"CORRELATION OF IMMUNOHISTOCHEMICAL EXPRESSION OF BRCA1 AND BRCA2 IN BREAST CARCINOMA PATIENTS - A CROSS SECTIONAL STUDY AT KLE's DR. PRABHAKAR KORE HOSPITAL AND MRC, BELAGAVI."

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LIST OF ABBREVIATIONS USED

WHO	-	World Health Organisation
IHC	-	Immunohistochemistry
BRCA	-	BReast Cancer gene
TNM	-	Tumor stage, Nodal status, Metastasis
H & E	-	Haematoxylin and eosin
mRNA	-	Messenger Ribonucleic acid
TDLU	-	Terminal duct lobular unit
DCIS	-	Ductal carcinoma in situ
IDC	-	Invasive ductal carcinoma
LCIS	-	Lobular carcinoma in situ
NOS	-	Not otherwise specified
PTEN	-	Phosphatase and TENsin homologue
PALB2	-	Partner and localiser of BRCA2
CHEK2	-	Checkpoint kinase 2
CDH1	-	Cadherin-1
HRT	-	Hormone replacement therapy
AJCC	-	American Joint Committee on Cancer
SBR	-	Scarff-Bloom-Richardson

ABSTRACT

"CORRELATION OF IMMUNOHISTOCHEMICAL EXPRESSION OF BRCA1 AND BRCA2IN BREAST CARCINOMA PATIENTS- ACROSS SECTIONAL STUDY AT KLE'S DR. PRABHAKAR KORE HOSPITAL AND MRC, BELAGAVI"

Background and Objectives: Breast cancer is the second most commonly diagnosed cancer and is the fifth leading cause of death worldwide. Among the genes associated with breast cancer, the genes BRCA1 and BRCA2 are strongly involved in pathogenesis.It is seen that on IHC there is decreased or absent expression of these genes protein in familial and sporadic breast cancer. The purpose of this study to the immunohistochemical expression of BRCA1 and BRCA2 in breast carcinoma patients and to correlate their expression with various clinicopathological parameters.

Methods: The present study was a prospective as well as retrospective study which included 40 cases from January 2018 to December 2019. Clinical details pertaining to the patient with breast carcinoma admitted to the tertiary care hospital were obtained from the case papers, and from the medical records department of the hospital. The details of the gross and histopathological findings were obtained from the requisition forms and report forms archived in the department ofpathology. Paraffin embedded histologically proven invasive breast tissue sections that were obtained and the adjacent normal breast tissue sections used as controls to determine breast carcinoma specific changes in the expression of BRCA1 and BRCA2 by immunohistochemistry (IHC).Statistical analysis using chi square test was applied. A p value of <0.05 was considered statisticallysignificant.

Results: Most of the cases showed either low or no detectable level of BRCA1 expression in tumor tissues. The decline in BRCA1 expression was found to be more prominent in modified Bloom Richardson grade III showing that tumors with higher histological grade have lower BRCA1 protein expression.

Conclusion: This study results demonstrated BRCA1 positivity decreased with presence of family history, increase in age, lymph nodes showing metastasis and higher grade of tumor. However, no significant correlation was seen among all the parameters except modified Bloom Richardson grade of tumor.

Key words:BRCA1 gene, BRCA2 gene, immunohistochemistry, breast cancer

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INTRODUCTION

Breast cancer is the second most commonly diagnosed cancer in the world followed by lung cancer and is, by far the most frequent cancer among women with an estimated 2.1 million newly diagnosed female breast cancer cases in 2018, contributing for about 11.6% of the total cancer incidence burden, accounting for almost 1 in 4 cancer cases among women.[1]

Breast cancer ranks as the fifth leading cause of death worldwide (627,000 deaths, 6.6%). Among females, breast cancer is the leading cause of cancer death (15%), followed by lung cancer and colorectal cancer.[1,2]

In India, breast cancer is the most frequently observed cancer (14% of the total cases) and it is the leading cause of cancer death (11.1% of the total cases) followed by cancers of lip and oral cavity.[3,4]

Among females, breast cancer is the most commonly diagnosed cancer (27.7%) followed by the cancers of cervix uteri. It is also the leading cause of cancer death in women (23.5%) followed by cancers of cervix uteri in india.[3,5]

In the state of Karnataka state the incidence is as high as 36.6 per 100 000 women.[6]

Overall breast cancer has emerged as the leading cause of morbidity and mortality in India in 2018. The main reasons for this observed hike in mortality is due to lack of inadequate breast cancer screening, unavailability of appropriate medical facilities and diagnosis of disease at advanced stage.[1,2]

There are many genetic and non-genetic factors that are linked with breast cancer growth. For example, non genetic factors include excessive exposure to oestrogen due to early menarche, oral contraceptive usage, late menopause, sedentary lifestyle and high fat diet play a significant role in breast cancer development.[7]

Almost 12% of breast carcinoma cases are attributable to inheritance of an identifiable susceptibility gene or genes. Among the genes associated with breast cancer, the genes BRCA1 and BRCA2 are strongly involved in pathogenesis; both genes are now cloned and have been fully characterised.[8,9]

BRCA1 and BRCA2 are two tumor suppressor genes. BRCA1 located on human chromosome 17q21 and encodes a protein of 220 kDa with 1863 amino acids; whereas BRCA2 is present on 13q12 and produces a 384 kDa protein having 3418 amino acids. Their mutations are responsible for 80% to 90% of single gene familial breast cancers and about 3% of all breast cancers.[9,10]

The probability of familial breast cancer increases when there are multiple affected first degree relatives, early onset, multiple cancers or family members with other specific cancers. However, the genetic testing of BRCA1 and BRCA2 is difficult and restricted to individuals having a strong family history and certain ethnic groups.[8]

Therefore immunohistochemical identification of BRCA1 and BRCA2 should facilitate early diagnosis of breast cancer susceptibility.[11]

The BRCA1 protein is found in the nucleus of normal and malignant breast tissue exclusively. Several authors have documented that on immunohistochemical analysis, it is seen that there is decreased or absent expression of BRCA1 protein in

familial and sporadic breast cancer.[12,13] Altered expression may include process other than direct mutation of the BRCA1 gene, such as loss of allele or methylation of the BRCA1 promoter region.[14-17]

Similarly, few Indian studies also indicate that reduced expression of BRCA1 and BRCA2 protein can play an important role in Indian cases of breast carcinomas and that mechanisms other than mutation can lead to diminished expression of BRCA1 and BRCA2 protein.[11]

Present study has been devised to look for BRCA1 and BRCA2 proteins expression in breast cancer cases and also to correlate their expression with various clinicopathological variables.

AIMS AND OBJECTIVES

1. To study the immunohistochemical expression of BRCA1 and BRCA2 in breast carcinoma patients.
2. To study the correlation of BRCA1 and BRCA2 expression with clinical parameters like family history and age.
3. To study the correlation of BRCA1 and BRCA2 expression with tumor grade, tumor size and lymph node metastasis.

REVIEW OF LITERATURE

Embryology and Development of Human Breast

The breasts are a secondary sexual feature in females and the neonate's source of nutrition.[18] The human mammary gland is comprised of the parenchyma and stroma. The parenchyma forms a system of branching ducts which eventually leads to the development of secretory acini, and the stroma mainly consists of adipose tissue which provides the environment for the parenchymal development and support.[19, 20]

The course of development of the acini and ductal system is called as the branching morphogenesis and while it begins in the fetus, it ceases until puberty when hormonal stimulation prompts further differentiation. Under the control of hormones, the epithelium and mesenchyme undergoes a complex reciprocal interactivity leading to differentiation of the prenatally formed rudimentary framework to develop a mature mammary gland.[21,22]

In the 1st trimester, mammary-specific progenitor cells appear as soon as 4 to 6 weeks of gestation.[23] In the epidermis of the thoracic region, around day 35 of gestation, paired areas of epithelial cells proliferates and is extended in a line from the fetal axilla to inguinal region. The two elevations formed are termed as the mammary crests or the milk line (Fig.1). These paired epithelial masses continue to grow in the pectoral region at the 4th intercostal space forming the primary mammary buds while the remaining part of the mammary crest atrophies.[24,25]

By the end of the three months of gestation, the primary mammary buds start to enlarge and develop downwards into the underlying mesenchyme, under the control of regulating factors secreted by the mesenchyme and moves from dorsal to ventral position. Indentations around its basolateral margin arise, forming sites for the future secondary mammary outgrowths.[26,27] The core of these cells evaginates into the underlying stroma and gets surrounded by a fibroblast like cells within a collagenous mesenchyme forming a more cellular zone. In the end of this trimester, a well-defined mammary bud is noticed penetrating the upper dermis.[21]

During the second trimester, the main mammary bud indentations form the secondary epithelial buds which grow vertically into the mesenchyme encircling the primary bud. The secondary epithelial proliferates, canalizes and coalesces forming secondary buds which later give rise to lactiferous ducts (Fig. 1).[27] The lactiferous ducts are lined by the epithelial cells which are arranged in two layers, with the layer abutting to the lumen have secretory function and the basal layer differentiates into myoepithelial cells. By six months of gestation, the basic structure of the mammary gland is formed.[21,22]

In the third trimester, the secondary epithelial buds undergo repeated branching and canalization. The epidermis gets depressed in the area of the future nipple, forming the mammary pit (Fig. 1)[21,28] and these lactiferous ducts drain into retroareolar ampullae that converges into this pit. The nipple is further delineated by mesoderm proliferation, caused by the invagination of the ectodermis in this area. The nipple is formed with smooth muscle fibers arranged in a longitudinal and circular fashion. The surrounding areola is formed by the ectoderm throughtout the fifth month of gestation.[27]

Towards the final weeks of gestation, the vascularity increases in the loose fibroconnective tissue stroma.

At term, approximately 15 to 20 lobes of glandular tissue have formed, each containing a lactiferous duct that opens onto the breast surface through the mammary pit. Both the fibrous suspensory ligaments of Cooper that anchor the breast to the fascia of pectoralis major and the surrounding skin support the mammary gland.[21]

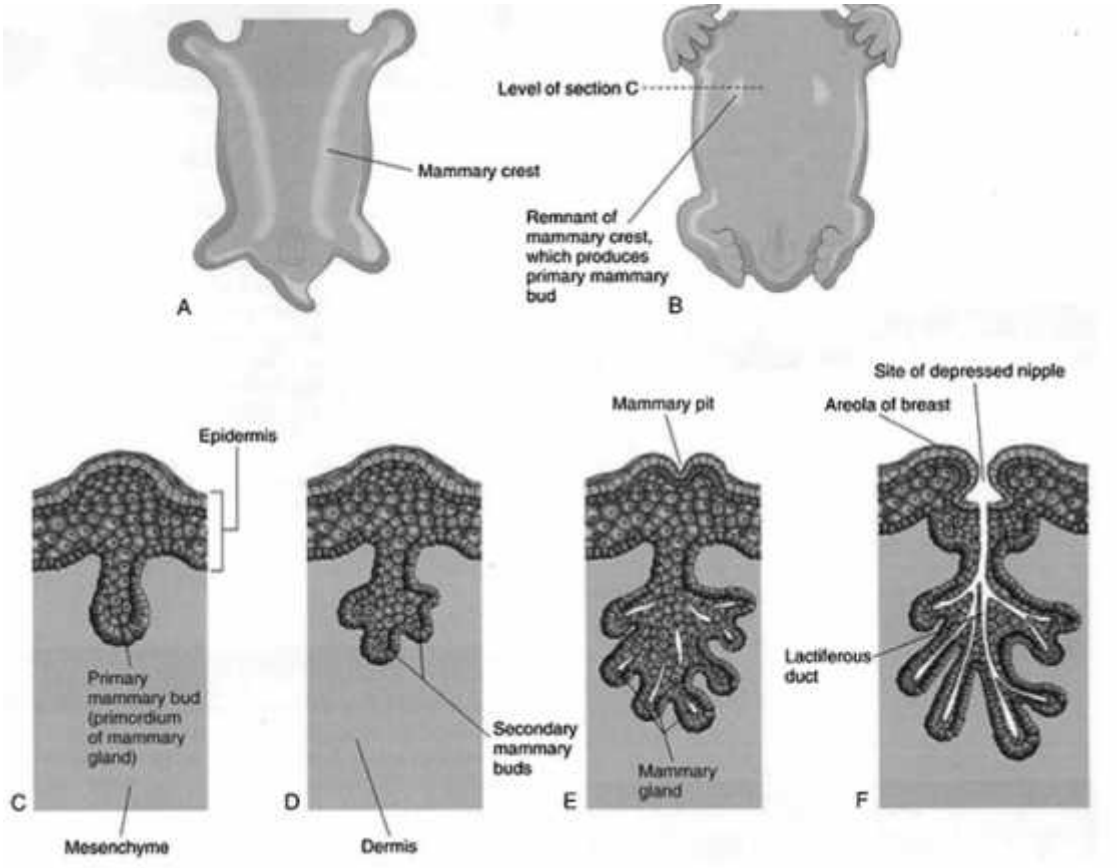


Fig1: Development of mammary gland [21]

The first 2 years of life are an important period for both breast maturation and involution. The normal mammary gland remains inactive from the age of two upto puberty.[29] Soon after birth, the nipples are everted. As a result of proliferating mesoderm, the areolar pigmentation increases and an increase in vascularity of the

stroma creates a visible difference between the light periductal connective tissue and the surrounding denser stroma.[27,30]

Female Breast Development and its Control

Breast development begins at puberty with the onset of periodic oestrogen and progesterone secretion. Oestrogen helps in the differentiation of the periductal stroma and along with the growth hormone and glucocorticoids participates in ductal growth. Progesterone, insulin and growth hormone are responsible for lobulo-alveolar growth and differentiation. Though the significant amount of breast glandular differentiation takes place during puberty, it continues during the second decade and is more enchanced in pregnancy.[31]

In males: At puberty, rising testosterone levels stops further development of the mammary gland.[32]

Anatomy of Breast

The mammary glands are a highly specialised type of modified sweat glands consisting of glandular and supporting fibrous tissue embedded within fatty matrix, together with blood vessels, nerves and lymph vessels.[33]

The breast lies on the deep fascia covering the pectoralis major. It is divided into four quadrants – upper medial, lower medial, upper lateral, and lower lateral. The upper lateral quadrant has a small extension termed as the Axillary tail of Spence which traverse through an opening in the deep fascia and ends in the axilla. Vertically it extends from the second to the sixth rib and horizontally from lateral border of sternum to mid axillary line.[18]

Structure: The breast is made up of 15-20 lobules of glandular tissue embedded in fat. These lobules are separated by fibrous septa running from the subcutaneous tissue to the fascia of the chest wall.[34]

The morphological and functional unit of the breasts are the lobes which are comprised of glands, ducts and terminal lobules in a fibrocollagenous connective tissue stroma. It has been labelled as the terminal duct lobular unit (TDLU). TDLUs are the functional milk secreting component of the breast and is responsive to hormonal changes and is also the most common site for a primary malignancy of the breast.[35,36]

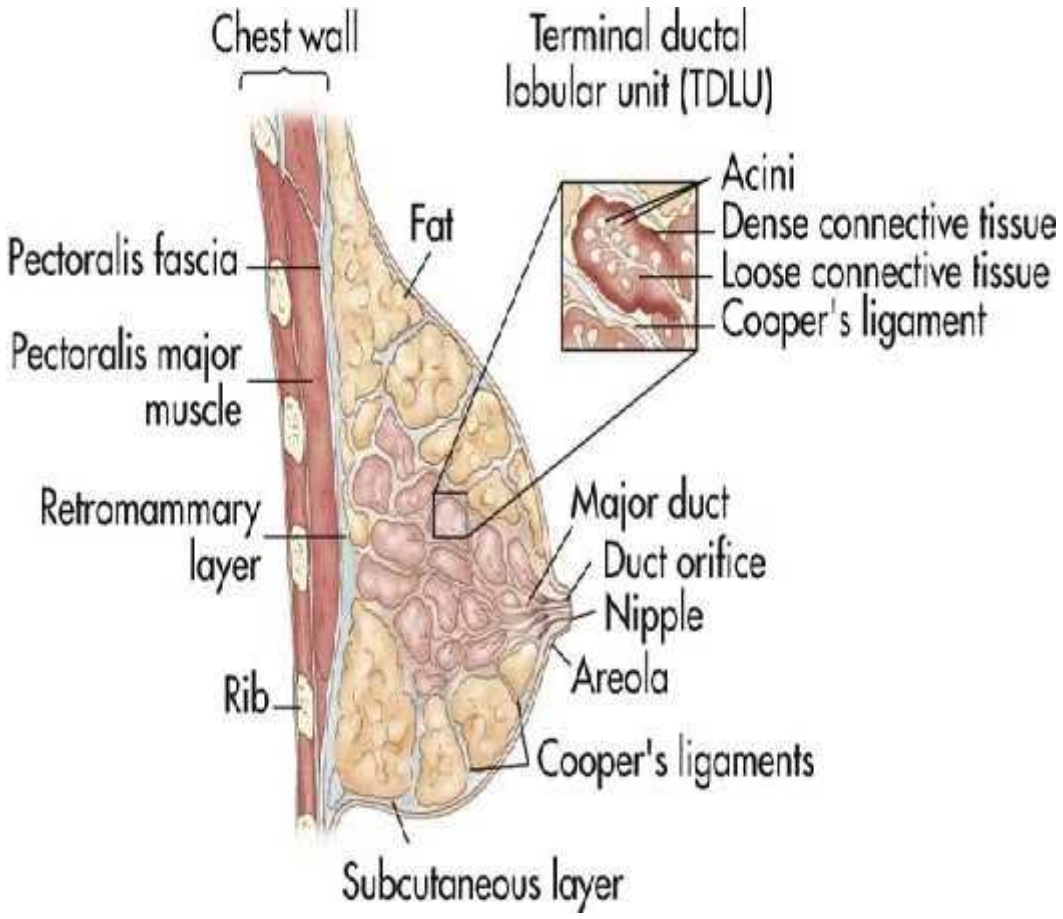


Fig 2: Normal anatomy of breast[21]

Nipple Areolar 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 known as the areola. It contains numerous sweat glands which open into the skin surface. These glands can become grossly visible near term pregnancy or in lactating mothers as circumferentially arranged elevations known as Montgomery's tubercles.[35]

Arterial supply:

- Lateral thoracic artery
- Internal thoracic Artery (branch of subclavian artery)
- Pectoral branches of thoraco-acromial artery
- Lateral branches of posterior intercostal arteries.

Venous drainage:

- Internal mammary vein
- Tributaries of the axillary vein
- Posterior intercostal veins.

Lymph from the breast drains into the axillary lymph nodes (around 75%), mainly from the anterior {or pectoral} group and a part from apical, central, lateral and posterior groups of nodes either directly or indirectly.

Some of the lymphatic fluid also drains into the following:

- Internal mammary (parasternal) nodes.
- Supraclavicular nodes
- Cephalic (deltopectoral) nodes

- Posterior intercostal nodes
- Subdiaphragmatic and subperitoneal lymph plexuses.

Breast is supplied by the lateral and anterior cutaneous branches of the fourth and sixth intercostal nerves.[34,35]

Histology

Nipple: Covered by pigmented squamous epithelium. Ducts dilate forming lactiferous sinuses beneath the nipple. Sinuses have serrated contours and these are supported by elastic fibres, collagen, and smooth muscles. Basement membrane encircles the entire mammary ductal/lobular system separating the epithelial cells from the stromal tissue.

Areola: Pilosebaceous units and hair are not present except at periphery. Many sensory nerve endings are also seen.

Large Duct System: 15-20 major duct systems empty at nipple and additional smaller ductal systems open onto areola. The ducts are subdivided to form TDLUs.

Lobules: Terminal duct branches into multiple rounded acini (TDLU) to form lobules. They have Lobulocentric architecture.

Epithelial Cell Types: Two types of epithelial cells are seen in breast which are: luminal cells and myoepithelial cells.

Luminal Cells: They form the innermost lining of ducts and acini. Cells are cuboidal to columnar in shape having small, round to oval nuclei and inconspicuous nucleoli. Cytoplasm is moderate and eosinophilic.

Myoepithelial Cells: They form the outermost layer between the basement membrane and luminal cells. The cells form a contractile meshwork which does not cover the entire basement membrane. Cells are flattened with clear and abundant amount of cytoplasm having small round nuclei.

Stroma: It is composed of varied amount of fibrous connective tissue and adipose tissue. Breast stromal tissue to parenchymal tissue ratio varies depending on the age, menstrual status, pregnancy history and lactation.[33]

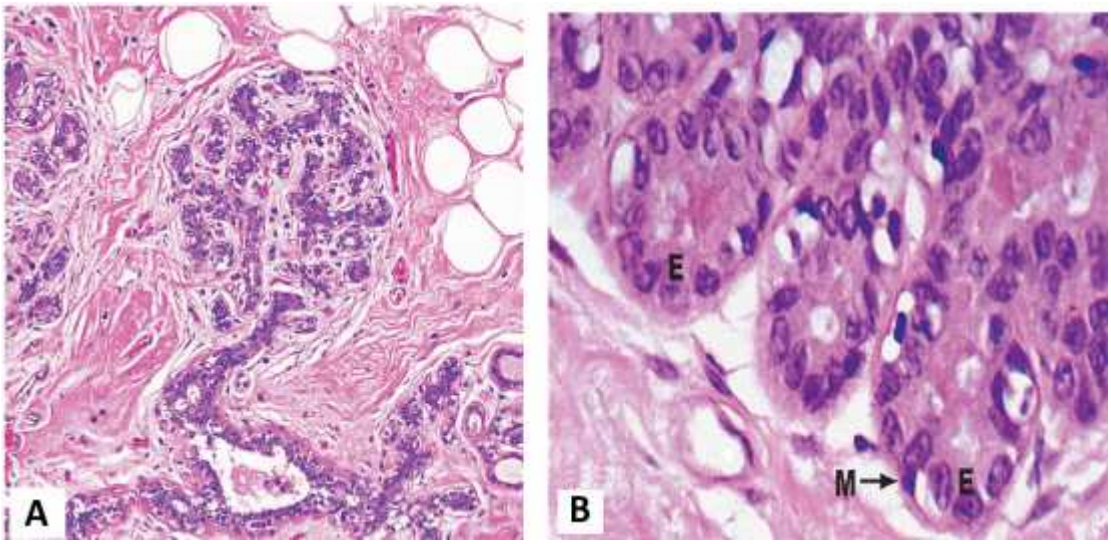


Fig 3: Histology of breast[33] A) Shows terminal duct–lobular units (TDLU) .

B) E- luminal epithelial cells M- myoepithelial cells

WHO Classification of Breast Tumors, 2019 5th edition (Annexure I)

Morphology of Breast Carcinoma

Breast cancer can be classified into biological and clinical subgroups according to histological grade (Elston and Ellis, 1991)[37] and histological type (Ellis et al, 1992).[38]

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).[37]

Histological type refers to the growth pattern of the tumors. Pathologists identified specific morphological and cytological patterns that were consistently associated with distinctive clinical presentations and outcomes. These patterns are called 'histological types'

Ductal carcinoma in situ (DCIS)

The ductal term 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.

In non comedo category, cribriform DCIS has round spaces within the ducts or has a solid DCIS pattern and micropapillary DCIS produces bullous protrusions

which lack a fibrovascular core. Focal calcifications, focal necrosis and intraluminal secretions maybe seen in non comedo DCIS.[39]

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 having markedly pleomorphic nuclei with irregular contour, coarse clumped chromatin and distinct nucleoli.[40]

Lobular carcinoma in situ (LCIS)

It is usually 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 a uniform population of cells which have round to oval nuclei and small nucleoli. Signet ring cells are commonly seen. Pagetoid spread is commonly seen. The cells do not form cribriform spaces or papillae.[39]

Invasive lobular carcinoma

Invasive lobular carcinoma represents 5 to 15% of invasive breast tumors. They are usually seen as irregular and poorly defined tumors, and have diffuse growth pattern. Microscopically, there is proliferation of small cells, arranged in Indian file arrangement invading the stroma.

Invasive ductal carcinoma, not otherwise specified (NOS)

It is the most common 'type' and the largest group of invasive carcinoma of breast comprising upto 40% to 75%, it is the most common diagnosed subtype.[41]

It is a heterogenous group of tumors that fail to exhibit sufficient characteristics to achieve classification as a specific histological type, such as lobular or tubular carcinoma. Tumor size varies from 0.1 to 1cm having irregular or nodular configuration.[40] Classically, they are firm or even hard on palpation and may have a gritty feel when cut with the knife.

Microscopically, tumor cells may be arranged in clusters, cords, trabeculae, solid or syncytial infiltrative pattern. Cells have abundant eosinophilic cytoplasm.

Nuclei may be regular, uniform or highly pleomorphic with multiple prominent nucleoli and mitotic activity may be virtually absent or extensive.

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 scaling or crusting. The lesion mimics eczema. Pruritus 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.[39]

Risk Factors

Age: The risk of carcinoma breast increases with advancing age. Reproductive influences include young age of menarche (age lesser than 12 years), late primigravid (age more than 30 years), and late age of cessation of menstrual cycles (age greater than 55 years) may also be a factor for cause of breast cancer. [42]

Family history: The risk of breast cancer approximately doubles among women whose mother has been previously diagnosed as a case of breast cancer or who have a sister with a history of breast cancer.[43]

Genetic Factors: The most common cause of hereditary breast cancer is the BRCA1 located on human chromosome 17q21 and BRCA2 located on 13q12. The risk of carcinoma is dependent on the position of 13 q chromosome and the mutation of the same. The other genes which cause breast cancer include gene critical for DNA repair namely the ATM gene. Inheritance of one mutated copy is linked with the risk of breast cancer. Other genes associated with breast cancer are PTEN {Phosphatase and tensin homologue function as tumor suppressor gene}, PALB2 {interacts with BRCA1 and also localiser and partner for DNA repair}. CHEK2 {important for DNA repair}, CDH1 {E-Cadherin gene plays a role of tumor suppressor gene}.[44]

Weight: Obesity is associated with an increased risk of breast cancer in postmenopausal women whereas lower incidence is seen before menopause.[45]

Exercise: It has been seen that exercise is beneficial in reducing level of hormone.[44] Some studies show that physically active lifestyle after cancer treatment prevents relapse and reduces the overall risk of mortality. Favorable lifestyle, which includes, low calorie diet, increase in exercise, reduction in alcohol intake and less environmental exposures to disturb circadian rhythm can reduce breast cancer by one-third.[45]

Breast feeding: Prolonged breast feeding reduces the chances of breast cancer. Also, the risk of breast carcinoma is decreased as the number of children breastfed increases.[39,46]

Parity: Low parity and late age of primiparity are significant and independent determinants of increased risk of breast cancer. Nulliparity is associated with a 30%

increase risk as compared to the parous women. There is also a significant trend of increase in risk with increasing age at first birth.[47]

Smoking: There is a higher risk of breast cancer with increased exposure.[44]

Radiation exposure: Radiation exposure at a younger age (lesser than 30 years) such as in cases of patients undergoing treatment for lymphoma are at a greater risk . The risk of developing breast cancer is directly proportional with dose of radiation and gives a linear line on plotting.[44]

Hormone replacement therapy (HRT) : The risk of breast cancer is higher in combined estrogen and progestogen combinations. HRT increases the breast density, and reduces the sensitivity and specificity of screening of the breast. Cancers diagnosed in women taking HRT tend to be less advanced clinically than those diagnosed in women who have not used HRT. However, current evidence suggests that HRT does not increase breast cancer mortality.[45]

Bone density: Higher values of bone density act as an accessory indicator for high estrogen levels in the blood corresponding to a greater risk of development of breast cancer.[44]

Precancerous breast disease: Patients with atypical ductal hyperplasia or lobular hyperplasia are at quadruple time danger of developing a breast cancer. History of even a benign breast disease is associated with an increased risk.[39]

Geographic influence: White women are slightly more likely to develop breast cancer than African-American women. But African-American women 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 women are relatively safer from development of and death due to breast carcinoma.[48]

Prognostic Factors

Tumor size: Small tumors restricted to the breast, with no metastasis hold a greater probability of getting cured. Big tumors which invade the axillary group of lymph nodes with an indolent spread to other organs are a marker for late stage of cancer.[49]

Lymph node status: Involvement of the axillary group of lymph nodes is an important prognostic factor particularly in individuals with early onset of breast cancer. Although there are discoveries of newer tumour markers, the status of the axillary node still remains a sole critical indicator for prognosis in breast carcinoma.[50,51]

Tumor type: The histopathological characteristics indicate the prognosis. Tubular, mucinous, and medullary subtypes of breast carcinoma have better prognosis than breast cancer of non specific origin.[52,53]

TNM staging: TNM staging by AJCC considers the size of the cancer mass and the lymph node involvement as a strong prognostic value.[54]

Scarff-Bloom-Richardson (SBR) classification: It is the most widely accepted grading system. Three parameters- Tubule formation, Mitotic index and pleomorphism are scored between 1 to 3 and the total score is calculated. Tumors are graded as following:

Score of 3 to 5 are well differentiated (grade 1)

Score of 6 to 7 - moderately differentiated (grade 2)

Score of 8 to 9 - poorly differentiated (grade 3).

Another popular prognostic system is - The Nottingham prognostic index which includes Tumor size and lymph node status along with grade.[55]

Lymphatic and Vascular Invasion: Studies have demonstrated that recurrence rate for women with LVI-positive stage I disease are higher as compared to LVI-negative disease.[56] Another study demonstrated that patients with LVI associated disease have 5-year recurrence risk increased by 15%, and is independent of whether adjuvant therapy is received or not.[57]

Histologic Grading

Histologic grading is done on the basis of Nottingham histological score, also referred to as Scarf Bloom Richardson scoring system.

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.

Table 1: Histologic Grading Using Nottingham Modification of Scarff Bloom Richardson System [58]

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(0.44mm) filled diameter	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.[59]

With the advancement in imaging techniques, treatment and prognosis, periodic revisions are made. Currently, the TNM staging includes tumor size, lymph node involvement, and presence of metastatic disease. The eighth edition, which is effective as of January 2018, incorporates biologic biomarker which would improve the prognostic discrimination.[60]

TNM staging is the most widely accepted staging system which is used in clinical practice and to provide information to the patients.

TNM Staging of Malignant Tumors, 2017, 8th Edition

TNM Clinical Classification (Annexure II)

BRCA and its role in breast cancer

BRCA1 and BRCA2 present on human chromosome 17q21 and 13q12 respectively and act as tumor suppressor genes. BRCA1 encodes 220KDa protein containing 1863 amino acids and BRCA2 encodes a protein of 384KDa consisting of 3418 amino acids. Protein products of both genes are localised to the nucleus and are believed to be involved in transcription regulation.[39,61]

BRCA1 and BRCA2 participate in the process of homologous recombination of DNA repair; they bind to RAD51, a gene involved in repair of double strand DNA breaks and are also involved in chromatin remodelling. They are also involved in protecting the genome from damage by halting the cell cycle.

In hereditary carcinoma, one mutant BRCA allele is inherited and the second allele is inactivated by somatic mutation. Although BRCA1 and BRCA2 mutation are rarely involved in sporadic tumors, about fifty percent of such tumors have decreased or absent expression of BRCA1.[62,63]

At these loci tumors with germline mutations of BRCA1 show loss of heterozygosity, which causes loss of wild type BRCA1 allele, indicate its role as tumor suppressor gene. In sporadic cases of breast carcinoma, still somatic mutations are not clearly elucidated but loss of heterozygosity, depleted BRCA1 mRNA levels and reduced protein expression and methylation of the BRCA1 promoter region indicates that BRCA1 gene in sporadic form also work as a tumor suppressor gene. This is indicative of interference with gene expression causing abnormal expression of the protein.

Although the incidence of BRCA1 and BRCA2 mutations may vary due to geographical location perhaps, BRCA2 is not as commonly mutated in breast cancer in contrast with BRCA1.[63]

However, studies have shown substantial role of both these genes in the development of the sporadic breast cancers.[64] BRCA2 is localised either in the nuclear or perinuclear compartments for example, golgi vesicles and ER. Limited information regarding BRCA2 gene expression is known except that BRCA2 and BRCA1 mRNA has similar tissue specific expression and that both of them are regulated in a coordinated way during mammary epithelial proliferation and differentiation although they do not exhibit homology .[65]

Overall, the degree to which these two genes expressions are modulated in the sporadic carcinomas is not yet clear. The BRCA1 protein is localised solely in the nucleus of normal and malignant mammary tissue. Several researchers have confirmed that BRCA1 protein expression in both hereditary and sporadic breast carcinomas is decreased or absent by immunohistochemical analysis.[66,67]

Cancers associated with BRCA1 and BRCA2 mutation other than breast and ovarian carcinomas are:

BRCA1 mutation: Fallopian tube carcinoma, primary papillary serous carcinoma of the peritoneum, prostate cancer, pancreatic cancer, male breast cancer, uterine body cancer and cervical carcinomas.

BRCA2 mutation: Fallopian tube carcinoma, primary papillary serous carcinoma of the peritoneum, prostate cancer, pancreatic cancer, male breast cancer, gallbladder cancer, bile duct cancer, stomach cancer and melanoma.

MATERIALS AND METHODS

The present study was conducted at the Department of Pathology of KLE Academy of Higher Education and Research (KAHER) University, Jawaharlal Nehru Medical College (JNMC), Belagavi.

Study design: This is a Cross Sectional study

Inclusion criteria:

1. Malignant breast lesions

Exclusion criteria:

1. Specimens which had not been fixed optimally.
2. Benign breast lesions
3. Male breast carcinoma

Sample Size Calculation: The sample size was calculated using the formula $4pq/d^2$

p– Expected prevalance

q- 100-p

d- Sample error (10)

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

Ethical clearance: The present study was approved by Jawaharlal Nehru Medical College's Institutional Ethics Committee on Human subjects Research. (Ref.MDC/DOME/36) (annexure IV)

Sampling Procedure: All surgically resected specimens of carcinoma breast during the period of January 2018 to December 2019 were included in the study.

Case Selection:

Fourty surgically resected specimens of breast carcinoma were collected from KLES Dr. Prabhakar Kore Hospital and Medical Research Centre and Department of Pathology JNMC during the period of 2018 to 2019 and were studied.

The clinicopathological parameters, including age, family history, tumor size, Lymph nodes status, clinical diagnosis were obtained from the patient's outpatient and inpatient records and requisition forms as per proforma given. 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 staining.

The Hematoxylin and Eosin stained slides were evaluated for

- Diagnosis of lesion
- Pattern of growth
- Invasion

Immunohistochemistry:

All cases were studied for the expression of BRCA1 and BRCA2.

For immunohistochemical analysis, 3-4 micron thick serial tissue sections were prepared from formalin fixed, paraffin embedded blocks, on saline coated slides. Slides were air dried for 2 hours at 58^{o}C. Slides were deparaffinised, dehydrated and rehydrated. The rehydrated slides were subjected to heat induced Antigen retrieval in a decloaking chamber. Slides were incubated for 15mins on high heat after adding distilled water. After 15mins, the chamber 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. Slides were incubated with respective optimized primary antibody for 60 min. A brown precipitate is produced on addition of substrate chromogen. Slides were removed. Appropriate controls were run with each batch of slides.

Antibodies used in IHC

Sr. No.	**Antibody**	**Company**	**Positive Control**
1	BRCA 1	Dako	Breast tissue
2	BRCA 2	Dako	Breast tissue

Assessment of expression BRCA1 and BRCA2

Evaluation of staining intensity and distribution of the tissue sections was done under a microscope. The scoring for the same was done in the absence of any clinical data.

Similarly, in each case another observer analysed all the sections and the results were recorded as the mean of two observations.

The sections were analysed at the target antigen site for the presence of a brown colored end-product.

The staining was compared with the adjacent normal breast tissue.

The immunoreactivity was observed and recorded as nuclear. Only nuclear expression was taken positive in this study.

Scoring of Immunoreactivity:

According to the staining distribution and intensity each tumour was given one of the following scores, a score from 0 to 3 similar to Weberpals et al study.[68]

0: Negative

1+: mild

2+: moderate

3+: strong

Statistical Analysis of the collected data was done using chi-square tests.

Mean and standard deviations were calculated for the continuous quantitative variables. Categorical data were expressed in terms of rates, ratios and percentages.

Microsoft excel was also used for the formulation of graphs.

RESULTS

A cross sectional study was conducted in the Department of Pathology of KLE Academy of Higher Education and Research's JNMC, and Dr. PrabhakerKore Hospital, and Medical Research Centre, Belagavi to study the expression BRCA1 AND BRCA2 in breast carcinoma.

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.

AGE DISTRIBUTION:

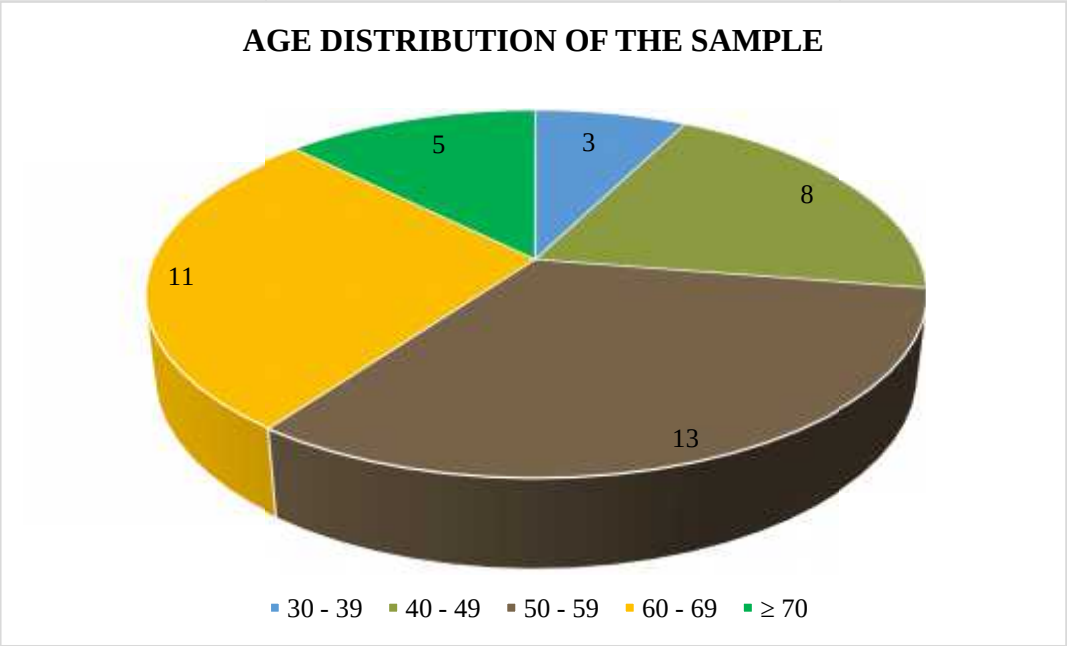
Table 3: Age distribution of patients with breast carcinoma (n=40)

AGE(YEARS)	NUMBER	PERCENTAGE
30 – 39	3	7.50
40 – 49	8	20.00
50 – 59	13	32.50
60 – 69	11	27.50
≥ 70	5	12.50
TOTAL	40	100.00

	MEAN	S.D.	MINIMUM	MAXIMUM
AGE	56.48	13.22	30	87

The age of the patients with carcinoma breast ranges from 30 to 87 years. Mean age was 56.48 years. Majority of the patients, 13 (32.50%) belonged to age group of 50-59 years.

Graph 1: Age distribution of patients with breast carcinoma (n=40)



FAMILY HISTORY:

Table 4: Distribution of patients according to family history (n=40)

FAMILY HISTORY	NUMBER
YES	16
NO	24
TOTAL	40

The family history was present in 16 cases(40%) and was absent in 24 cases(60%) out of 40 cases.

DISTRIBUTION OF SIZE OF TUMOR

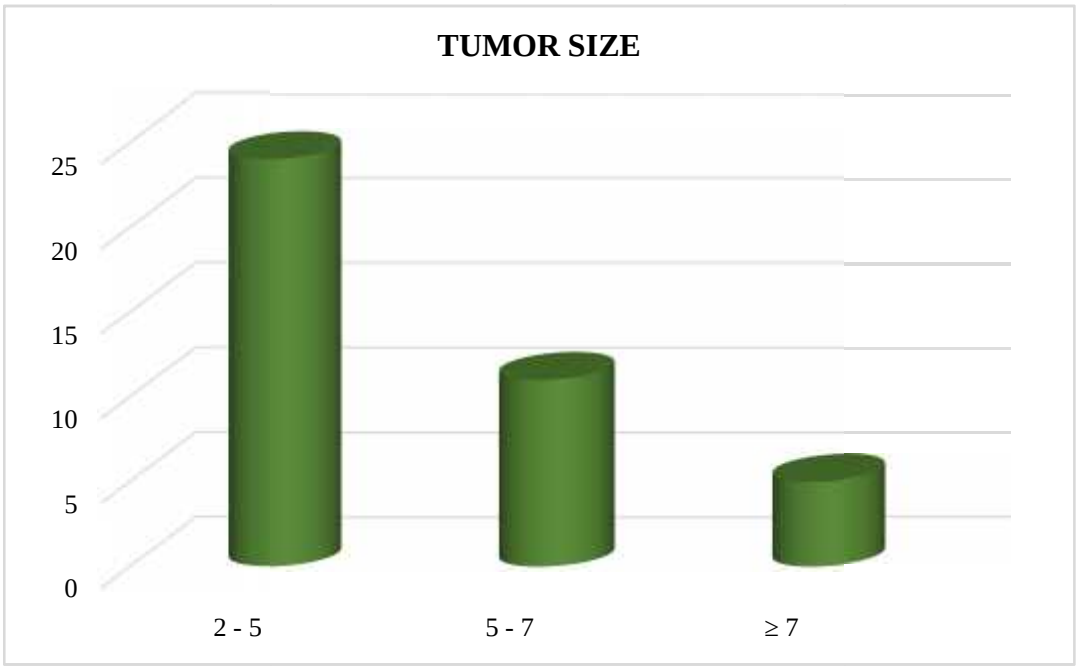
Table 5: Distribution of patients according to size of tumor (n=40)

TUMOR SIZE(cm)	NUMBER	PERCENTAGE
2 – 5	24	60.00
5 – 7	11	27.50
≥ 7	5	12.50
TOTAL	40	100.00

	MEAN	S.D.	MINIMUM	MAXIMUM
TUMOR SIZE	4.67	2.27	2	13

In the Present study of the 40 cases, the size of the tumor ranged from 2 to 13 cm considering the largest dimension of the tumor. Most of the cases (24 cases) were of the size between 2 cm to 5 cm. Mean tumor size was 4.67 cm.

Graph 2: Distribution of patients according to size of tumor (n=40)



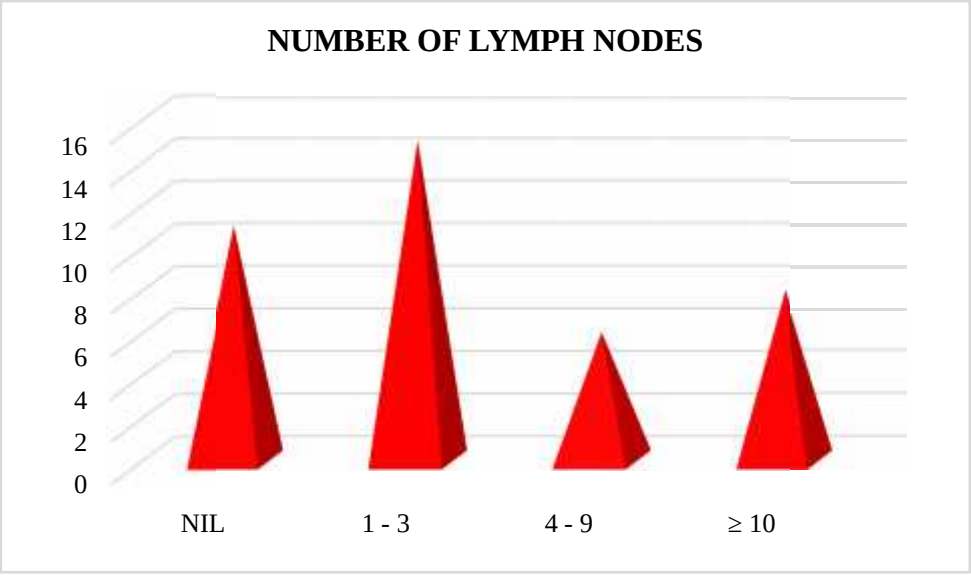
DISTRIBUTION OF LYMPH NODAL METASTASIS:

Table 6: Distribution of patients according to number of lymph nodes showing metastatic deposits (n=40)

LYMPH NODES	NUMBER	PERCENTAGE
NIL	11	27.50
1 - 3	15	37.50
4 - 9	6	15.00
≥ 10	8	20.00
TOTAL	40	100.00

Of the 40 cases, 11 cases (27.50%) had no lymph node metastasis. 15 cases (37.50%) had metastatic deposits in 1-3 lymph nodes and, 6 cases in 4-9 lymph nodes and 8 cases showed metastatic deposits in more than 10 lymph nodes.

Graph 3: Distribution of patients according to number of lymph nodes showing metastatic deposits (n=40)



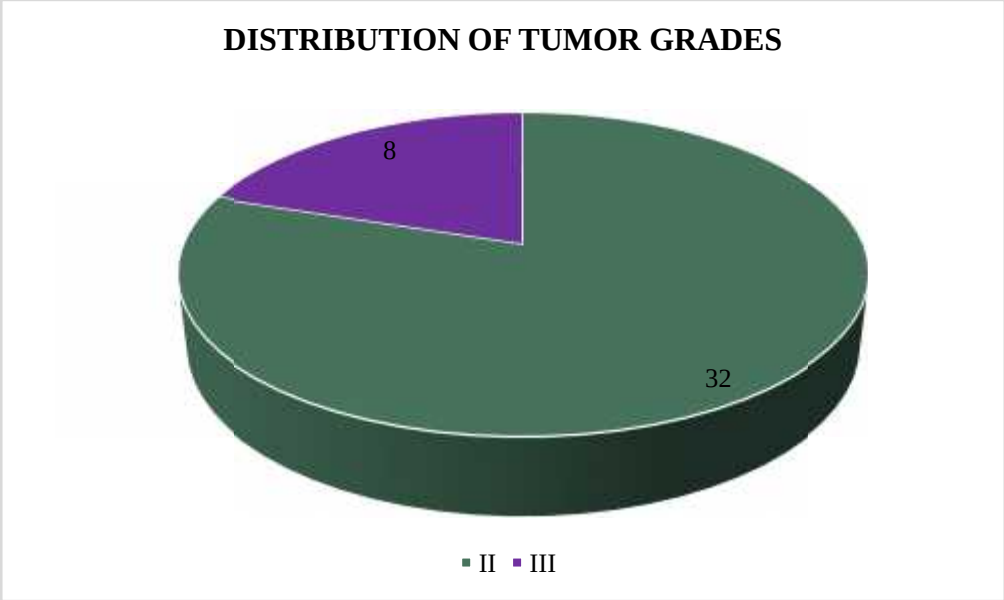
GRADING OF TUMORS:

Table 7: Distribution of patients according to histological grade of tumor (n=40)

TUMOR GRADE	NUMBER	PERCENTAGE
I	0	0.00
II	32	80.00
III	8	20.00
TOTAL	40	100.00

Among 40 cases of invasive carcinoma breast studied, 32 (80%) cases were of Bloom Richardson grade II and 8 (20%) cases belonged to grade III.

Graph 4: Distribution of patients according to histological grade of tumor (n=40)



BRCA 1 IHC EXPRESSION:

Table 8: Distribution of patients according to BRCA1 IHC expression (n=40)

BRCA 1 IHC SCORE	No of cases
0	11
1+	22
2+	5
3+	2

To evaluate the expression of BRCA1 and BRCA2 proteins, the distribution of IHC was checked in the segment of cancer tissue and was compared to their protein expression in normal adjacent tissue.

Most of breast cancer cases studied showed 1+ score (22/40 i.e 55.0%) or no expression (11 out of 40) of BRCA1. Remaining 7 out of 40 cases, five cases showed 2+ score and two cases had a score of 3+.

COMPARISON OF BRCA1 EXPRESSION WITH VARIOUS CLINICOPATHOLICAL AND PROGNOSTIC PARAMETERS

Table 9: Comparison of BRCA1 expression with age distribution of cases of carcinoma breast (n=40)

	BRCA1 IHC SCORE				
AGE (YEARS)	**0**	**1+**	**2+**	**3+**	**TOTAL**
30 – 39	1	2	0	0	3
40 – 49	3	3	1	1	8
50 – 59	3	6	4	0	13
60 – 69	2	8	0	1	11
≥ 70	2	3	0	0	5
TOTAL	11	22	5	2	40

Most of the cases belonged to the age group 50-59 years and out of which majority had decreased expression.

Table 10: Comparison of BRCA1 expression with mean age (n=40)

		AGE (YEARS)	
BRCA1 IHC SCORE	**NUMBER**	**MEAN**	**S.D.**
0	11	57.55	17.35
1+	22	56.68	12.50
2+	5	53.80	6.06
3+	2	55.00	14.14

Cases showing score 1+ had a mean age of 56.68, cases of score 2+ had a mean age of 53.80, cases of score 3+ had a mean age of 55.00 and mean age of cases showing no expression was 57.55.

Overall in all age groups most of the cases had 1+ or no expression particularly with age 50 years onwards. The p-value was 0.5659(not significant) which showed that BRCA1 expression is not associated with age.

Graph 5: Comparison of BRCA1 expression with mean age (n=40)

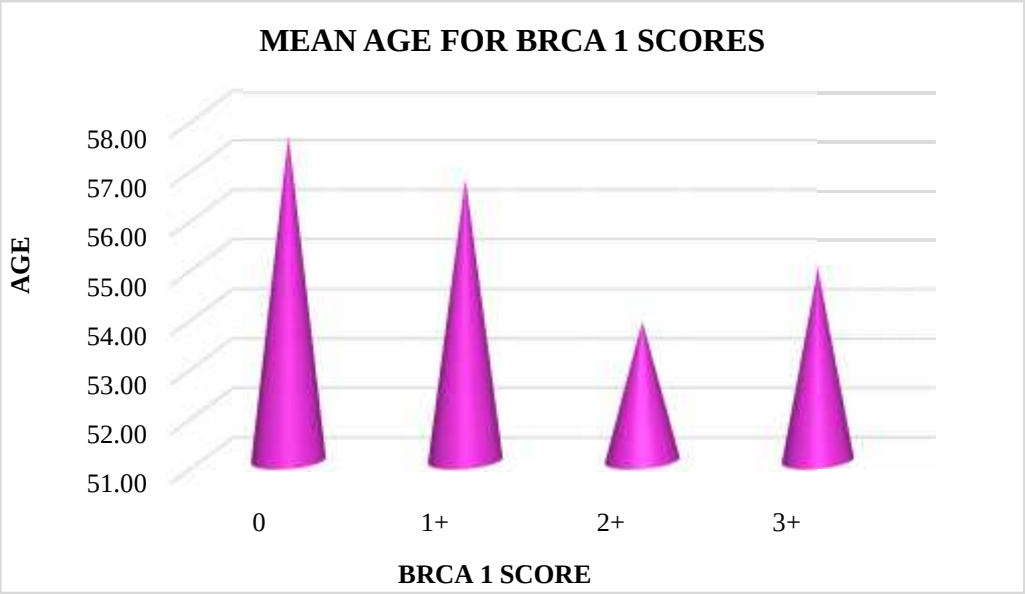


Table 11: Family history wise distribution of cases with BRCA1 expression (n=40)

	FAMILY HISTORY	
BRCA 1 SCORE	**YES**	**NO**
0	4	7
1+	8	14
2+	4	1
3+	0	2

Of the 40 cases, 16 (40%) patients were found to have family history and 24 (80%) cases with no family history. Eight cases having family history showed 1+ score and 4 cases had no expression. Four cases with family showed 2+ score. In cases with no family history, majority showed decreased expression.

The p-value was 0.1832 which was statistically insignificant. No significant association between BRCA1 expression and family history could be identified.

Table 12: Tumor size wise distribution of cases with BRCA 1 expression (n=40)

	BRCA1 SCORE				
TUMOR SIZE(cm)	**0**	**1+**	**2+**	**3+**	**TOTAL**
2 – 5	8	13	2	1	24
5 – 7	3	6	2	0	11
≥ 7	0	3	1	1	5
TOTAL	11	22	5	2	40

Out of 40 cases, maximum number of cases are of size between 2-5 cm and among those cases, majority showed 1+ score.

Table 13: Comparison of BRCA 1 expression with mean tumor size in cases of carcinoma breast (n=40)

		TUMOR SIZE (cm)	
BRCA 1 SCORE	**NUMBER**	**MEAN**	**S.D.**
0	11	4.30	1.32
1+	22	4.50	2.08
2+	5	6.20	4.09
3+	2	5.30	3.18

Among the 40 cases studied, cases with no expression and 1+ score had a lower mean tumor size of 4.30 and 4.50 cm respectively as compared to cases with 2+ and 3+ score which had a higher mean tumor size of 6.20 and 5.30 cm respectively. The p-value was 0.4968 which was not statistically significant. No significant correlation was found between BRCA1 expression and tumor size.

Graph 6: Comparison of BRCA 1 expression with mean tumor size in cases of carcinoma breast (n=40)

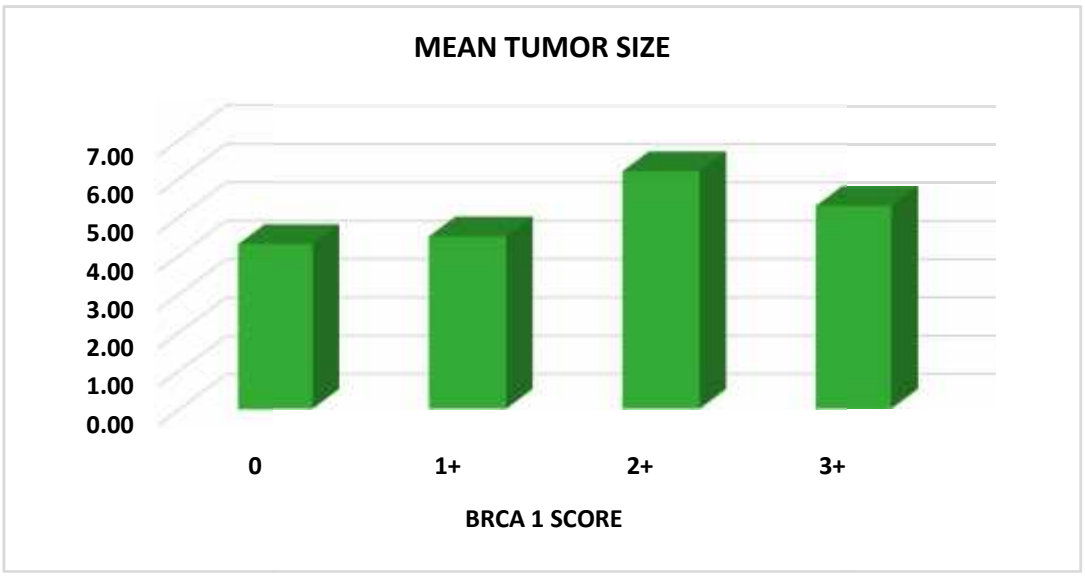


Table 14: Comparison of BRCA 1 expression and histological grade of tumor (n=40)

	TUMOR GRADE			
BRCA 1 SCORE	**I**	**II**	**III**	**TOTAL**
0	0	10	1	11
1+	0	18	4	22
2+	0	4	1	5
3+	0	0	2	2
TOTAL	0	32	8	40

Among the 40 cases studied, 8 cases belonged to BR grade III, 32 cases belonged to BR grade II. Among 8 cases of grade III, 4 cases had expression of 1+ score.

Among 32 cases of grade II, 18 cases had expression of 1+ score and 10 cases showed no expression and remaining showed 2+ score. This shows that tumors with higher histological grade have lower BRCA1 protein expression. The p-value was 0.0312 which was statistically significant establishing association with BRCA 1 expression and tumor grade

Table 15: Comparison of BRCA1 expression with regional lymph node status (n=40)

	METASTASIS		
BRCA 1 SCORE	**WITH**	**WITHOUT**	**TOTAL**
0	9	2	11
1+	14	8	22
2+	4	1	5
3+	2	0	2
TOTAL	29	11	40

Among 40 cases studied, 29 cases had metastatic deposits in the regional lymph nodes and 11 cases had no lymph node metastasis. Out of the 29 cases, 14 cases showed 1+ score, 9 cases showed no expression and remaining 6 cases were of higher score. This shows that majority of the tumors having metastatic deposits have decreased protein expression. The p- value was 0.1357 which was statistically insignificant. Overall no significant association was seen between BRCA1 expression and lymph node status.

OVERALL COMPARISON OF BRCA 1 EXPRESSION:

Table 16: Overall comparison of BRCA1 expression with the parameters (n=40)

						TUMOR GRADE			FAMILY HISTORY	
BRCA 1 SCORE	**NUMBER**	**MEAN AGE (YRS)**	**MEAN TUMOR SIZE (cm)**	**LN METASTASIS**	**NO METASTASIS**	**I**	**II**	**III**	**YES**	**NO**
0	11	57.55	4.30	9	2	0	10	1	4	7
1+	22	56.68	4.50	14	8	0	18	8	8	14
2+	5	53.80	6.20	4	1	0	4	1	4	1
3+	2	55.00	5.30	2	0	0	0	2	0	2

All the samples that were checked for BRCA1 were subsequently screened for BRCA2 expression by IHC.

The results of current study showed that none of the total of fourty cases showed BRCA2 protein nuclear expression in tumour tissue. Some of these cases however, showed mild to moderate cytoplasmic expression for the same.

While taking criteria for the study only nuclear expression was taken as positivity criteria and cytoplamic expression was not included.

Therefore, no correlation was done between BRCA1 and BRCA2 protein expression and also between BRCA2 protein expression with various clinicopathological parameters such as age, family history, tumor size, lymph node status and tumor grade.

DISCUSSION

Breast carcinoma is one of the commonest cause of cancer and mortality among women. Breast cancer can be hereditary or sporadic, hereditary are the ones associated with family history and germline mutations which are responsible for approximately 5 – 10% of all breast carcinomas.[69-71]

BRCA1 and BRCA2 genes mutations are associated with familial breast and ovarian carcinomas and about 30–40% of sporadic cancers are linked with reduced or loss of BRCA1 expression.[72-74]

The overall lifetime risk of developing cancer of breast is nearly 45-80% in mutated BRCA1 and BRCA2 gene carriers.[75,76] Individuals carrying mutation of BRCA gene are at a higher risk for development of other cancers like pancreatic cancer, genitourinary cancer, melanoma , colorectal cancer and cancer of prostate.[77-79]

Eighty percent of people with BRCA1 mutation develop cancer early in life that is before menopause. In the fifth decade of life, females with BRAC 1 mutation are at an additional 10 % to 15 % risk of developing both breast and ovarian cancer and the risk increases by 10–15 % every 10 years thereafter .[80-82]

There is loss of heterozygosity in the germline tumours with mutation of BRCA 1 which causes depletion of wild type BRCA1 allele , thereby establishing BRCA1 gene as a tumour suppressor gene.[83-85]

In sporadic cases of carcinoma breast, though no somatic mutation is seen but there is loss of the heterozygosity, reduced levels of mRNA of BRCA1 and the

methylation of the BRCA1 promoter gene thus establishing the role of BRCA1 in the sporadic forms.[86-88]

Purpose of BRCA1 protein is not clear although a few studies show that the RING finger domain at the NH2 end and BRCT motif at COOH terminus of BRCA -1 gene play the role of a transactivator.[89,90]

BRCA1 has an important role in maintaining the stability of the genome by direct interaction with p53, Rad 51 which plays a role in activating p21waf1 through transcription, and helps in the repair of breaks in dsDNA respectively. The exact role of expression of BRCA 1 protein remains unclear in the breast tissue although literature has demonstrated different location at the subcellular level.[91-93]

When in 1994 BRCA1 gene was cloned, another susceptibility locus for breast carcinoma was mapped on human chromosome 13q12 consisting of 27 exons encoding protein comprising of 3418 amino acids termed as breast carcinoma susceptibility gene 2 i.e BRCA2 and was fully cloned in 1995.[94,95]

On exon 3, there is a portion of BRCA2 protein sharing homologue with a transcription factor. It is able in activating transcription thereby proving a possible role of this protein. Siddique et al[9] study reported a strong positive Histone Acetyl Transferase {HAT} activity at the N terminal of BRCA2 protein. However for HAT activity to function the role of protein encoded by 3rd exon is not required. So, most probably this activity is enabled by some other functional domain.

Results also showed that BRCA2 protein acetylates H3 and H4 fraction of these free histones. HAT activity may have a critical function as a tumor suppressor gene. Various literature also shows BRCA2 association with RAD51 which helps in

ATP dependent DNA strand exchange reaction and function in recombination and gene for DNA repair.

So, pertaining to multiple possible function of BRCA2 protein including activating transcription, HAT activity, DNA repair gene and its large size, it is postulated that BRCA2/Rad51 complex is responsible for genes expression which regulates growth control, differentiation and apoptosis.[96-99]

Thus, main function of BRCA2 protein was seen in the DNA repair accountable for majority of hereditary breast cancers.

BRCA2 mRNA expression is regulated by cell cycle and is linked with proliferation of normal and tumor derived mammary epithelial cells. Their level are lowest in early G1 phase and reaches maximum in the late G1 phase and throughout S phase. Similar kinetics is observed between BRCA2 and BRCA1 mRNA up-regulation proposing that both genes may be controlled and regulated commonly.[100-102]

Although various studies have reported the significance of BRCA1 expression in familial breast cancer, there exist a controversy with its expression at different subcellular location and its function in sporadic tumors which is not clearly described.[103-106]

Many studies have demonstrated BRCA1 subcellular localization ranges from nuclei of normal and cancerous mammary cells, to cytoplasmic invaginations in the nucleus of normal cells, and to the aberrant cytoplasmic location .[107-110]

Immunohistochemical assessement of BRCA1 mutation is less expensive and time consuming than genetic testing.

Immunohistochmeical studies on formalin fixed, paraffin was embedded tumours have demonstrated a loss of reduction of the protein expression not only in BRCA1 associated breast carcinoma is but also in one BRCA1 associated family and sporadic breast carcinoma.[111-113]

In the present study nuclear expression was taken as the positive end result.

In the present study 40 mastectomies diagnosed histologically as invasive carcinoma of the breast were studied for immunohistochemical analysis for BRCA1/2 protein expression.

Table 17 : Comparing age distribution with other studies

Age group (Years)	**Present study (No of cases)**	**Suresh Hedau et al[11] (No of cases)**
20 - 29	0	7
30 - 39	3	9
40 - 49	8	14
50 - 59	13	6
60 - 69	11	4
≥ 70	5	0
TOTAL	40	40

In the present study, majority of the patients were above 50 years of age and mean age was 56 years. The youngest patient was aged 30 years and the oldest patient, 87 years. Majority of the patients - 13 cases (32.50%) were seen to be in the age group of 50-59 years.

When compared to Suresh Hedau et al[11] study who had a mean age of 41 years, who studied 40 cases of invasive breast carcinoma and most of their cases - 14 cases (35%) were of the age group 40–49 years. The study by Pérez-Vallés et al[114], who studied 45 patients with breast cancer had 25 patients upto 45 years and 20 patients above 45 years.

Family history:

In this study, family history was present in 16 cases (40%) and was absent in 24 cases(60%) out of 40 cases. Study done by Verma et al[71] shows 6 out of 54 cases (11.1%) with family history which is lower than the present study. In another study by Lambie H et al[69] family history was present in 69 out of 100 patients which was higher compared to the present study.

Tumor size:

In the Present study of 40 cases, the size of the tumor ranged from 2 to 13 cm considering the largest dimension of the tumor. Most of the cases (24 cases) were of the size between 2– 5 cm. Mean tumor size was 4.67 cm. In the study by Pérez-Vallés et al[114], 18 cases out of 45 cases were of tumor size of 2-5 cm.

Table 18: Comparing nodal metastasis status with other studies

Nodal metastasis Status	Present study (No of cases)	Suresh Hedau et al[11] (No of cases)
With metastasis	29(72.5%)	31(77.5%)
Without metastasis	11(27.5%)	9(22.5%)
Total	40(100%)	40(100%)

Out of 40 cases, 11 cases (27.5%) had no lymph node metastasis and 29 cases (72%) showed metastatic deposits in the regional lymph nodes. Of these 29 cases 15 cases (37.50%) had metastatic deposits in 1-3 lymph nodes and 6 cases in 4-9 lymph nodes and 8 cases showed metastatic deposits in more than 10 lymph nodes.

In the study by Suresh Hedau et al[11] 31 (77.5%) of 40 cases had regional lymph node metastasis and 9 of 40 cases (22.5%) had no regional lymph node metastasis and a similar observation was noticed in the present study. Study by Pérez-Vallés et al[114], 19 (42%) of 45 cases had regional lymph node metastasis and 26 of 45 cases (58%) had no regional metastasis which was having lower number of metastatic cases when compared to the present study.

Table 19: Comparing histological grade with other studies

Bloom Richardson Grade	Present study (No of cases)	Suresh Hedau et al[11] (No of cases)	Verma et al[71] (No of cases)
I	0	6(15%)	16(29.6%)
II	32(80%)	22(55%)	27(50%)
III	8(20%)	12(30%)	11(20.4%)
Total	40(100%)	40(100%)	54(100%)

The cases were evaluated for histological grading as per modified Bloom Richardson (BR) grading system. Out of the 40 cases, 32 (80%) cases were of grade II and 8 (20%) cases were of grade III. In Suresh Hedau et al[11] study, 6 (15%) cases of grade I, 22 (55%) cases of grade II and 12 (30%) cases of grade III out of 40 cases. Study by Verma et al[71] showed, 27 (50%) cases out of 54 cases were of grade II. In the study by Pérez-Vallés [114] et al, 21 (47%) cases out of 45 cases belonged to grade to II. These studies correlate with the present study that a maximum number of cases belong to grade II.

In the present study, immunohistochemical expression of BRCA1 and BRCA2 protein was studied in 40 cases.

Analysis demonstrated that nuclei of normal epithelial and myoepithelial cells expresses BRCA1 protein; and also that, a majority of the normal breast tissue sections showed a high expression of BRCA1.

In contrast, most of breast cancer cases showed 1+ score i.e 22/40 (constituting 55.0%) or negative expression (i.e 11/40) of BRCA 1. Remaining 7 out

of 40 cases, five cases showed 2+ score and two cases had a score of 3+. In tumor tissue BRCA1 expression was less in distribution and intensity in comparison with adjacent normal tissue.

Comparison of BRCA1 protein expression with clinicopathological features:

In the present study, cases showing score 1+ had a mean age of 56.68, cases of score 2+ had a mean age of 53.80, cases of score 3+ had the mean age of 55.00 and mean age of cases showing no expression was 57.55. Overall, in all age groups most of the cases had 1+ or no expression particularly with age 50 years onwards. This correlates with Suresh Hedau et al[11] who had similar results with decreasing BRCA1 protein expression with increasing age. But both the researches did not find any significant association between BRCA1 and age groups.

Study by Pérez-Vallés [114] et al, 7 patients had BRCA 1 mutation. Out of which five patients were <45 years and two patients were >45 years. These results were opposing to the present study.

Of the 40 cases, 16 (40%) patients were found to have family history and 24 (80%) cases had no family history. Majority of the cases with family history showed 1+ score or no expression. Four cases with family showed 2+ score. Cases with no family history, majority showed decreased expression. But significant association between family history and BRCA1 could not be established.

Study done by Verma et al[71] showed 24 (44.4%) cases out of 54 cases with altered BRCA1 expression. Six breast cancer had family history, out of which 4 had altered expression. There were fewer cases with family history, the altered BRCA1

expression was found to be (66.7 percent) higher relative to cases with no family background. But there was no significant association was seen between the two.

Among the 40 cases studied, cases with no expression and 1+ score had a lower mean tumor size of 4.30 and 4.50 cm respectively as compared to cases with 2+ and 3+ score which had a higher mean tumor size of 6.20 and 5.30 cm respectively. However no significant correlation was observed between BRCA1 expression and tumor size. Similar related findings were reported by Lambie et al[69], Yang et al[102] and Lee et al[101] where no correlation could be established.

However other studies reported by Verma et al[71], Pérez-Vallés et al[114] and Rakha et al[111] found positive correlation with absence of nuclear expression of BRCA1 and larger tumor size.

Table 20: Comparing histological grade and BRCA1 expression with other studies

	Present study			Suresh Hedua et al[11]		
BRCA 1 IHC score	I	II	III	I	II	III
0	0	10	1	3	5	1
1+	0	18	4	2	11	5
2+	0	4	1	0	3	3
3+	0	0	2	1	3	3

Out of the 40 cases studied, 8 cases belonged to BR grade III, 32 cases belonged to BR grade II. Among 8 cases of grade III, 4 cases had expression of 1+ score. Among 32 cases of grade II, 18 cases had expression of 1+ score and 10 cases

showed no expression and remaining showed 2+ score. This shows that tumors with higher histological grade have lower BRCA1 protein. The p-value was 0.0312 which was statistically significant establishing association with BRCA 1 expression with tumor grade.

Similar results were seen in the study done by Suresh Hedua et al[11] who observed in their study that 12 cases were of grade III, 5 cases had 1+ score and 3 cases had 2+ score. Of the 22 cases of grade II, 11 cases had 1+score and 5 cases had no expression. Majority of cases with grade II and III had decreased protein expression. This was also consistent with results of Verma et al[71], Lambie et al[69], Yang et al[102] and Rakha et al[111] indicating decreased protein expression with higher tumor grade.

However, in patients without germline mutation, Pérez-Vallés et al[114] found no correlation of negative BRCA1 expression with tumor grade. Patients with germline mutations, however, were of Grade II (2/7) and Grade III (5/7) respectively. Similarly, Yoshikawa et al[108] did not identify any significant association between tumor grade and BRCA1 expression.

Among the 40 cases studied 29 cases had metastatic deposits in the regional lymph nodes and 11 cases had no lymph node metastasis. Out of the 29 cases, 14 cases showed 1+ score, 9 cases showed no expression and remaining 6 cases were of higher score. This shows that majority of the tumors having metastatic deposits have decreased protein expression. However, no significant association could be established between BRCA1 expression and involvement of lymph node. Similar results were seen in studies done by Verma et al[71], Pérez-Vallés et al[114] and Yoshikawa et al[108]. On the contrary, Yang et al[102] and Rakha et al[111] found a

significant opposite correlation between lymph node involvement and nuclear BRCA1 expression.

In this study, all BRCA1 samples were subsequently checked for BRCA2 expression.

The results showed that 100% of our cases did not reveal nuclear expression for BRCA2. Some cases however, showed mild to moderate cytoplasmic expression. But since only nuclear expression was taken as positivity criteria and cytoplamic expression was not included, no correlation could be done between BRCA1 and BRCA2 protein expression and also between BRCA2 protein expression with various clinicopathological parameters.

The clinical significance in this study is that the BRCA1 expression reduced with higher tumor grade. Lack of statistical significance with other parameters may be due to small sample size.

LIMITATIONS OF THE STUDY

1. The present study had smaller sample size.
2. The availability of literature is limited on BRCA1 IHC for comparison studies.
3. BRCA immunohistochemistry can show wide variation due to several factors like inter laboratory, inter and intra observer variations.
4. The available studies could not specifically immunolocalise BRCA protein expression in the cell whether nuclear, cytoplasmic or membranous and wide difference were noticed between different studies

CONCLUSION

In the present study we have attempted to see correlation of immunohistochemical expression of BRCA1 and BRCA2 in breast carcinoma with various clinicopathological parameters.

In conclusion, BRCA1 positivity decreased with presence of family history, increase in age, lymph nodes showing metastasis and higher grade of tumor. However, no significant correlation was seen among all the parameters except modified Bloom Richardson grade of tumor.

Present study indicates that BRCA1 is a predictor of prognosis as its association was seen with histological grade of tumor.

None of the cases in this study revealed nuclear expression for BRCA2 which was taken as a positivity criteria. Hence, no correlation could be done between BRCA1 and BRCA2 protein expression and also between BRCA2 protein expression with various clinicopathological parameters.

The combination of immunohistochemical expression of BRCA1 in breast carcinoma with histomorphological features, risk factors, prognostic parameters and clinical history is of valuable help in detection of individuals who are more likely to carry mutated BRCA1 gene.

A major limitation of our study is the limited sample size. And also, availability of literature is limited on BRCA1 IHC for comparison studies. Hence more studies with larger cohorts are needed to conclusively identify the efficacy of these markers.

SUMMARY

A Cross Sectional study of 40 cases was conducted at the Department of Pathology of KAHER's JNMC, Belagavi on correlation of immunohistochemical expression of BRCA1 and BRCA2 in breast carcinoma.

To summarize the study of 40 cases of invasive breast carcinoma were studied for BRCA1 and 2 protein expression using immunohistochemical markers for the same. Their expressions were compared with various clinicopathological parameters like age, family history, tumor size, Bloom Richardson grading and regional lymph node metastasis status.

The commonest age group for breast carcinoma was 50-59 (32.50%) years. The family history was present in 16 cases (40%) and absent in 24 cases (60%) out of 40 cases.

Among 40 cases, most of the cases (24 cases) were of the size between 2 to 5 cm, 29cases (72.50%) had metastatic deposits and majority cases 32(80%) were of Bloom Richardson grade II.

Of the 40 cases studied, predominant nuclear expression for BRCA1 was that of 1+ score (22 cases) followed by negative expression (11 cases).

No correlation was done for BRCA2 with BRCA1 or other clinicopathological parameters as none of the cases showed positive BRCA2 expression.

Of all the parameters studied with BRCA1 expression, positive statistical correlation was found with Bloom Richardson grade of tumor. This shows that tumors with higher histological grade have lower BRCA1 protein expression.

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ANNEXURE I

WHO CLASSIFICATION OF BREAST TUMORS, 2019 5TH EDITION

1. Epithelial tumors of Breast

A. Benign epithelial proliferations and precursors

a. Usual ductal hyperplasia

b. Columnar cell lesions

c. Atypical ductal hyperplasia

B. Adenosis and Benign sclerosing lesions

a. Sclerosingadenosis

b. Apocineadenosis and adenoma

c. Microglandularadenosis

d. Radial scar/ Complex sclerosing lesion

C. Adenomas

a. Tubular adenoma

b. Lactating adenoma

c. Ductal adenoma

D. Epithelial-myoepithelialtumors

a. Pleomorphic adenoma

b. Adenomyoepithelioma

c. Malignant adenomyoepithelioma

E. Papillary Neoplasms

a. Intraductal papilloma

b. Papillary ductal carcinoma in situ

c. Encapsulated papillary carcinoma

d. Solid papillary carcinoma (Insitu/Invasive)

e. Invasive papillary carcinoma

F. Non invasive lobular Neoplasia

a. Atypical lobular hyperplasia

b. Lobular carcinoma insitu

G. Ductal carcinoma Insitu

H. Invasive Breast Carcinoma

a. Invasive Breast Carcinoma, No special type

b. Microinvasive carcinoma

c. Invasive Lobular carcinoma

d. Tubular carcinoma

e. Cribriform carcinoma

f. Mucinous carcinoma

g. Mucinous cystadenocarcinoma

h. Invasive micropapillary carcinoma

i. Carcinoma with apocrine differentiation

j. Metaplastic carcinoma

I. Rare and Salivary gland tumors

a. Acinic cell carcinoma

b. Adenoid cystic carcinoma

c. Secretory carcinoma

d. Mucoepidermoid carcinoma

e. Polymorphous adenocarcinoma

f. Tall cell carcinoma with reversed polarity

J. Neuroendocrine neoplasms

a. Neuroendocrine tumor

b. Neuroendocrine carcinoma

2. Fibroepithelialtumors and hamartomas of Breast

a. Hamartoma

b. Fibroadenoma

c. Phyllodestumor

3. Tumors of Nipple

A. Epithelial tumors

a. Syringomatoustumor

b. Nipple adenoma

c. Paget's disease of breast

4. Mesenchymaltumors of Breast

A. Vascular tumors

a. Haemangioma

b. Angiomatosis

c. Atypical vascular lesions

d. Postradiationangiosarcoma of breast

e. Primary angiosarcoma of breast

B. Fibroblastic and Myofibroblastictumors

a. Nodular fascitis

b. Myofibroblastoma

c. Desmoidfibromatosis

d. Inflammatory myofibroblastictumor

C. Peripheral nerve sheath tumors

a. Schwannoma

b. Neurofibroma

c. Grannular cell tumors

D. Smooth muscle tumors

a. Leiomyoma

b. Leiomyosarcoma

E. Adipocytictumors

a. Lipoma

b. Angiolipoma

c. Liposarcoma

F. Other mesenchymaltumors and tumor like conditions

a. Pseudoangiomatous stromal hyperplasia

5. Hematolymphoidtumors of Breast

A. Lymphoma

a. MALT Lymphoma

b. Follicular Lymphoma

c. DLBCL

d. Burkitt's Lymphoma

e. Breast implant associated – anaplastic large cell lymphoma

6. Tumors of Male Breast

A. Epithelial tumors

a. Gynaecomastia

b. Carcinoma in situ

c. Invasive carcinoma

7. Metastasis to Breast

8. Genetic tumor syndromes of Breast

a. BRCA1/2-associated hereditary breast and ovarian cancer syndrome

b. Cowden syndrome

c. Ataxia-telangiectasia

d. Li-Fraumeni syndrome, TP53-associated

e. Li-Fraumeni syndrome, CHEK2-associated

f. CDH1-associated breast cancer

g. PALB2-associated cancers

h. Peutz-Jeghers syndrome

i. Neurofibromatosis type 1

j. The polygenic component of breast cancer susceptibility

ANNEXURE II

TNM STAGING OF MALIGNANT TUMORS, 2017, 8th Edition

TNM Clinical Classification

T- Primary Tumour

TX- Primary tumour cannot be assessed

T0- No evidence of primary tumour

Tis- Carcinoma in situ

Tis- (DCIS) Ductal carcinoma in situ

Tis- (LCIS) Lobular carcinoma in situ

Tis- (Paget) Paget disease of the nipple not associated with invasive carcinoma and/or carcinoma in situ (DCIS and/or LCIS) in the underlying breast parenchyma.

T1- Tumour 2 cm or less in greatest dimension

T 1mi- Microinvasion 0.1 cm or less in greatest dimension

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

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

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

T 2- Tumour more than 2 cm but not more than 5 cm in greatest dimension

T 3- Tumour more than 5 cm in greatest dimension

T 4- Tumour of any size with direct extension to chest wall and/or to skin (ulceration or skin nodules)

T4a- Extension to chest wall (does not.Includepectoralis muscle invasion only)

T4b- Ulceration, ipsilateral satellite skin nodules, or skin oedema (including peaud'orange)

T4c- Both 4a and 4b

T4d- Inflammatory carcinoma

pN - Pegional Lymph Nodes

pNX - Pegional lymph nodes cannot be assessed (e.g., previously removed, or not removed for pathological study)

pN0- No regional lymph node metastasis

pN1-Micrometastases; or metastases in 1 to 3 axillary ipsilateral lymph nodes; and/or in internal mammary nodes with metastases detected by sentinel lymph node biopsy but not clinically detected

pN1mi - Micrometastases (larger than 0.2 mm and/or more than 200 cells, but none larger than 2.0 mm)

pNla - Metastasis in 1-3 axillary lymph node(s), including at least one larger than 2 mm in greatest dimension

pNlb - Internal mammary lymph nodes not clinically detected

pNlc - Metastasis in 1-3 axillary lymph nodes and internal mammary lymph nodes not clinically detected

pN2 - Metastasis in 4-9 ipsilateral axillary lymph nodes, or in clinically detected ipsilateral internal mammary lymph node(s) in the absence of axillary lymph node metastasis

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

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

pN3

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

pN3b - Metastasis in clinically detected internal ipsilateral mammary lymph node(s) in the presence of positive axillary lymph node(s); or metastasis in more than 3 axillary lymph nodes and in Internal mammary lymph nodes with microscopic or macroscopic metastasis detected by sentinel lymph node biopsy but not clinically detected

pN3c - Metastasis in ipsilateral supraclavicular lymph node(s)

pM - Distant Metastasis

M0 - No distant Metastasis

pM1- Distant metastasis microscopically confirmed

TNM Classification of Breast tumors

Stage 0	Tis	N0	M0
Stage 1a	T1	N0	M0
Stage 1b	T0	N1mi	M0
	T1	N1mi	M0
Stage 2a	T0	N1	M0
	T1	N1	M0
	T2	N0	M0
Stage 2b	T2	N1	M0
	T3	N0	M0
Stage 3a	T0	N2	M0
	T1	N2	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
Stage 3b	T4	N0	M0
	T4	N1	M0
	T4	N2	M0
Stage 3c	Any T	N3	M0
Stage 4	Any T	Any N	M1

ANNEXURE III

INFORMED CONSENT FORM

CORRELATION OF IMMUNOHISTOCHEMICAL EXPRESSION OF BRCA1 AND BRCA2 IN BREAST CARCINOMA PATIENTS- A CROSS SECTIONAL STUDY AT KLE'S DR. PRABHAKAR KORE HOSPITAL AND MRC, BELAGAVI.

Purpose of the study: The purpose of this study to look for BRCA1 and BRCA2 proteins expression in breast cancer cases and also to correlate their expression with various clinicopathological variables.

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.

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 a better way to diagnose invasive cancers which is essential for providing appropriate 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 KAHER University, Belagavi as a part of requirement towards the completion of MD degree, review and publishing.

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.

Principal Investigator: _____

Guide : _____

Name of the participant:

(signature/thumbprint)

Name of the witness : (signature)

Name of the investigator: (signature)

Date:

Address:

Phone no:

ANNEXURE IV

ETHICAL CLEARANCE CERTIFICATE

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/36 **Date: 24/11/2018**

To.

REG. NO: BN0118004

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"CORRELATION OF IMMUNOHISTOCHEMICAL EXPRESSION OF BRCA1 AND BRCA2 IN BREAST CARCINOMA PATIENTS – A CROSS SECTIONAL STUDY AT KLE'S DR. PRABHAKAR KORE HOSPITAL AND 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 V

PROFORMA

- **NAME:**
- **AGE:**
- **GENDER:**
- **IP NO.**
- **DATE OF COLLECTION:**
- **CLINICAL DETAILS:**
- **WEIGHT**(H/O RECENT WEIGHT LOSS)
- **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
- H/O ORAL CONTRACEPTIVES(if yes- which and for how long)

- **PAST HISTORY**
  - H/O CA BREAST
  - H/O CA ENDOMETRIUM
  - H/O CA OVARY
  - OTHERS
- **H/O PREVIOUS SURGERY**
- **FAMILY HISTORY**(CA BREAST)-(if yes-what is the relationship: first/second degree/third degree relative)
- **H/O MEDICAL ILLNESS**
- **EXAMINATION:**
  - SIDE
  - SITE
  - SIZE
  - SKIN CHANGES

- AXILLARY LYMPH NODE INVOLVEMENT
- NIPPLE
- AREOLA
- OTHERS

- **INVESTIGATIONS(IF ANY)**
  - FNAC
  - MAMMOGRAPHY

- **HISTOPATHOLOGICAL REPORT:**
  - TUMOR SIZE
  - TUMOR GRADE
  - LYMPH NODE METASTASIS

- **BRCA1 AND BRCA2 EXPRESSION:**

ANNEXURE VI

HAEMATOXYLIN AND EOSIN STAINING PROTOCOL

- Deparaffinize in Xylene I and II and III changes. [III change use warmedxylene] (5 minutes in each)
- Rehydrate using:
    - Absolute Ethanol 100% (5 minutes)
    - Absolute Ethanol 100% (5 minutes)
- Rinse in distilled water (5 minutes)
- Rinse in running tap water (5 minutes)
- Stain in Harris's hematoxylin by progressive method (2 minutes) Fresh and filtered
- Rinse in running tap water (20 minutes)
- Decolorize in 1% acid alcohol (1 second)
- Rinse well in tap water (5 minutes)
- Immerse in hot water bath, 550 C for bluing (3 Seconds)
- Rinse in tap water (5 minutes)
- Counterstain in Eosin (15 seconds)
- Dehydrate absolute alcohol 100 % (2-4 dips)
- Clear in Xylene I and II (5 minutes)
- Mount with DPX.

Stock Solutions – EOSIN:

Stock – 1% aqueous Eosin-Y

Stock – 1% aqueous Phloxin B

Working Solutions – Eosin:

100ml stock Eosin

10 ml stock Phloxin B

780 ml 95% Ethanol

4 ml glacial Acetic Acid

Working Solution: - Hematoxylin

Harris Hematoxylin, 1 Liter

Working Solution: - 0.25% Acid Alcohol

95% Ethanol, 2578 ml

dH2O, 950ml

HCL, 9ml

Result: Nuclei – Blue, Cytoplasm – Pink, RBCs – Red.

Reference: Bancroft D, Laylon C. The haematoxylin and eosin, In: Kim SS Ed, Bancroft'sTheory and practice of histopathological techniques. 7th Ed., China, Churchill Livingstone; 2013: p173-87

ANNEXURE VII

IMMUNOHISTOCHEMICAL STAINING PROTOCOL

- 3-4µ thick sections to be taken on Poly l lysine coated slides.
- Bake slides for 30minutes at 80 degrees Celsius.
- Deparaffinize and rehydrate the tissue in series of xylene (3 changes) and graded alcohol (100%, 90%,70% ethyl alcohol) 5minutes each and wash in running tap water for 5minutes.
- Soak the slides in PBS* buffer for 2minutes
- Antigen retrieval to be done using Heat induced epitope retrieval (HIER)method using BIOGENX EZ- RETRIEVER System V.3 microwave. Slides tobe kept in retrieval jar containing TRIS EDTA**/ citrate buffer.
- Antigen retrieval done in 2 cycles -1st cycle (Preheat cycle) - 85 degrees Celsius for 5minutes.2nd cycle (Retrieval cycle) - 98 degree Celsius for 15minutes. Take precaution to note evaporation of the buffer during this heat cycle.
- The slides are then allowed to cool at room temperature for 15 minutes.
- Humid chamber to be prepared using a wooden box, moist cotton and glass rods.
- Wash the slides with PBS buffer 3 times. The area on the slide containing the tissue to be marked using paraffin wax.
- Peroxidase block to be added to the tissue for 10minutes.
- Wash slides with PBS 3times.
- Add power block for 5minutes. Excess to be drained out after 5minutes.

- Add primary antibody for BRCA1 and keep for 1.5 hours / Add primary antibody for BRCA2 and keep for 1.5 hours
- Wash slides with PBS 3 times. Add super enhancer and keep it for 20minutes.Slides to be washed with PBS 4 times.
- Prepare DAB working solution using the chromogen and substrate15minutes prior use.
- Add DAB substrate and allow for 7-10minutes.
- Wash with PBS 4 times.
- Wash with distilled water 4 times.
- Counterstain with Gill's/Meyer's commercial haematoxylin (1-3minutes) following which wash in running tap water for 1minute.
- Dehydrate the slides in graded alcohol and clear in xylene.
- Mount the slides using DPX

Preparation of phosphate buffer [PBS] (pH 7.1-7.4)

Solution A – 2.1g of sodium monobasic phosphate + 100ml distilled water.

Solution B – 2.9g of sodium dibasic phosphate + 100ml of distilled water.

Solution C – 0.9g sodium chloride + 100 distilled water.

Working solution is prepared using 68ml solution B + 32ml Solution A + 100ml of solution C

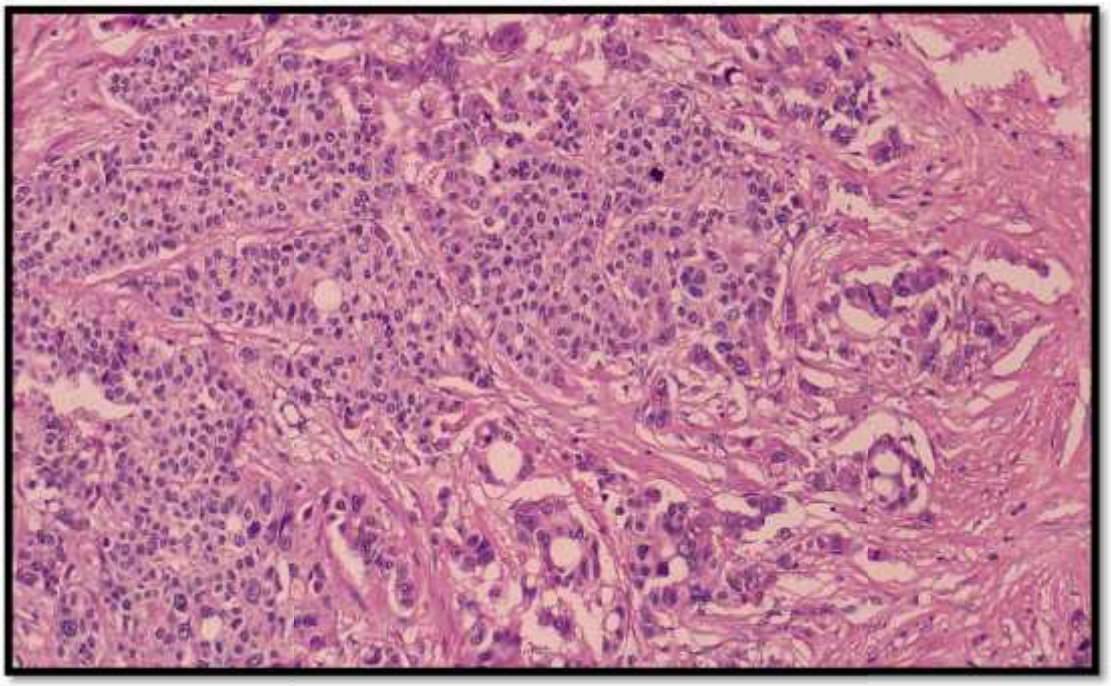
Preparation of TRIS EDTA antigen retrieval buffer (pH – 9)

- Take 1.21g of TRIS base and 0.37g of EDTA.
- To this add 1000ml of distilled water.

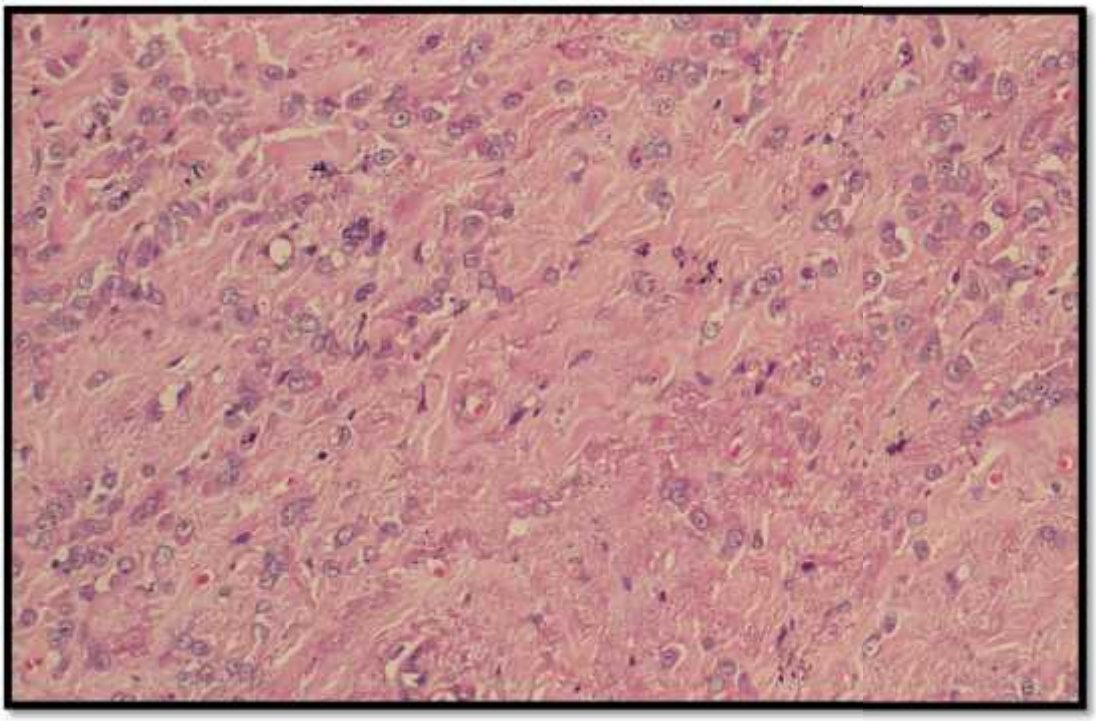
Preparation of DAB [3,3 –Diaminobenzidine substrate] working solution

- 1ml DAB substrate + 1-2 drops of DAB chromogen.
- To be prepared fresh 15minutes prior to use

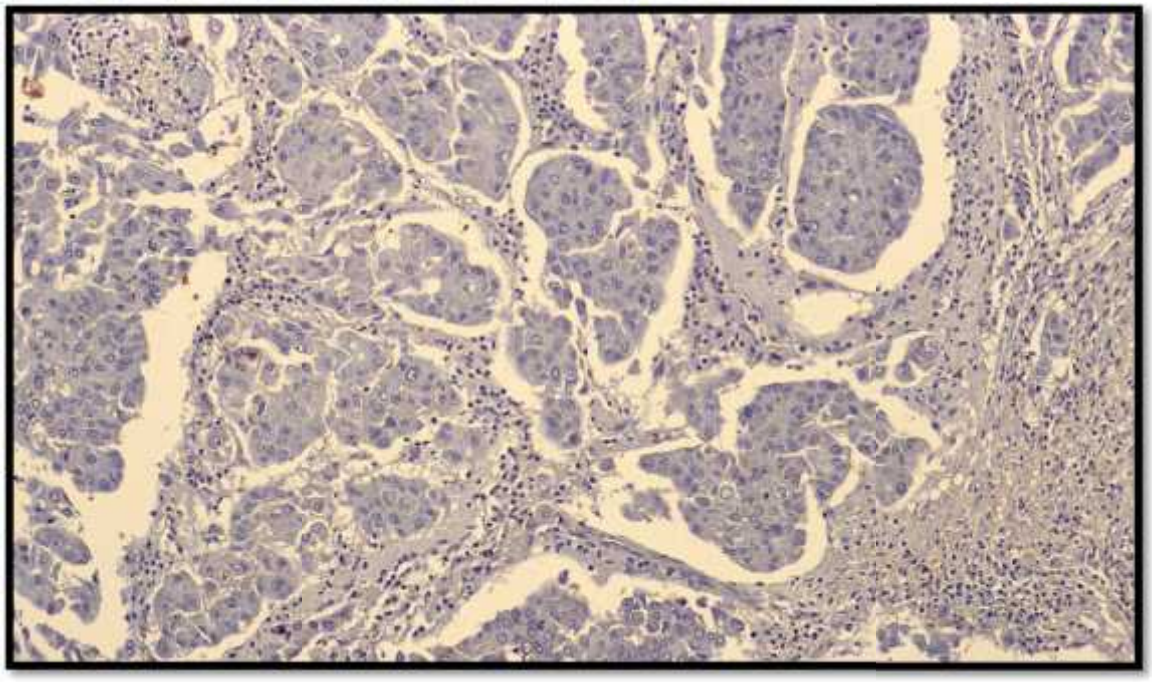
ANNEXURE-VIII-PHOTOMICROGRAPH



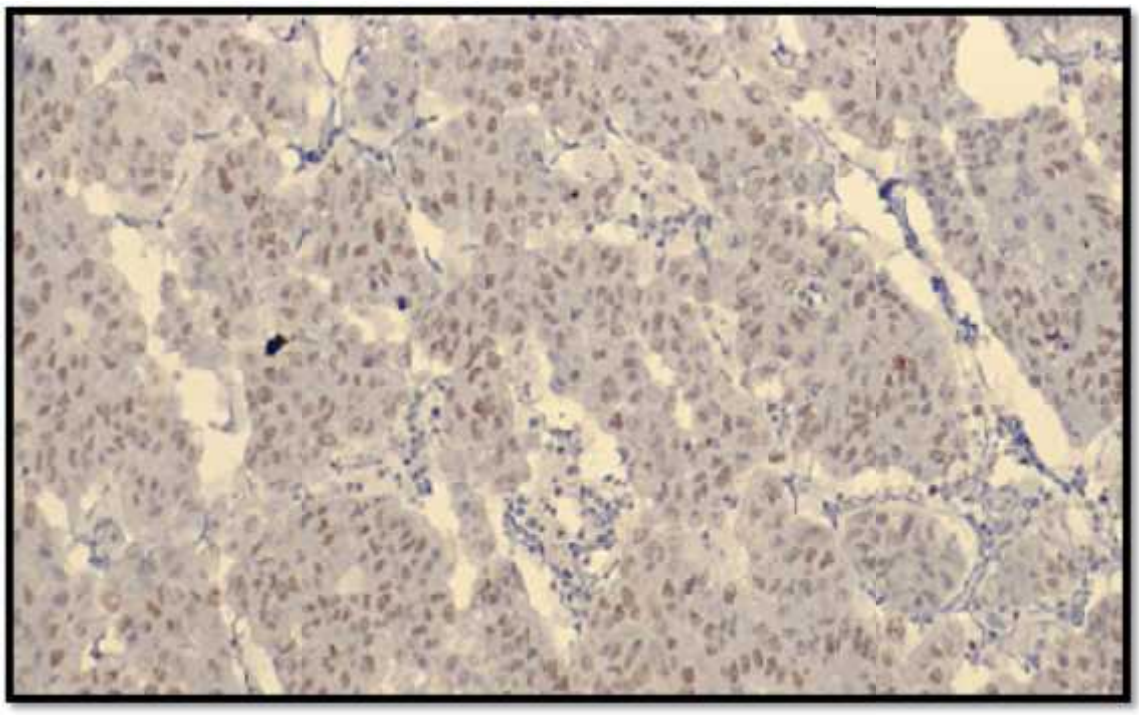
Photomicrograph 1: Invasive ductal carcinoma (IDC) NOS grade 2 (H&E, X 20)



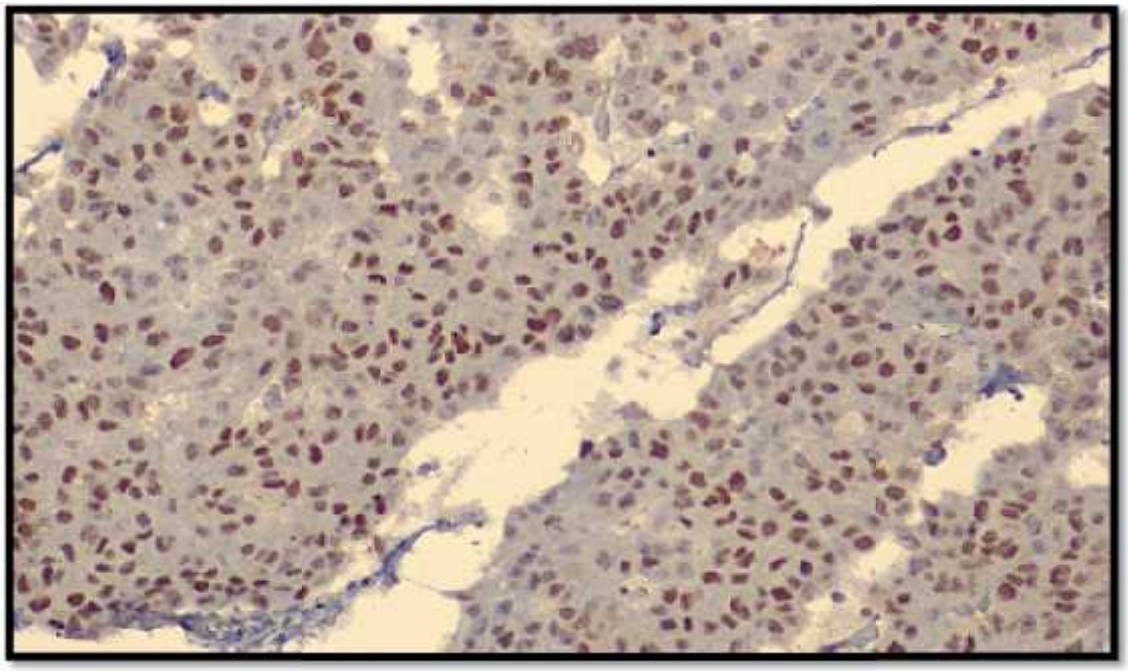
Photomicrograph 2: Invasive ductal carcinoma (IDC) NOS grade 3 (H&E X40)



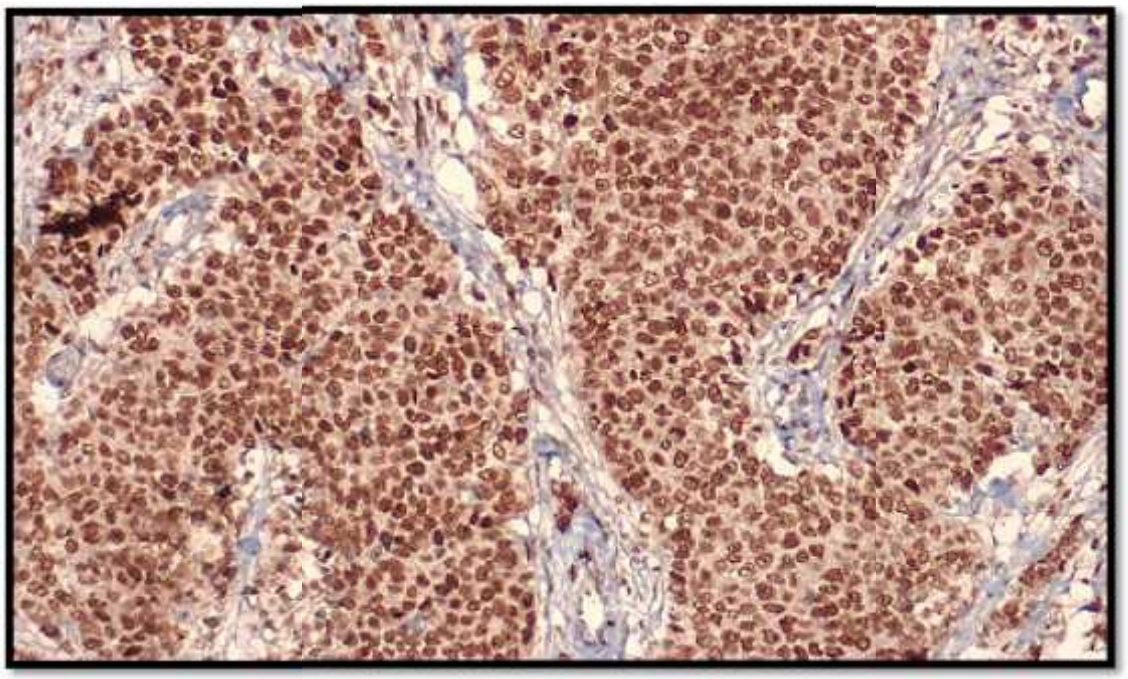
Photomicrograph 3: BRCA1 IHC score 0 (Negative) (IHC, X 20)



Photomicrograph 4: BRCA1 IHC score 1+ (Mild) (IHC, X 40)

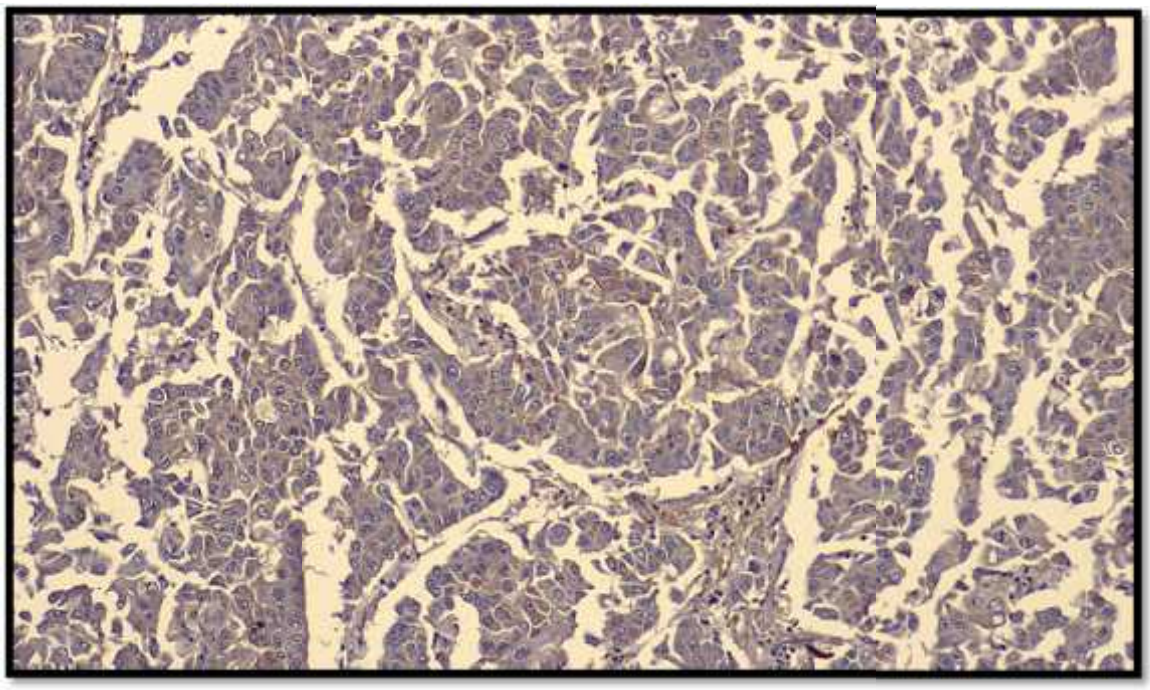


Photomicrograph 5: BRCA1 IHC score 2+ (Moderate) (IHC, X 40)



Photomicrograph 6: BRCA1 IHC score 3+ (Strong) (IHC, X 40)

Photomicrograph 7: BRCA1 IHC control (IHC, X 40)



Photomicrograph 8: BRCA2 IHC score 0 (Negative) (IHC, X 20)

ANNEXURES - IX

KEY TO MASTERCHART

- **FH –** Family History
- **TS –** Tumor Size
- **TG –** Tumor Grade
- **LN –** Lymph Node
- **Dist -** Distribution
- **Inten -** Intensity
- **Comp -** Composite

ANNEXURE-X

MASTER CHART

Sr no.	IP no./ Biopsy no.	Age(yrs)	FH	TS(cm)	TG	LN	BRCA1 Stain Dist	BRCA 1 Stain Inten	BRCA1 Comp Score	BRCA1 Final Score	BRCA2 Stain Dist	BRCA2 Stain Inten	BRCA2 Comp Score/ Final Score
1	993227/35	55	No	7	II	1	2	1	2	1	0	0	0
2	3009731/224	50	No	2	II	0	1	1	1	1	0	0	0
3	924002/289	41	Yes	4.5	II	0	0	0	0	0	0	0	0
4	3009496/394	49	No	4	II	2	1	2	2	1	0	0	0
5	999107/415	57	Yes	13	II	4	2	2	4	2	0	0	0
6	999112/458	83	No	5	II	6	1	1	1	1	0	0	0
7	38839/510	57	Yes	10	II	0	1	1	1	1	0	0	0
8	19064730/515	63	No	3	II	0	1	1	1	1	0	0	0
9	925152/544	53	Yes	2.5	III	1	1	1	1	1	0	0	0
10	932111/835	50	No	2	II	2	1	1	1	1	0	0	0
11	932714/870	36	Yes	2.5	II	3	1	1	1	1	0	0	0
12	19114736/911	65	No	3	III	24	3	3	9	3	0	0	0
13	933057/951	30	Yes	3	II	0	1	2	2	1	0	0	0
14	935202/1062	68	No	4	II	4	0	0	0	0	0	0	0
15	19163253/1347	60	No	6	III	0	1	1	1	1	0	0	0
16	397724/1573	86	No	3	III	2	0	0	0	0	0	0	0
17	13228929/1788	61	No	4.5	II	0	1	1	1	1	0	0	0
18	19236447/1984	55	No	3	III	10	1	1	1	1	0	0	0
19	3012153/2739	72	Yes	6	II	0	1	2	2	1	0	0	0
20	958816/2792	68	No	6	II	5	1	1	1	1	0	0	0
21	3012324/2856	65	Yes	5.5	II	3	1	1	1	1	0	0	0
22	9614715/2937	59	Yes	6	II	18	2	2	4	2	0	0	0

23	961202/2951	54	Yes	3	II	13	0	0	0	0	0	0	0
24	19363762/3024	62	No	3	II	1	1	1	1	1	0	0	0
25	963752/3061	58	No	4.5	II	2	0	0	0	0	0	0	0
26	965969/3186	60	No	2.5	II	0	2	1	2	1	0	0	0
27	19391366/3210	45	Yes	3	II	0	3	2	6	2	0	0	0
28	969132/3338	45	No	7.5	III	19	3	3	9	3	0	0	0
29	969337/3351	58	Yes	3	III	1	2	2	4	2	0	0	0
30	19419975/3380	46	No	2.5	II	3	0	0	0	0	0	0	0
31	19425866/3435	40	Yes	3	II	6	1	2	2	1	0	0	0
32	983405/4262	50	No	6	II	3	2	2	4	2	0	0	0
33	19565569/4485	74	No	4	II	22	1	2	2	1	0	0	0
34	19565715/4490	63	No	6.5	II	2	2	1	2	1	0	0	0
35	19581992/4578	87	No	6.5	II	0	0	0	0	0	0	0	0
36	19585431/4628	43	Yes	4	II	6	0	0	0	0	0	0	0
37	988827/4633	64	No	3.5	II	14	0	0	0	0	0	0	0
38	3014130/4646	41	Yes	7	III	1	1	1	1	1	0	0	0
39	3014245/4707	35	No	5.8	II	2	0	0	0	0	0	0	0
40	3014364/4834	51	Yes	6	II	17	0	0	0	0	0	0	0