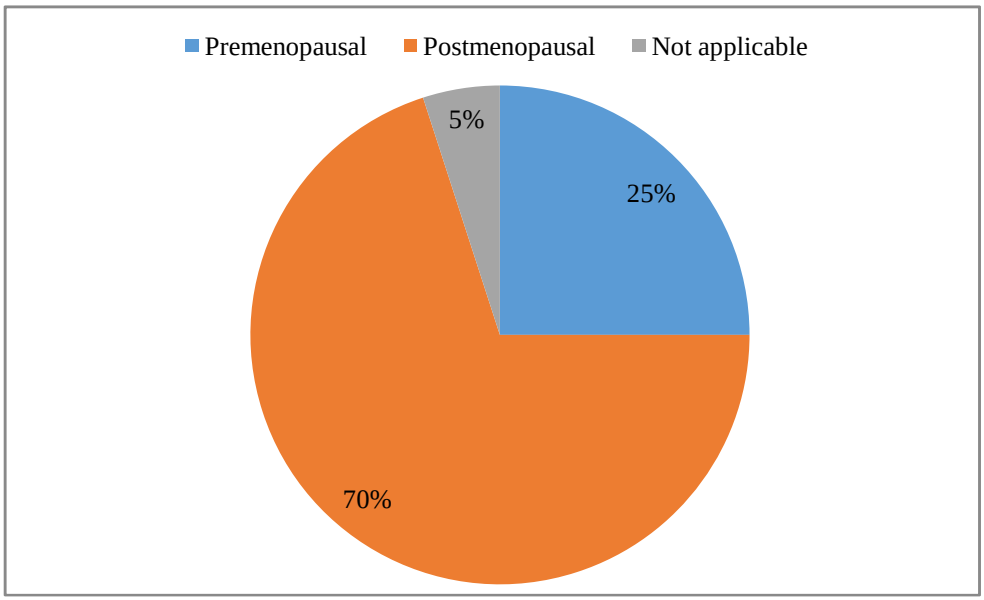
MENOPAUSAL STATUS-

Among a total of 38 cases of females out of 40, 25% were premenopausal and 70% were postmenopausal.

Table 9- Menopausal status wise sample distribution

	Number	Percentage
Premenopausal	10	25
Postmenopausal	28	70
Not applicable	2	5
TOTAL	40	100

Graph 5- Menopausal status wise sample distribution



13 patients were positive for COX-2 expression, out of which 3 belonged to premenopausal group and 10 to the postmenopausal group.

Table 10- Menopausal status wise COX-2 expression

	COX-2 Positive	COX-2 Negative	TOTAL
Premenopausal	3 (7.5%)	8 (20%)	11 (27.5%)
Postmenopausal	10 (25%)	17 (42.5%)	27 (67.5%)
Not applicable	0 (0)	2 (5%)	2 (5%)
TOTAL	13 (32.5%)	27 (67.5%)	40 (100%)

7.5% of premenopausal and 25% of postmenopausal females were positive for COX-2 immunohistochemical expression. The chi-square statistic is 0.33. The p-value is 0.56. The result is not statistically significant .

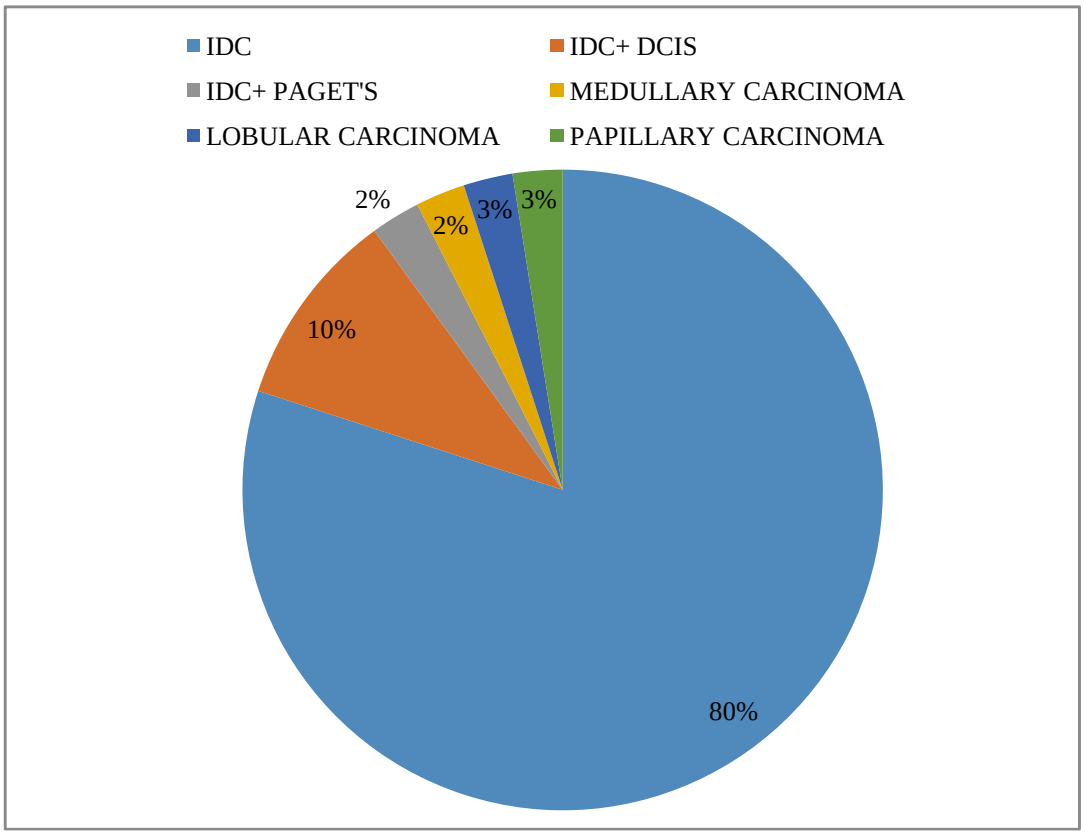
HISTOLOGICAL TYPE-

In our study of 40 cases, there were 33 i.e. majority cases of Invasive ductal carcinoma (IDC), 3 cases of IDC with DCIS component, 1 each of IDC with Paget's disease, Medullary carcinoma, Papillary carcinoma and invasive lobular carcinoma respectively.

Table 11- Histological type wise sample distribution

Type	Number	Percentage
IDC	32	80
IDC+ DCIS	4	10
IDC+ PAGET'S	1	2.5
MEDULLARY CARCINOMA	1	2.5
LOBULAR CARCINOMA	1	2.5
PAPILLARY CARCINOMA	1	2.5
TOTAL	40	100

Graph 6- Histological type wise sample distribution



On evaluating for COX-2 expression, 11 out of 13 COX-2 positive cases belonged to IDC and only 1 case of Medullary carcinoma was positive for COX-2 immunohistochemical expression. Rest of the cases were not seen to express any reactivity and were considered as negative.

Table 12- Histological type wise COX-2 Expression

	COX-2 Positive	COX-2 Negative	TOTAL
IDC	11	22	33
IDC+ DCIS	0	3	3
IDC+ paget's	0	1	1
ILC	0	1	1
Medullary Carcinoma	1	0	1
Papillary Carcinoma	0	1	1
TOTAL	12	28	40

For ease of evaluation, we divided COX-2 expression into IDC and others, as the majority of cases in the study group belonged to IDC variety i.e. 12 out of 13 positive cases for COX-2 immunohistochemistry.

Table 13- Histological type wise COX-2 expression

	COX-2 Positive	COX-2 Negative	TOTAL
IDC	12 (30%)	21 (52.5%)	33 (82.5%)
OTHERS	1 (2.5%)	6 (15%)	7 (17.5%)
TOTAL	13 (32.5%)	27 (67.5%)	40 (100%)

30% of IDC cases showed COX-2 positivity and and 52.5% did not. For evaluation of significance, the chi-square statistic is 0.99. The p-value is 0.31. The result is not statistically significant

TUMOR SIZE-

All the breast carcinoma specimens included in the study were measured three dimensionally before grossing. The tumor tissue was also measured in three dimensions viz. length, breadth and height. However during evaluation of data and statistical analysis, the largest dimension was considered and tabulated.

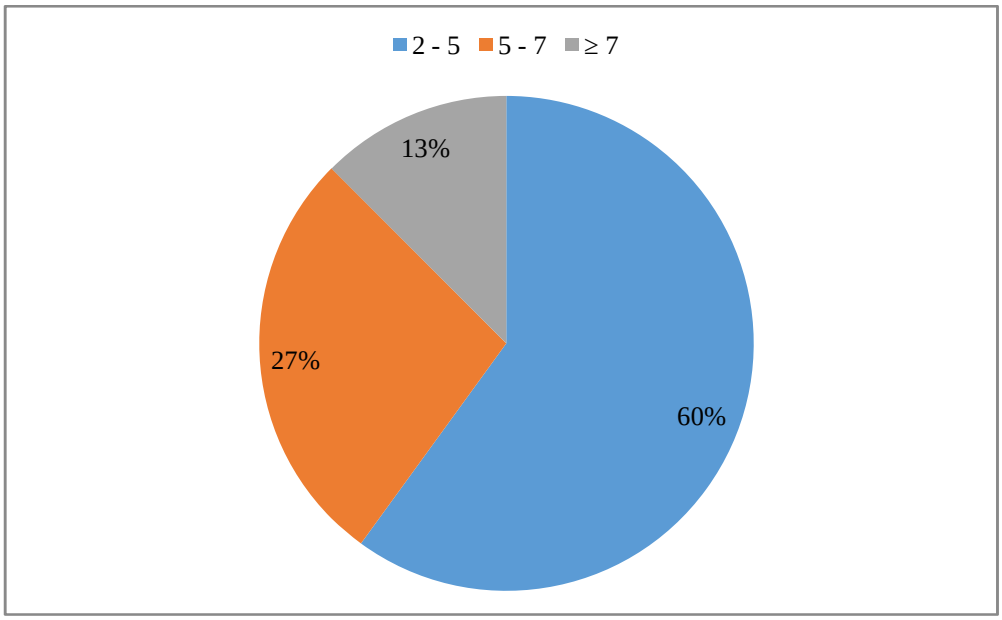
The tumor tissue was divided into groups in the difference of every 3 cms [(97)] and three such groups were formulated based on the size of the tumor tissue in our study sample that varied from as small as 2cm to as large as 13cm. The average size was 4.6cm with a standard deviation of 2.2cm.

Table 14- Tumor size wise sample distribution(in cms)

Tumor size	Number	Percentage
2 - 5	24	60.00
5 - 7	11	27.50
≥ 7	5	12.50
TOTAL	40	100.00

In this study 60% of tumors were small in size ranging from 2cm to 5cm in largest dimension.

Graph 7- Tumor size wise sample distribution(in cms)



Out of 32.5% of total positive cases, 25% of cases with small tumor size of 2-5cm were positive for COX-2 expression and only 3% cases with larger size of greater than or equal to 7cm showed COX-2 positivity.

Table 15- Tumor size wise COX-2 expression

Tumor size (in cm)	COX-2 Positive	COX-2 Negative	TOTAL
2 - 5	10 (25%)	19 (47.5%)	29 (72.5%)
5 - 7	0 (0)	5 (12.5%)	5 (12.5%)
≥ 7	3 (7.5%)	3 (7.5%)	6 (15%)
TOTAL	13 (32.5%)	27 (67.5%)	40 (100%)

While looking for positivity for COX-2 expression, i.e. if increase or decrease in size of tumor tissue has any relationship with COX-2 reactivity. The p-value was 0.72. The result is not statistically significant .

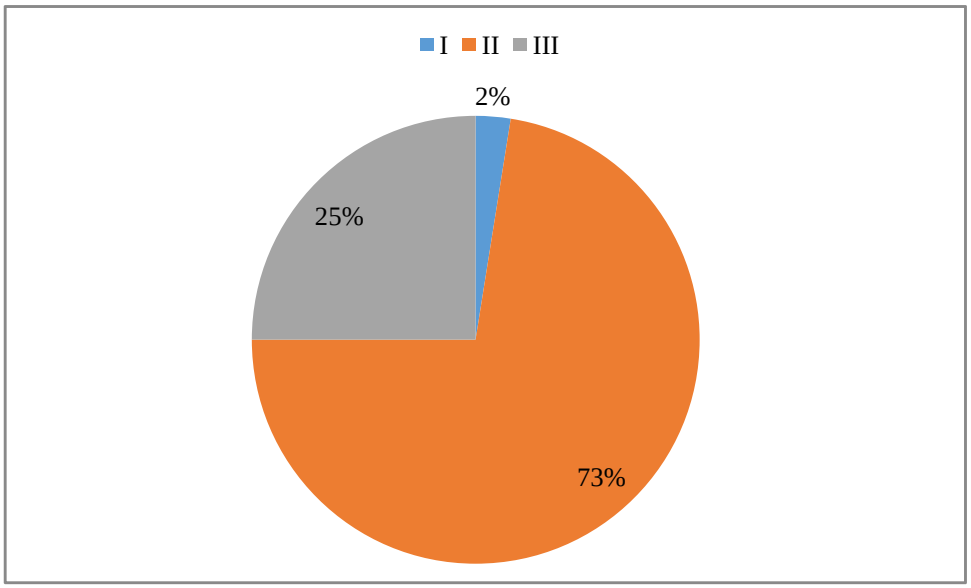
TUMOR GRADE-

The entire sample of 40 cases were also segregated on the basis of three grades of Modified Scarff Bloom Richardson Histologic Grading. Majority i.e. 72.5% cases belonged to Grade II and 25% cases to Grade III tumor.

Table 16- Tumor grade wise sample distribution

Tumor Grade	Number	Percentage
I	1	2.50
II	29	72.50
III	10	25.00
TOTAL	40	100.00

Graph 8- Tumor grade wise sample distribution (Grade I,II&III)



All these cases were then studied for Immunohistochemical expression of COX-2.

Table 17- Tumor grade wise COX-2 expression

	COX-2 Positive	COX-2 Negative	TOTAL
Grade I	0 (0)	1 (2.5%)	1 (2.5%)
Grade II	7 (17.5%)	22 (55%)	29 (72.5%)
Grade III	6 (15%)	4 (10%)	10 (25%)
TOTAL	13 (32.5%)	27 (67.5%)	40 (100%)

Out of 32.5% of COX-2 positive cases 17.5% belonged to Grade II, 15% belonged to Grade III and none to Grade I. The statistical significance was calculated and the p value was 0.051 which is just over the arbitrary threshold. The result is not statistically significant.

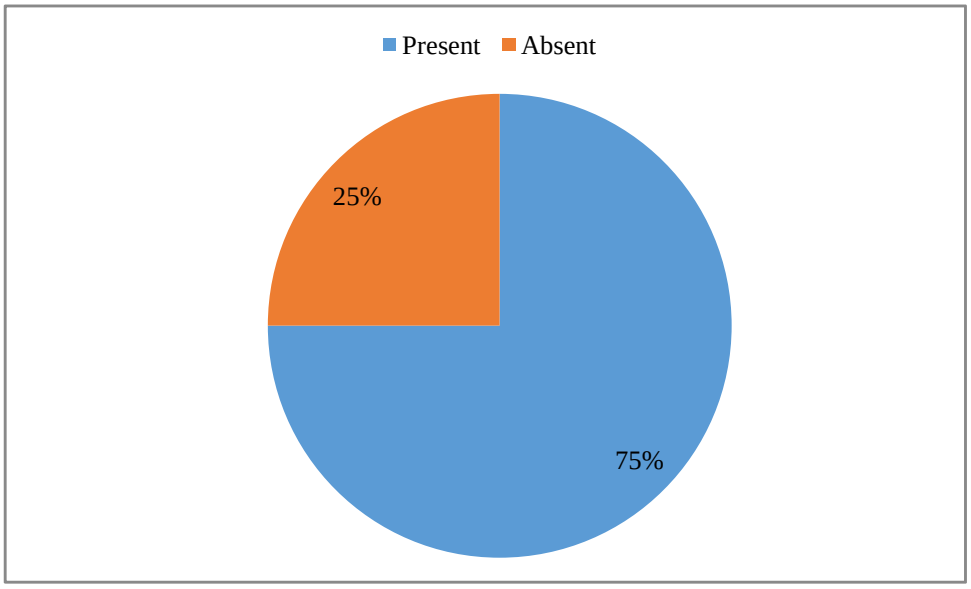
LYMPHOVASCULAR INVASION-

Among the total of 40 cases selected for the study, 75% cases showed lymphovascular invasion(LVI) and 25% did not show any evidence of LVI.

Table 18- LVI wise sample distribution

LVI	Number	Percentage
Present	30	75
Absent	10	25
TOTAL	40	100

Graph 9- LVI wise sample distribution



Among the 32.5% cases that were positive for expression of COX-2, 25% were positive for lymphovascular invasion and 7.5% did not show any feature of lymphovascular invasion.

Table 19- LVI wise COX-2 expression

	COX-2 Positive	COX-2 Negative	TOTAL
LVI Present	10 (25%)	20 (50%)	30 (75%)
LVI Absent	3 (7.5%)	7 (17.5%)	10 (25%)
TOTAL	13 (32.5%)	27 (67.5%)	40 (100%)

On calculating significance of the same, the chi-square statistic is 0.03. The p-value is 0.84. The result is not statistically significant.

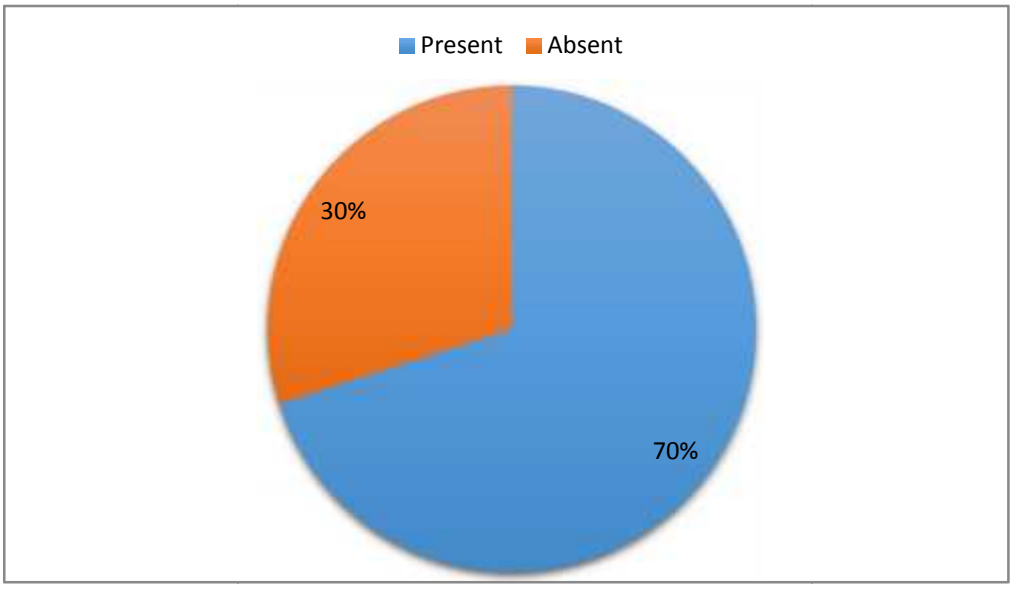
LYMPH NODE METASTASES-

A majority of cases, most of the cases i.e. 70% cases showed presence of lymph node metastases and 30% cases did not show any evidence of lymph node metastases (LNM).

Table 20- Lymph node metastases wise sample distribution

LNM	Number	Percentage
Present	28	70
Absent	12	30
TOTAL	40	100

Graph 10- Lymph node metastases wise sample distribution



For COX-2 positivity, out of a total of 13 positive cases, 9 cases also showed presence of lymph node metastases. 8 cases out of 40 were neither positive for COX-2 expression nor for the presence of lymph node metastases.

Table 21- Lymph node metastases wise COX-2 expression

LNM	COX-2 Positive	COX-2 Negative	TOTAL
Present	9 (22.5%)	19 (47.5%)	28 (70%)
Absent	4 (10%)	8 (20%)	12 (30%)
TOTAL	13 (32.5%)	27 (67.5%)	40 (100%)

22.5% cases were positive for both lymph node metastases as well as COX-2 expression. 10% cases did not show presence of lymph node metastases but were positive for COX-2 immunohistochemistry. On calculation of significance, the chi-square statistic is 0.005. The p-value is 0.94. The result is not statistically significant .

DISCUSSION

The prevalence of breast cancer is more in developed countries and it accounts for almost half of breast cancer cases worldwide. In case of females, cancer of the breast can be taken as the most commonly diagnosed cancer throughtout the world. Its estimation is over 1.7million cases in the year 2012 alone along with recorded deaths of more than 521,900. [72, 73]

In an attempt to look for possible targets that can be exploited for prognostic, diagnostic or therapeutic use, new markers are being studied, one of which is COX-2. Only few studies have been done to understand the possible part played by C0X-2 in breast cancer and still fewer are done in Indian setting.

Numerous molecular as well as biological agents have a role to play in the prognosis of breast malignancies [74,75].

In this study, the correlation of COX-2 positivity with various clinic pathological parameters have been studied which are known to have prognostic significance in many other settings.

However, none of those parameters revealed any association with COX-2 positivity in our study.

There are studies suggesting that the metabolites which are derived from COX-2 may contribute significantly in maintaining viability of tumor tissue,its proliferation, growth patterns, invasive properties and transformation or spread [76,77,78] . It is known to be highly expressed in many malignant cancers affecting humankind [68].

The present study aimed to study the immunohistochemical expression of COX-2 in proven cases of breast carcinoma and its correlation with various clinico-pathological factors, individually each of which, may or may not affect the prognosis of the patients in the long run.

In this study, the expression of COX-2 is seen in 32.5% of the cases. This finding is in accordance with a study done by Mosalpuria et. al. in the year 2014 where they showed positive COX-2 activity in 33% of their cases. [97] Similar finding has also been shown by Denkert et. al, Ristimaki et.al, Schmitz et.al, Kim et.al and several other studies on COX-2 in breast cancer [59,86,99-104]. Few other studies like Costa et. al., Lee et al., Davies et al. & Misron et al.,have demonstrated either a higher or lower value of expression shown by COX-2 marker. ([85,90,105-106]. The variation in the expression of COX-2 in different studies could be attributed to the type of tissue sampling used eg. Frozen/ paraffin section. Other causes can be the method of analysis of data, threshold variation and most importantly the size of the sample studied.

In the current study, 15% of the total COX-2 positive cases belonged to sixth decade of their life which is the most number of cases positive in any definite age group. However, on performing analysis there was no significant correlation between the immunohistochemical expression of COX-2 and the age at which patients present themselves. This finding was seen to be in agreement with findings of Costa et. al,Singh et. al and several other researchers [70,85,105,107,108]. Their study also did not show any positive relation between age at presentation and COX-2 reactivity of tumor.

This study comprises of 95% cases of females and5 % cases that of males. However COX-2 activity was observed only in female patients. Among female cases that were positive for the immunohistochemical expression of COX-2, 7.5 % are premenopausal and 25 % are postmenopausal taking into account that the sample positivity was 32.5% . Thus, majority of postmenopausal cases show positivity for COX-2 expression. This result is shared by study done by Jana et. al, where the activity of COX-2 was enhanced in postmenopausal patients [(76)] .

In this study the expression of COX-2 is detected in the epithelial cells of human breast carcinomas. 80% cases belonged to IDC type and 27.5% cases of IDC were positive for COX-2 reactivity. Major chunk of COX-2 positive cases was formed by IDC in our study. In studies done by Ristimaki et. al, Williams et.al also COX-2 has been shown to be activated in invasive carcinoma of ductal type which is in corcordance with our study [(59,79,80).] Since in this study other special types of breast cancers were very less in number and still lesser showed COX-2 posivity which is only 2.5%. Due to insufficient cases it is difficult to comment on their part in the expression of COX-2 regarding malignancies of breast.

Among a total of 40 cases, 60% cases in our study were found to have a smaller tumor size of 2cm to 5cms. 25% of total positive cases belonged to the group having smaller size and only 7.5% cases positive for COX-2 expression were of larger size i.e. >7cms. This states that majority cases in our study that are positive for COX-2 immunohistochemical expression are of small size i.e tumor size of 2-5cm. Statistical analysis did not reveal any positive correlation between tumor size and positivity for COX-2. There are indeed studies, demonstrating COX-2 expression to be correlated with larger size [(81-84).]However, our result corresponds to the results

published by Lee et. al.& Misron et. al. where they too did not observe any positive correlation between these two entities. [105,106]

When taking tumor grade into account, in this study 72.5% cases belonged to grade II and 25% belonged to grade III according to Modified Scarff Bloom Richardson Histological grading. 17.5% of Grade II tumors showed COX-2 positivity and 15% of Grade III expressed positive COX-2 expression ,but grade I tumor positive cases showed no activity towards COX-2. However, on analysis of data, no correlation could be deduced between grade of tumor and positive expression for COX-2 marker.Similar results for tumor grade were observed in study done by Solanki et. al,Costa et. al & Lee et. al. [87,105,106]. Their studies also did not infer any positive correlation in tumor grade and COX-2 marker reactivity.However Jana et. al. and few other researchers found significant correlation between the two entities [76].

This study consists of 70 % cases having lymph nodal metastases. It has been reported that elevated COX-2 expression was found to be common in tumours having axillary lymph nodal metastasis than those that do not.[85,86] In our study the expression of COX-2 was seen in tumors with lymph node metastases as well as without. Out of the total 32.5% positive cases for COX-2 expression 22.5% cases were positive for both lymph node metastases as well as COX-2 expression which comprises a major chunk. However, no positive correlation was observed between the two in our study. These findings were supported by the studies done by Costa et. al. and Misron et. al. that also commented that there were no positive relating factors between the lymph nodal status and COX-2 positivity [85,89]. Few other studies have commented upon the correlation between the two where they have mentioned that these two are positively correlated [107]. Such difference of opinion can be due to the

choice of threshold for p-value as well as because of the variation in sample sizes among different studies.

In our study,75 % cases demonstrated lymphovascular invasion. Out of 32.5% of positive COX-2 cases,22.5 % were ones that showed presence of lymphovascular invasion which forms the major bulk of the study. On statistical analysis, however, there was no positive correlation observed between LVI and COX-2 immunohistochemical positivity.There are several studies that have shown a significantly positive correlation between COX-2 expression and lymphovascular invasion [(87).] A study has also stated that this positive correlation indicates aggressive biological behaviour [(88).] However, there are studies like one done by Misron et. al, Davies et. al. that obtained a non significant P value and no correlation between COX-2 expression and lymphovascular invasion,thereby supporting our findings for the same [(89,90).]

The results depicted in our study thus have no supporting eveidence indicating an association of COX-2 immunohistochemical expression to the factors that are known to be associated with a poor outcome in malignancies of breast, such as large tumor size,higher histological grade, positive lymph nodal status & lymphovascular invasion.Further studies are therefore necessary to ascertain the conclusive prognostic significnce of COX-2. Studies of such kind would be helpful in evaluating the importance of selective COX2 inhibitors not only for therapy but also in the chem oprevention of breast malignancies in human race.

LIMITATION

A major limitation of our study was smaller sample size. Hence more studies with larger study population can be undertaken in future to establish a significant correlation among these entities.

CONCLUSION

To conclude, in the present study we have attempted to study the immunohistochemicalexpression of COX-2 in breast carcinoma and its correlation with various clinicopathologicalparameters that affect long term survival and prognosis of patient. COX -2 was seen to be expressed comparatively higher in sixth decade, female patients.

The expression was seen to be more in IDC type of histology and higher tumor grade with positive lymph node metastases and presence of lymphovascular invasion. However, no statistically significant correlation of COX-2 could be obtained with any of the parameters.

SUMMARY

A descriptive observational study of 40 cases were performed in Pathology Department of KAHER's Jawaharlal Nehru Medical College, Dr Prabhakar Kore Hospital & Research Centre,Belagavi.

The aim of present study was to evaluate the expression of COX-2 in histologically diagnosed cases of breast carcinoma and to study its relation with clinical parameters and histological features of breast carcinoma.

In this study, 32.5% cases showed COX-2 expression positivity among which 27.5% were weak positive cases and 5% were strong positive. Patients ranging from a minimum age of 30 years to a maximum of 87 years were in the study. A majority, 32.5% of the cases belonged to sixth decade of their lives and 15% showed positivity for COX-2 marker.

Females formed the major portion of the study group accounting for 95% cases, whereas 5% cases belonged to that of males. Among females, 25% were premenopausal with COX-2 positivity of 7.5% and 70% were postmenopausal with COX-2 positivity of 25% . 80% of the cases in the current study belonged to IDC type & 27.5% of IDC were positive for expression of COX-2. The study comprised of 60% of tumors with small size ranging from 2cm to 5cm in their largest dimension. 25% of these tumors were positive for the expression of COX-2. 72.5% of cases in this study belonged to Grade II and 25% cases to Grade III tumor. Among COX-2 positive cases, 17.5% belonged to Grade II & 15% belonged to Grade

III. Most cases, 75% , showed lymphovascular invasion among which 25% expressed positivity for COX-2 marker. Lastly,70% cases showed presence of lymph node metastases and among those cases 22.5% showed positivity for COX-2 immunohistochemical expression.

However, on statistical analysis, none of the above mentioned parameters showed any correlation with the expression of COX-2.

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ANNEXURE I – CONSENT FORM

INFORMED CONSENT

EVALUATION OF IMMUNOHISTOCHEMICAL EXPRESSION IN COX-2 ASSOCIATED WITH BREAST CARCINOMA.

Purpose of the study: You are being asked to enroll in this study as you are eligible for participation in this study. If you undergo mastectomy or lumpectomy for a breast lesion you will be included in this study.

The purpose of this study is to determine the prognosis associated with COX-2 in breast carcinoma.

Procedure: During this study, you will be asked questions regarding history and background and you are supposed to answer to the best of your knowledge. The principal investigator of the study is Dr. _____ under the guidance of Dr. _____(guide).

If you agree to enroll yourself in this study, you will be interviewed regarding your present, past and family history and your clinical manifestations.

Risks and benefits: There are no risks involved in taking part in this study and benefit is we will be able to know the prognosis for providing appropriate prevention and treatment.

Alternatives: Taking part in this study is voluntary. You may choose not to take part in this study or if you decide to take part now, you can later change your mind and withdraw from the study. The study doctor or sponsor may terminate your participation in this study anytime.

Privacy and confidentiality: All information collected about you during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify you in this research record. Information from this study will be published but your identity will be confidential in any publication. No information about you or information provided by you during research will be disclosed to other without your written permission except:

1. In emergency to protect your rights and welfare.
2. If required by law.

Financial incentives for participation: You will not be paid / offered any gift /incentives for participating in this study.

Authorization to publish results: The results of this study would be forwarded to the KLE University, Belagavi as a part of requirement towards the completion of MD degree, review and publishing.

Questions: In case you have any questions related to the study in future you can contact:

1. If you have any queries about your rights as a study subject, you may call Dr. RoopaBellad, Professor, Department of Paediatrics, Chairman of J.N. Medical College Institutional Ethical Committee of Human Subjects Research, Ph No-9448113403, at J.N. Medical College, Belagavi

CONSENT STATEMENT

I voluntarily agree to take part in this study by signing below. I may withdraw at any time. I am not giving up any legal rights by signing this form. My signature below indicates that I have read, or it has been read to me, this entire consent form and have had all my questions answered.

In case of the queries during the study or in future you may contact following person.

Name of the witness : (signature)

Name of the investigator: (signature)

Date:

Address & Phone no:

ANNEXURE – II

PROFORMA

NAME:

AGE:

GENDER:

IP NO.

DATE OF COLLECTION:

CLINICAL DETAILS:

- **DIET:**VEG/NON VEG
- **WEIGHT**(H/O RECENT WEIGHT LOSS)
- **PHYSICAL ACTIVITY**
- **MENSTRUAL HISTORY**

  AGE AT MENARCHE

  CYLCE LENGTH

  DURATION

  AGE AT MENOPAUSE
- **OBSTETRIC HISTORY**

  NUMBER OF LIVING CHILDREN

  AGE AT THE TIME OF FIRST BABY

  H/O BREAST FEEDING

  IF YES,HOW LONG BREAST FEEDING DONE?

H/O ORAL CONTRACEPTIVE INTAKE

IF YES,HOW MANY YEARS?

- **PAST HISTORY**

  H/O CA BREAST

  H/O CA ENDOMETRIUM

  H/O CA OVARY

  OTHERS

- **H/O PREVIOUS SURGERY**
- **FAMILY HISTORY**(CA BREAST)
- **H/O MEDICAL ILLNESS**

EXAMINATION:

- **SITE**
- **SIDE**
- **SIZE**
- **SKIN CHANGES**
- **AXILLARY LYMPH NODE INVOLVEMENT**
- **NIPPLE,AREOLA**
- **OTHERS**

INVESTIGATIONS

- **FNAC**
- **MAMMOGRAPHY**
- **HPR**
- **NODE STATUS**

- **ER/PR/HER2NEU**
- **OTHERS**

CLINICAL STAGING:

TREATMENT ADVISED-

HORMONAL

RADIOTHERAPY

CHEMOTHERAPY

HISTOPATHOLOGICAL CLASSIFICATION

COX-2 EXPRESSION:

ANNEXURE-III-ETHICAL CLEARANCE LETTER

K.L.E. ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Deemed – to- be- University)
Accredited 'A' Grade by NAAC (2nd Cycle) Placed in Category 'A' by MHRD (GoI)
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)
Website: http//www.jnmc.edu
E-Mail : dome@jnmc.edu
Phone: (+ 91-(0)831 Office : 2472550
Principal: 2471701
Fax No. +91 (0)831 – 2470759

Ref: MDC/DOME/37 **Date: 24/11/2018**

To,

Dr.
PG student in Pathology,
J.N.Medical College,
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"EVALUATION OF IMMUNOHISTOCHEMICAL EXPRESSION OF COX-2 IN BREAST CARCINOMA PATIENTS – A HOSPITAL BASED STUDY AT KLES DR. PRABHAKAR KORE HOSPITAL & MRC, BELAGAVI"**, is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Arathi Darshan)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Roopa M Bellad)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

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ANNEXURE – IV

HEMATOXYLIN AND EOSIN STAINING PROTOCOL

Bancroft D, Laylon C. The haematoxylin and eosin, In: Kim SS Ed, Bancroft's

Theory and practice of histopathological techniques.&th Ed., China, Churchill

Livingstone; 2013: p173-187.

1. Deparaffinize in Xylene I and II and III changes. (III change use warmedxylene) (5 minutes in each)

2. Rehydrate using

- Absolute ethanol 100% (5 minutes)
- Absolute Ethanol 100% (5 minutes)

3. Rinse in distilled water (5 minutes)

4. Rinse in running tap water (5 minutes)

5. Stain in Harris's haematoxylin by progressive method (2 minutes) Fresh andfiltered

6. Rinse in running tap water (20 minutes)

7. Decolorize in 1% acid alcohol (1 second)

8. Rinse well in tap water (5 minutes)

9. Immerse in hot water bath, 55˚C for blueing (3 seconds)

10. Rinse in tap water (5 minutes)

11. Counterstain in Eosin (15 seconds)

12. Dehydrate with absolute alcohol 100% (2-4 dips)

13. Clear in xylene I and II (5 minutes)

14. Mount with DPX.

Stock solution – Eosin:

Stock – 1% aqueous Eosin – Y

Stock – 1% aqueous Phloxin B

Working Solution – Eosin:

100ml stock Eosin

10 ml stock Phloxin B

780 ml 95% Ethanol

4 ml glacial acetic acid

Working Solution – Hematoxylin

Harris Hematoxylin, 1 litre

Working solution – 0.25% Acid alcohol

95% Ethanol, 2578 ml

dH2O, 950 ml

HCl, 9ml

Result: Nuclei – blue, cytoplasm – pink, RBCs – red.

ANNEXURE - V

Procedure for IHC staining for COX-2 antibody

1. Cut the sections at approximately 2- microns

2. Float on to the positive charged slides

3. Slides were air dried for 2 hours at 58 ºC.

4. Two changes of xylene of 10 minutes each for deparaffinization

5. Two changes of absolute alcohol of 5 minute each for rehydration

6. Wash in distilled water for 5 minutes

7. Antigen retrieval by heat, using microwave using TRIS EDTA Buffer

8. Cooling of sections to room temperature

9. Rinse in distilled water for 3 minutes

10. Wash in TBS buffer two times for 3 minutes each

11. Treatment with peroxide block for 10 minutes to block endogenous peroxidaseenzyme

12. Wash in TBS buffer two times for 3 minutes each

13. Treatment with primary antibody of COX-2 for 60 minutes to identify the endothelial cells by antigen-antibody reaction

14. Wash in TBS buffer two times for 3 minutes each

15. Treatment with Target binder for 10 minutes

16. Wash in TBS buffer two times for 3 minutes each

17. Treatment with HRP Polymer for 10 minutes

18. Wash in TBS buffer two times for 3 minutes each

19. Treatment with DAB (secondary antibody) for 3-5 minutes to give browncolor to antigens

20. Wash in distilled water for 3 minutes

21. Counter stain with Harris haematoxylin for 30 seconds to 1 minute

22. Wash in tap water for 3 minutes to remove excess stain

23. Two changes of absolute alcohol for 2 minute each for dehydration

24. Clearing with xylene for two minutes. Dry the slides and mount with DPX

Preparation of reagents

1. Antigen retrieval Buffer

TRIS EDTA Buffer- pH: 8.5 to 9.0

Preparation:

TRIS Base- 1.21 gram

EDTA (atomic number:372)- 0.37 gram

Dissolve in 1000ml of water

2. Wash buffer

TRIS BUFFERED SALINE (TBS)-pH: 7.2 to 7.6

Preparation:

TRIS Base- 8.6 gram

NaCl- 9.6 gram

Dissolve in 1000ml of water.

Adjust pH by using concentrated HCl

ANNEXURE VI

2019 WHO classification of tumors of the breast

- Epithelial tumors
- Invasive breast carcinoma
    - Infiltrating duct carcinoma (NOS)
    - Oncocytic carcinoma
    - Lipid rich carcinoma
    - Glycogen rich carcinoma
    - Sebaceous carcinoma
    - Lobular carcinoma NOS
    - Tubular carcinoma
    - Cribriform carcinoma NOS
    - Mucinous adenocarcinoma
    - Mucinous cystadenocarcinoma NOS
    - Invasive micropapillary carcinoma of breast
    - Metaplastic carcinoma NOS
- Rare and salivary gland type tumors
    - Secretory carcinoma
    - Acinar cell carcinoma
    - Mucoepidermoid carcinoma
    - Polymorphous adenocarcinoma
    - Adenoid cystic carcinoma
        - Classic adenoid cystic carcinoma
        - Solid basaloid adenoid cystic carcinoma

        - Adenoid cystic carcinoma with high grade transformation
    - Tall cell carcinoma with reversed polarity

- Neuroendocrine neoplasms
    - Neuroendocrine tumor, NOS
    - Neuroendocrine tumor, grade 1
    - Neuroendocrine tumor, grade 2
    - Neuroendocrine carcinoma NOS
    - Neuroendocrine carcinoma, small cell
    - Neuroendocrine carcinoma, large cell

- Epithelial - myoepithelial tumors
    - Pleomorphic adenoma
    - Adenomyoepithelioma NOS
    - Adenomyoepithelioma with carcinoma
    - Epithelial-myoepithelial carcinoma

- Non invasive lobular neoplasia
    - Atypical lobular hyperplasia
    - Lobular carcinoma in situ NOS
        - Classic lobular carcinoma in situ
        - Florid lobular carcinoma in situ
    - Lobular carcinoma in situ, pleomorphic

- Ductal carcinoma in situ (DCIS)
    - Ductal carcinoma, non infiltrating, NOS
        - DCIS of low nuclear grade
        - DCIS of intermediate nuclear grade
        - DCIS of high nuclear grade
- Benign epithelial proliferations and precursors
    - Usual ductal hyperplasia
    - Columnar cell lesions including flat epithelial atypia
    - Atypical ductal hyperplasia
- Adenosis and benign sclerosing lesions
    - Sclerosing adenosis
    - Apocrine adenoma
    - Microglandular adenosis
    - Radial scar / complex sclerosing lesion
- Papillary neoplasms
    - Intraductal papilloma
    - Ductal carcinoma in situ, papillary
    - Encapsulated papillary carcinoma
    - Encapsulated papillary carcinoma with invasion
    - Solid papillary carcinoma in situ
    - Solid papillary carcinoma with invasion
    - Intraductal papillary adenocarcinoma with invasion

- Adenomas
    - Tubular adenoma NOS
    - Lactating adenoma
    - Duct adenoma NOS
- Mesenchymal tumors
- Vascular tumors
    - Hemangioma NOS
        - Perilobular hemangioma
        - Venous hemangioma
        - Cavernous hemangioma
        - Capillary hemangioma
    - Angiomatosis
    - Atypical vascular lesion
        - Lymphatic atypical vascular lesion resembling lymphangioma
        - Vascular atypical vascular lesion resembling hemangioma
    - Postradiation angiosarcoma
        - Epithelioid angiosarcoma
    - Angiosarcoma
        - Epithelioid angiosarcoma
- Fibroblastic and myofibroblastic tumors
    - Nodular fasciitis
    - Myofibroblastoma
    - Desmoid type fibromatosis
    - Inflammatory myofibroblastic tumor

- Peripheral nerve sheath tumors
    - Schwannoma NOS
    - Neurofibroma NOS
    - Granular cell tumor NOS
    - Granular cell tumor, malignant
- Smooth muscle tumors
    - Leiomyoma NOS
        - Cutaneous leiomyoma
        - Leiomyoma of the nipple and areola
    - Leiomyosarcoma NOS
- Adipocytic tumors
    - Lipoma NOS
    - Angiolipoma NOS
    - Liposarcoma NOS
- Other mesenchymal tumors and tumor-like conditions
    - Pseudoangiomatous stromal hyperplasia
- Fibroepithelial tumors
    - Fibroadenoma NOS
    - Phyllodes tumor NOS
        - Periductal stromal tumor
    - Phyllodes tumor, benign
    - Phyllodes tumor, borderline
    - Phyllodes tumor, malignant
    - Hamartoma

- Tumors of the nipple
    - Nipple adenoma
    - Syringoma NOS
    - Paget disease of the nipple
- Malignant lymphoma
    - Diffuse large B cell lymphoma NOS
    - Burkitt lymphoma NOS/Acute leukemia, Burkitt type
        - Endemic Burkitt lymphoma
        - Sporadic Burkitt lymphoma
        - Immunodeficiency associated Burkitt lymphoma
    - Breast implant associated anaplastic large cell lymphoma
    - Mucosa associated lymphoid tissue lymphoma
    - Follicular lymphoma NOS
- Metastatic tumors
- Tumors of the male breast
    - Gynaecomastia
    - Carcinoma
        - Invasive carcinoma
        - In situ carcinoma

ANNEXURE VII

TNM STAGING (AGCC)

Primary tumor (pT)

- **pTX**: cannot be assessed
- **pT0**: no evidence of primary tumor
- **pTis**: ductal carcinoma in situ, Padget disease, encapsulated papillary carcinoma and solid papillary carcinoma
    - **pTis (DCIS)**: ductal carcinoma in situ without invasive carcinoma
    - **pTis (Paget)**: Paget disease without invasive carcinoma
- **pT1mi**: tumor ≤ 1 mm
- **pT1a**: tumor > 1 mm but ≤ 5 mm
- **pT1b**: tumor > 5 mm but ≤ 10 mm
- **pT1c**: tumor > 10 mm but ≤ 20 mm
- **pT2**: tumor > 20 mm but ≤ 50 mm
- **pT3**: tumor > 50 mm
- **pT4a**: extension to chest wall (not including pectoralis muscle)
- **pT4b**: edema (including peau d'orange), ulceration of skin or ipsilateral satellite skin nodules

- **pT4c**: both T4a and T4b
- **pT4d**: inflammatory carcinoma (involves > 1/3 of the breast skin, primarily a clinical diagnosis)

Regional lymph nodes (pN)

- **pNX**: cannot be assessed
- **pN0**: no regional lymph node metastasis histologically
- **pN0(i-)**: no regional lymph node metastasis by histology or immunohistochemistry
- **pN0(i+)**: isolated tumor cells (cluster ≤ 0.2 mm and < 200 cells)
- **pN0(mol+)**: RT-PCR positive but negative by light microscopy
- **pN1mi**: micrometastasis (tumor deposit > 0.2 mm and ≤ 2.0 mm or ≤ 0.2 mm and > 200 cells)
- **pN1a**: metastasis in 1 - 3 axillary lymph nodes with at least 1 tumor deposit > 2.0 mm
- **pN1b**: metastasis in internal mammary sentinel lymph node with tumor deposit > 2.0 mm
- **pN1c**: pN1a and pN1b
- **pN2a**: metastasis in 4 - 9 axillary lymph nodes with at least 1 tumor deposit > 2.0 mm

- **pN2b**: metastasis in clinically detected internal mammary nodes with pathologically negative axillary nodes
- **pN3a**: metastasis in ≥ 10 axillary lymph nodes with at least 1 tumor deposit > 2.0 mm or metastasis to infraclavicular lymph node
- **pN3b**: positive internal mammary node by imaging with pN1a or pN1b
- **pN3c**: metastasis in ipsilateral supraclavicular lymph node

Distant metastasis (M)

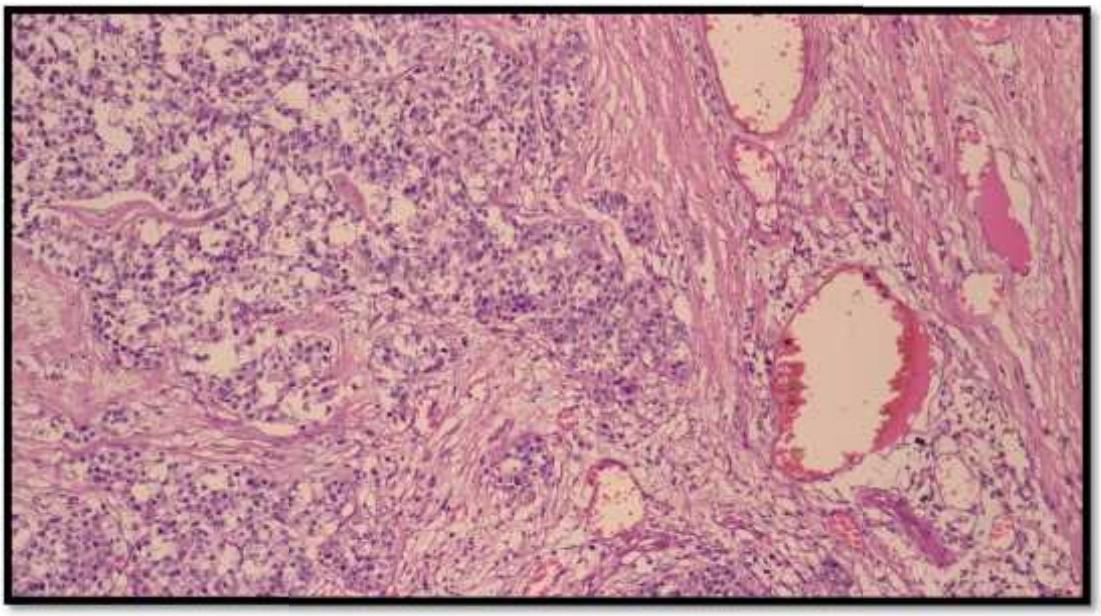
- **pM1**: distant metastasis histologically proven > 0.2 mm

Prefixes:

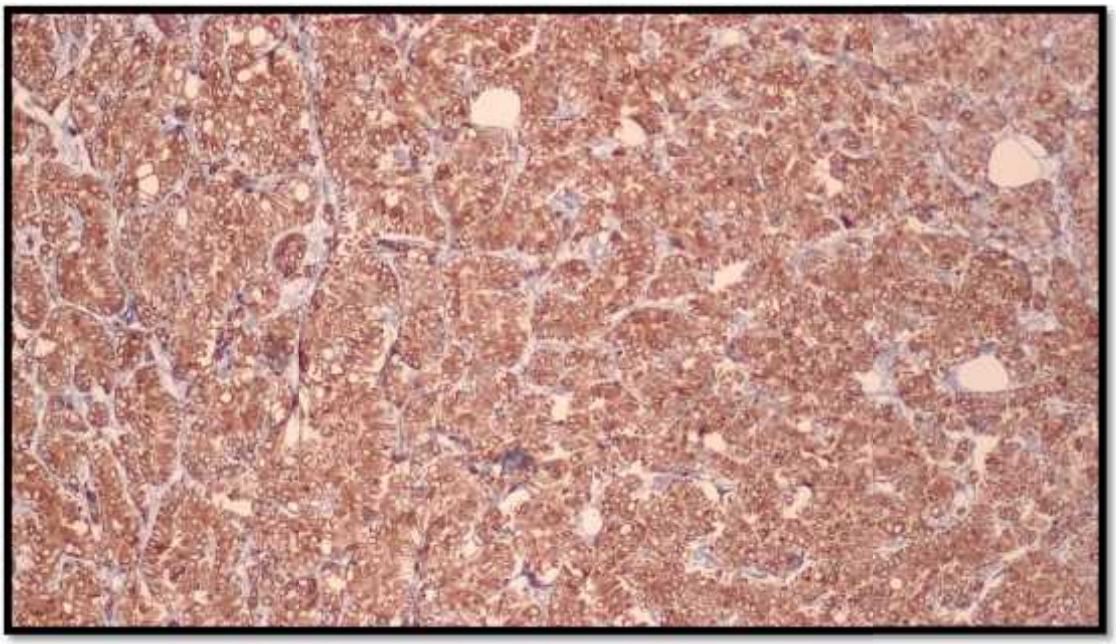
y: preoperative radiotherapy or chemotherapy

r: recurrent tumor stage

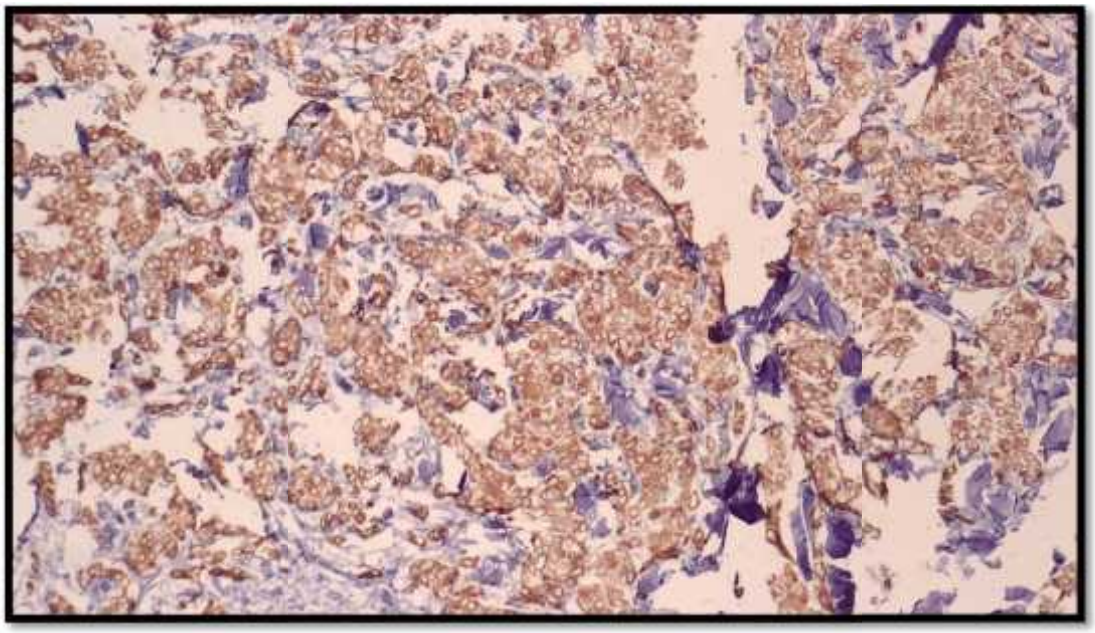
ANNEXURE-VIII-PHOTOGRAPHS



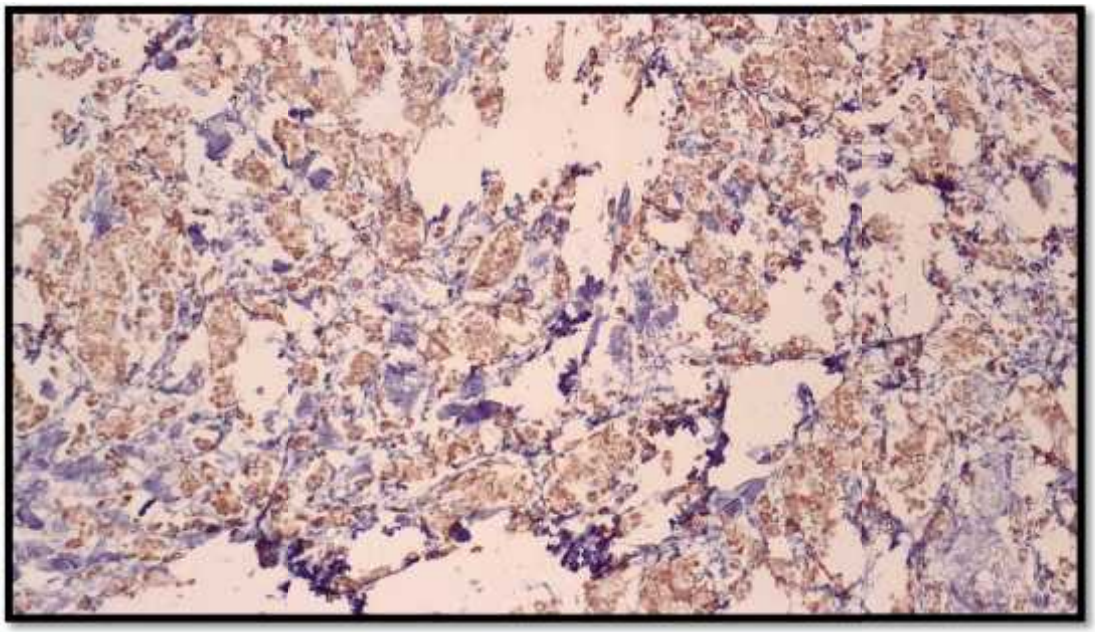
<u>Microphotograph A</u> - H & E; 10x ; Tumor tissue (IDC)



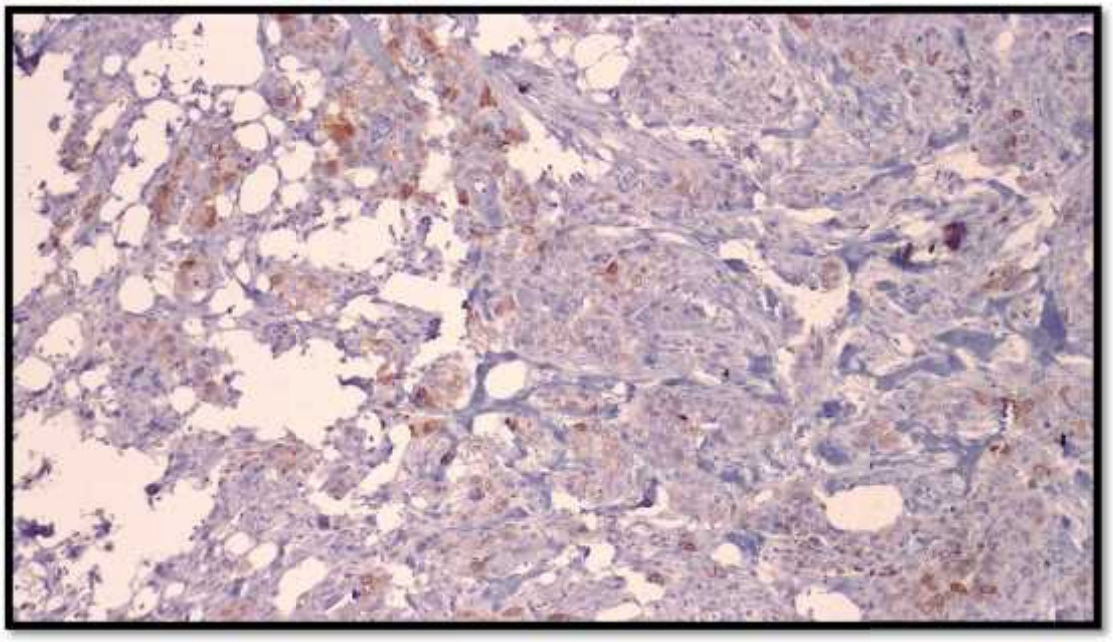
<u>Microphotograph B-</u> COX-2 immunohistochemistry; 10x; Strong positive



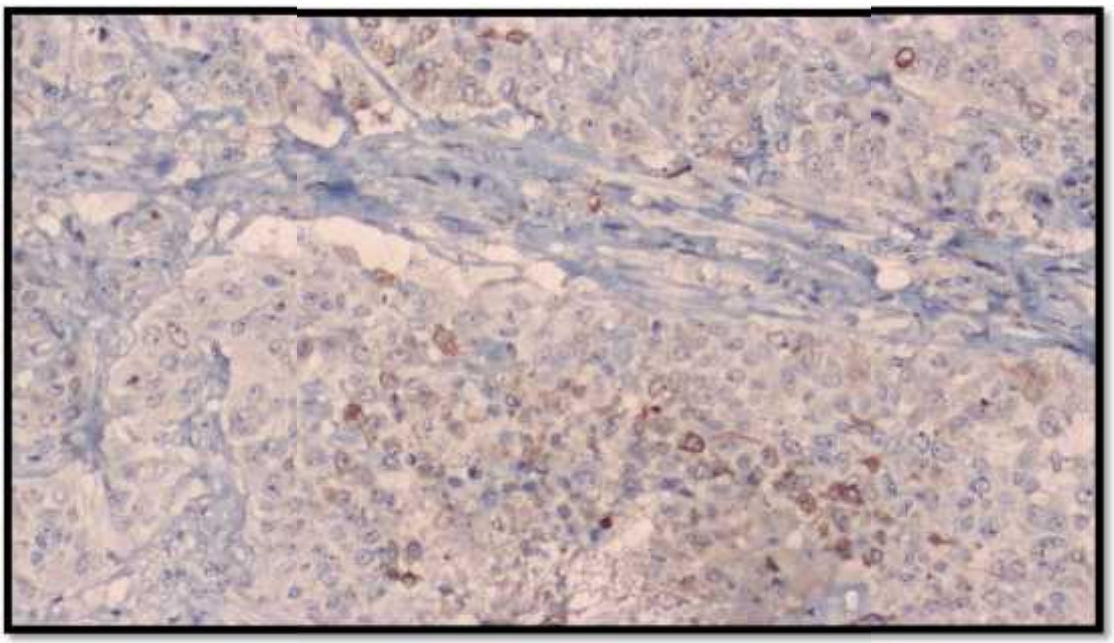
<u>Microphotograph C-</u> COX-2 immunohistochemistry;10x; Strong positive



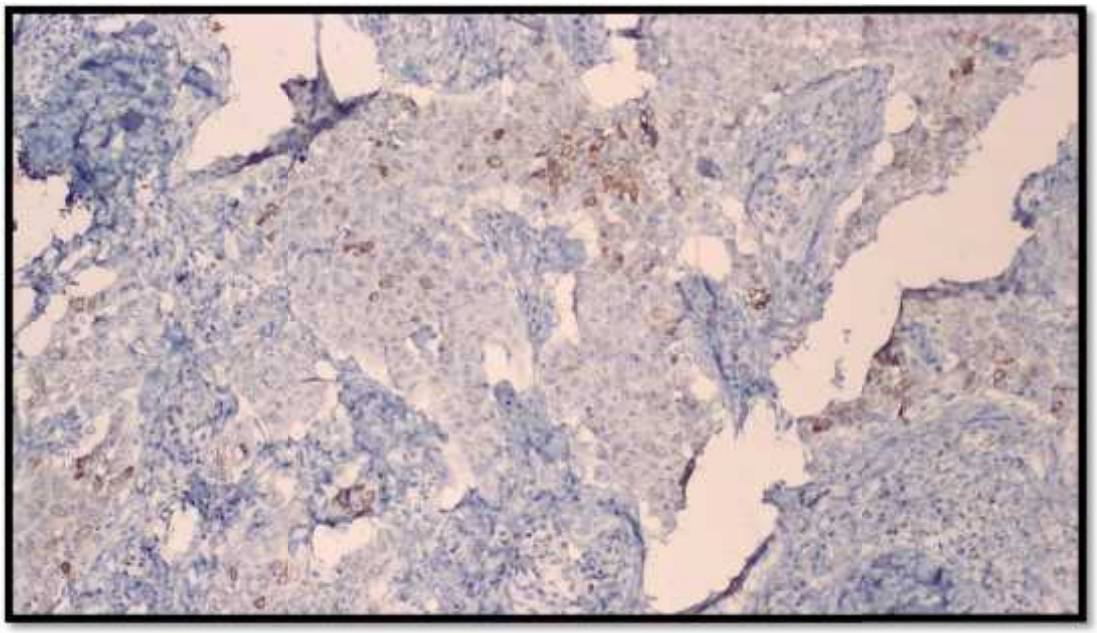
<u>Microphotograph D-</u> COX-2 immunohistochemistry;10x; Strong positive



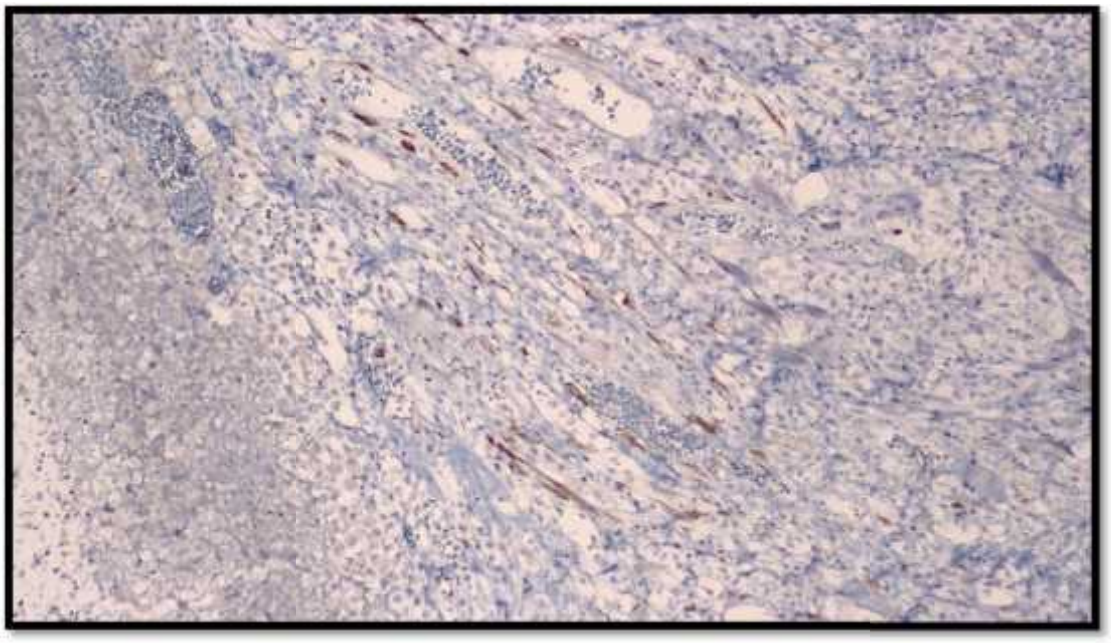
<u>Microphotograph E-</u> COX-2 immunohistochemistry; 10x; Weak positive



<u>Microphotograph F-</u> COX-2 immunohistochemistry; 20x; Weak positive



<u>Microphotograph G-</u> COX-2 immunohistochemistry; 10x; Weak positive



<u>Microphotograph H-</u> COX-2 immunohistochemistry; 10x; Negative

ANNEXURE-IX

KEY TO MASTER CHART

Menopausal status-

0: Premenopausal

1: Postmenopausal

LVI (lymphovascular invasion)

0-Absent

1-Present

LNM (lymph node metastases)

0-Absent

1-Present

COX-2 expression

0-Negative

1-Weak positive

2-Strong positive

ANNEXURES - X - MASTER CHART

Sr No.	Patient Id.	Age	Gender	Menopausal St	Histological type	Tumor grade	Tumor size	LVI	Lymph node Metastasis	COX-2
				0/1				0/1	0/1	0 1+ 2+
1	993227	55	F	1	IDC	2	7	0	1	0
2	3009791	50	F	1	IDC	2	2	1	0	1
3	924002	41	F	0	IDC	2	5	1	0	0
4	3009496	49	M	NA	IDC	2	4	1	1	0
5	999107	57	F	1	IDC	2	13	1	1	0
6	999112	83	F	1	IDC + DCIS	2	5	0	1	0
7	986103	55	F	1	IDC	2	4	0	0	1
8	19064730	63	F	1	IDC	3	3	1	0	0
9	925152	53	F	1	IDC	3	3	1	1	1
10	932111	50	F	1	IDC	2	2	1	1	1
11	932714	36	F	0	IDC	2	3	1	1	1
12	19114736	65	F	1	IDC	3	3	1	1	1
13	933057	30	F	0	IDC	2	3	1	0	0
14	935205	68	F	1	IDC	2	4	0	1	0
15	19163253	60	F	1	IDC	3	6	1	0	0
16	934115	71	F	1	LOBULAR CA	2	3	0	0	0
17	921082	80	F	1	PAPILLARY CA	2	5	0	0	0
18	19236447	55	F	1	IDC	3	3	1	1	0
19	3012153	72	F	1	IDC	2	5	1	0	0
20	958816	68	F	1	IDC	2	6	0	1	0
21	3012324	65	F	1	IDC	1	6	1	1	0
22	961415	59	F	1	IDC	2	6	1	1	0
23	961202	59	F	1	IDC	2	3	1	1	0
24	19363762	62	F	1	IDC+ DCIS	2	3	1	1	0
25	963752	58	F	1	IDC	2	5	1	1	0
26	965969	60	F	1	IDC	2	3	0	0	1
27	19391366	45	F	0	IDC	2	3	1	0	0
28	969132	45	F	0	IDC	3	8	1	1	1
29	969337	58	F	1	MEDULLARY CA	3	3	1	1	1
30	19475866	40	F	0	IDC	2	3	1	1	0
31	983405	50	F	1	IDC	3	17	1	1	2
32	19565569	74	M	NA	IDC	2	20	1	1	0
33	19565715	83	F	1	IDC	2	7	0	1	2
34	19581952	87	F	1	IDC	2	7	1	0	1
35	19585431	43	F	0	IDC+ DCIS	2	4	1	1	0
36	988827	64	F	1	IDC + PAGET'S	2	4	1	1	0
37	3014130	41	F	0	IDC+ DCIS	2	7	1	1	0
38	3014245	35	F	0	IDC	3	6	1	1	0
39	3014364	51	F	1	IDC	2	5	0	1	0
40	19419975	46	F	0	IDC	3	3	1	1	1