" EXPRESSION OF BRCA1 BY IMMUNOHISTOCHEMISTRY AND ITS ASSOCIATION WITH ER,PR, HER2NEU STATUS IN IDC OF BREAST"

BY
DR. PRINCY.S.SOMAN, MBBS

DISSERTATION SUBMITTED TO
SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION &RESEARCH
TAMAKA, KOLAR, KARNATAKA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR IN MEDICINE
IN
PATHOLOGY

UNDER THE GUIDANCE OF
DR. HEMALATHA A, MD
PROFESSOR
DEPARTMENT OF PATHOLOGY

DEPARTMENT OF PATHOLOGY
SRI DEVARAJ URS MEDICAL COLLEGE, KOLAR
MAY 2022

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IN SRI DEVARAJ URS MEDICAL COLLEGE, KOLAR

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OUT UNDER THE DIRECT GUIDANCE OF

DR. HEMALATHA A

PROFESSOR,

DEPARTMENT OF PATHOLOGY,

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DATE
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PROFESSOR
DEPARTMENT OF PATHOLOGY

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SIGNATURE OF CO-GUIDE

DR. P.N SREERAMULU

PROFESSOR
DEPARTMENT OF SURGERY

ENDORSEMENT BY THE HOD,PRINCIPAL/HEAD OF THE INSTITUTION

THIS IS TO CERTIFY THAT THE DISSERTATION ENTITLED

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PROFESSOR
DEPARTMENT OF PATHOLOGY

Dr. KALYANI. R **Dr. P. N. SREERAMULU**

SEAL & SIGNATURE OF THE HOD

Date:

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Dr. PRINCY.S.SOMAN

POSTGRADUATE STUDENT IN THE DEPARTMENT OF PATHOLOGY OF SRI DEVARAJ URS MEDICAL COLLEGE TO TAKE UP THE DISSERTATION WORK ENTITLED

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TO BE SUBMITTED TO

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HIGHER EDUCATION AND RESEARCH, TAMAKA, KOLAR

MEMBER SECRETARY

PRINCIPAL

Dr.P.N.SREERAMULU

ACKNOWLEDGEMENT

I begin by expressing my immense gratitude to the Almighty Lord for his blessings.

My continued reverence and acknowledgment to the beloved teacher and guide **Dr. Hemalatha A**, Professor of Pathology, who handpicked this topic and graced study officially with her constant support and expert advice, her encouragement, wise constructive judgment the painstaking effort to weed out errors, and her affection during study leave permanently indebted to her. I dedicate a good part of the work to her.

Sincere thanks to **Dr. P. N. Sreeramulu**, Professor of Surgery and Dean, Faculty of Medicine, to consent to be the co-guide and give timely help throughout the PG career.

I take this opportunity to express my humble and sincere gratitude and indebtedness to the teacher **Dr. Kalyani R**, Professor and Head of the Department, for her expert advice, constant support, encouragement, and timely help in every aspect.

I would like to express my gratitude to **Dr. Harendra Kumar M.L,** Professor, for his constant guidance, support, and encouragement.

I express my sincere and humble gratitude to **Dr. CSBR Prasad,** Professor, for his support, constructive advice, and constant encouragement.

I express my deep, immense gratitude and humble thanks to **Dr. T.N. Suresh,** Professor. For his support, advice, and encouragement.

I express my deep, immense gratitude and humble thanks to **Dr. Subhashish Das**, Professor, for his advice and encouragement throughout the study.

I want to convey my sincere thanks to **Dr. Manjula K**, Professor, **Dr. Swaroop Raj B.V,** and **Dr. Sushan Shweta Jayekar**, Associate Professors, for their kind help, constant support, and expert advice in preparing this dissertation.

I express my sincere thanks to **Dr. Supreetha M S**, **Dr. Shilpa M D**, **Dr.Sindhu C**, Assistant Professors, for their constant guidance and encouragement in preparing this dissertation.

My family **Dr.E Kaizer Ennis, Johann Ebey Ennis, Juwanna Ebey Ennis,**will always be the most significant source of strength and inspiration for their unconditional support in every aspect of life. I am infinetly thankfull to my mother **Mrs.SELIN, Mrs. SHARADHA EBENEZER** and my sister **Mrs.PRIYA SEIHETH** whom I am forever indebted.

I express my sincere thanks to my batchmates and friends, **Dr.Nikhil, Dr.Sowjanya, Dr.Soumya** for their support and love in every aspect of life. I express my immense gratitude and special thanks to seniors **Dr. VarshaSree and Dr. Preethi Utnal and Dr.Gaurav** for their support.

I enjoyed working with my juniors –**Dr. Ankita, Dr.Amrutha, Dr.Jahnavi Reddy, Dr.Nagaraju, Dr. Satadruti Chakraborty, Dr. K.Sudarshan, Dr. Snigdha, Dr. Ayswaria.** I thank you for your kind cooperation.

I am thankful to **Dr.Munilakshmi** for her guidance in statistics.

I am thankful to technical staff Mrs.Asha and Mrs.Sumathi and all non-teaching staff for their invaluable help, without whom this study would not have been possible.

Thank you, everyone.

Date : Signature of the Candidate

Place: KOLAR **DR. PRINCY.S.SOMAN**

List of Abbreviations

IHC – ImmunoHistoChemistry

BRCA1 – Breast Cancer gene 1

ER – Estrogen Receptor

PR – Progesterone Receptor

Her 2 – Human epidermal growth factor receptor 2

IDC – Infiltrating Ductal Carcinoma

TDLU – Terminal duct lobular unit

WHO – World Health Organisation

DCIS – Ductal carcinoma in situ

AJCC – American Joint Committee on Cancer

MBR – Modified Bloom Richardson

H&E – hematoxylin and eosin

NPI – Nottingham Prognostic index

TBS – Tris buffer Solution

ASCO – American Society of Clinical Oncology

LOH- Loss of heterozygosity

PARP – Poly (ADP –ribose) polymerase

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ABSTRACT

BACKGROUND: Carcinoma of breast is a heterogeneous disease which differs in their clinical behaviours and responses to treatment and outcome. Prognosis in breast cancer depends on many factors such as histological grade, molecular type, size of the tumor, lymphnode status, Estrogen receptor (ER), Progesterone receptor(PR) and Human epidermal growth factor 2 status (Her2neu). The prevalence of breast carcinoma in India is rising recently. Etiopathogenesis of breast cancer comprises both genetic and non-genetic causes. Among the more than 300 genes which leads to breast cancer the tumor suppressor gene like BRCA1 plays a vital role not only in germline mutation but also sporadic cases. It has an autosomal dominant trait of inheritance.BRCA1 revealed a role in breast cancer as well as increased risk of developing epithelial ovarian cancer and male breast cancer etc. Hence detection of BRCA1 mutation helps in screening of high risk family members.

AIMS: To evaluate the expression of BRCA1 in infiltrative Ductal carcinoma and to analyse the association of BRCA 1 with histopathological parameters and Estrogen Receptor, Progesterone Receptor and Human Epidermal Growth factor Receptor 2 neu expression.

MATERIALS AND METHODS: This is a laboratory-based exploratory study. Data has been collected from the Department of Pathology, Sri Devaraj Urs Medical College, Tamaka, Kolar from October 2019– July 2021. Retrospective cases were collected from January 2019 to september2019.We have selected 56 infiltrative ductal carcinoma of breast cases and patients subjected to chemotherapy & radiotherapy, trucut biopsies and with incomplete patient details were excluded. H&E slides were reviewed. Immunostaining for BRCA1 was performed. Individual

clinicopathological parameters were compared with the BRCA1 mutation. p-value of <0.05 considered statistically significant.

RESULTS: In our study among 56 cases 18 cases(32.1%) showed BRCA1 mutation. BRCA1 mutation were asscociated with postmenopausal age , larger tumorsize, lower tumor grade and higher tumor staging. When we analysed the biomarkers and BRCA1 mutation showed negative association with ER,PR and Her2 neu and high Ki67 proliferation index. Prognosis was done with the NPI and does not gave a significant result. Molecular subtyping showed 44.2% cases in luminal A type.

CONCLUSION: Our study showed BRCA1mutation was expressed in 32.1% and associated with postmenopausal age group, larger tumor size and higher staging and negative hormonal status of breast carcinoma. More data is needed to look in to the clinical outcome of BRCA1 mutation with PARP inhibitor therapy.

KEYWORDS: Infiltrating ductal carcinoma, BRCA1, Nottingham Prognostic Index.

INTRODUCTION

Breast is an apocrine gland located overlying the chest (pectoral) muscles. Terminal duct lobular units form the basic units of the breast. The main function of female breast is to provide nutrition to infants. There has been an increasing trend in non-neoplastic and malignant lesion of breast in western as well as in Indian population. Amongst the female population breast cancer is the most common malignant neoplasm with approximately 2.1 million (2,088,849 (11.6%)) of new cases in 2018 accounting for 1 in 4 cancer cases among women. Based on statistics from International Agency for Research on Cancer (IARC) information system, invasive breast carcinoma is 55.4% of all breast cancer.[1,2]

In Indian females incidence ranges from 19.3 to 89.7 per 100,000 population. In 2012, 144,937 females were newly diagnosed with breast carcinoma and a total of 70,218 succumbed to it (cancer registry data). In Karnataka, Breast cancer constitutes 27.5% of all cancers and is the most common cancer in women. The prevalence of breast cancer in Kolar region is around 6.41% of all malignancies.[3]

Etiopathogenesis of Breast carcinoma is well established and comprises of both genetic and non-genetic causes. Among non-genetic causes such as Age, Obesity, Radiation exposure, Race /Ethnicity, Late parity, Breast feeding, Early menarche and Late menopause, Hormone Replacement Therapy, Alcohol, Smoking have been implicated.[4,5]

Among the genetic causes the significance of breast carcinoma genes (BRCA) and P53 mutations remains undisputed. BRCA (Breast Carcinoma gene) is a Tumour suppressor Gene is associated with family history or germ line mutations and account for 5%-10% of all breast carcinoma. This susceptibility is generally inherited as an autosomal dominant trait.[4,5]

Detection of these mutations are usually done using expensive investigations such as DNA sequencing, microarray, and reverse transcriptase – polymerised chain reaction. Some studies have deduced that BRCA immunohistochemistry has 100% specificity and 80% sensitivity for detecting

germ line, somatic, or epigenetic mechanisms of BRCA1 loss.[6]

Reduced expression of BRCA1 protein may play an vital role in mammary carcinogenesis and mechanisms other than mutations like methylation may be involved in reduced expression of BRCA1 protein.[7] Hyper methylation of promoter gene CpG-rich areas results in silencing of tumour suppressor genes.[8]

The BRCA 1 protein plays a vital role in the development and progression of breast carcinomas, so it can be implemented as a promising biomarker to select targeted and effective chemotherapeutic regime for the patients with breast carcinoma.[9]

In view of paucity of data on the BRCA1 mutations in breast carcinomas, we aim to study the expression of BRCA1 mutation using immunohistochemistry in our population and compare the expression with histopathological parameters and hormone receptor status.

2. OBJECTIVES:

1) To evaluate the expression of BRCA1 in infiltrative Ductal carcinoma
2) To analyse the association of BRCA 1 with histopathological parameters and Estrogen Receptor, Progesterone Receptor and Human Epidermal Growth factor Receptor 2 neu expression.

3. REVIEW OF LITERATURE

Development of Breast

Breast is highly specialized and modified type of sweat gland. Mastogenesis starts at 5th to 6th week of foetal life as 2 ventral bands. At 9 months there is a clear linear elevation, called “milk line”. The mammary ridges extend from the axillary to the inguinal regions.[10] In humans mammary ridges disappear as the embryo develops. The primary bud formed in breast is as an outcome of ingrowth of ectoderm and later it leads to development to 15 to 20 secondary buds and then in to lactiferous ducts and its branches. Major lactiferous ducts open in to the nipple. Myoepithelial cells appear to arise from basal cells between weeks 23 and 28 of gestation. They play an vital role in the branching

morphogenesis of the mammary gland through the synthesis of basement membrane. The female breast development starts under the stimulus of estrogen and progesterone during puberty. These hormones help in the proliferation of epithelial and connective tissue elements and also in deposition of fatty tissue.[11,12]

Gross Anatomy of Breast

Breast is a modified apocrine sweat gland overlying the anterior chest wall. It extends from the second to the seventh rib and from the midaxillary line's anterior margin to the sternum's lateral margin. “Superolateral quadrant is extended towards the axilla along the inferolateral edge of pectoralis major from which it projects a little and may outspread through the deep fascia up to the apex of the axilla. It lies on the deep pectoral fascia, which overlies pectoralis major and serratus anterior superiorly and external oblique and its aponeurosis inferiorly.” The areola has a round shape and a varying size, on average 3 to 6 centimeters, normally situated around the forth rib level. Epidermis of nipple and areola is highly pigmented and it has sebaceous glands that make projections on its surface, forming tubercle of morgani, or areolar glands, which during pregnancy become enlarged giving rise to tubercles of Montgomery. The breast parenchyma contains ducts of 15 to 20 lobes, all of which enter the nipple and dilate to form milk sinuses, bonded together by septa of connective tissue known as interlobular connective tissue. Each lobe has a pyramidal shape with a base away from the nipple. The suspensory ligaments, connective tissue band reach out from interlobular connective tissue and gets attached to the dermis.

The blood supply to the breast is via internal mammary artery, the lateral thoracic artery and the intercostal arteries. Venous drainage is by perforating branches of the internal mammary vein, tributaries of the axillary vein and the perforating branches of posterior intercostal veins. The breast

is innervated by the lateral and anterior cutaneous branches of the 2nd to 6th intercostal nerves. About 75% of the lymphatic drainage of the breast is through the axillary lymph nodes. There are approximately 20-30 lymphnodes in the axillary region and these are located lateral, medial, superficial and deep to the pectoralis minor muscle. Superficial lymphatics drains the opposite breast and the anterior abdominal wall. Direct drainage may be there to the supraclavicular (deep cervical) nodes and its involvement is indicative of advanced disease. Lymphatic drainage of the epithelial and mesenchymal components of the breast is the primary route of metastatic spread of breast cancer.[13-15]

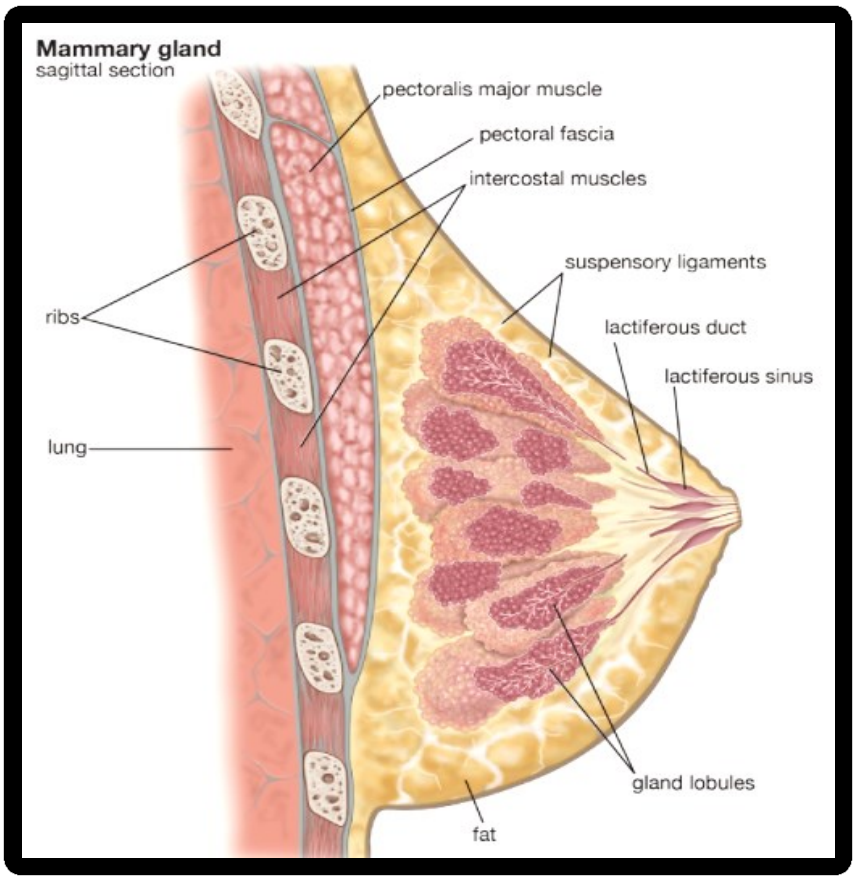

Figure1: Anatomy of normal breast [Source: Encyclopedia Britannica][12]

Histology of Breast

Histologically breast is consists of ducts and acini arranged in lobules and the stroma composed of

fibrous and abundant adipose tissue. Stromal and epithelial components constitutes the two main parts of the breast. The duct and lobular system's dual-layered epithelium lining rested on a basement membrane and enveloped by a stroma. The Inner Layer of ducts are lined by cuboidal to columnar type of epithelial cells, and myoepithelial cells line the outer layer. The basement membrane surrounds both the ducts, ductules in addition to the acini.[16]

Terminal Duct lobular units consists of 1) The terminal ductules whose epithelium differentiates into the secretory acini of the pregnant or lactating breast. 2) Intralobular collecting ducts and 3) the specialized intralobular stroma. Each of the lobes drains, with its own lactiferous duct which opens into the exterior of the nipple.[17]

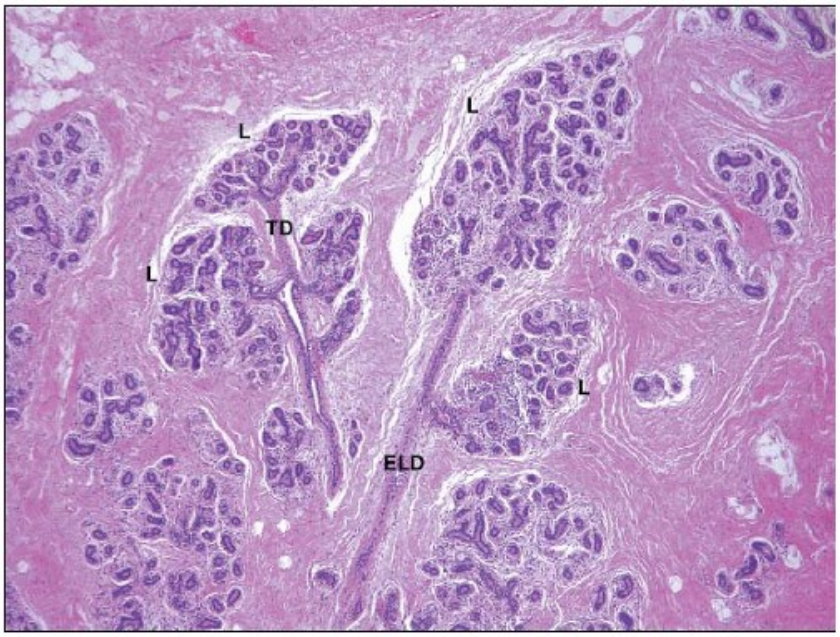

Figure 2 : Normal adult female breast tissue showing extralobular ducts (ELD), terminal ducts (TD), and lobules (L), the latter composed of groups of small glandular structures, the acini.[18]

Physiology

In females at puberty breast enlarges due to the development of the mammary glands and increased

deposition of fatty tissue. Under the stimulus of ovarian hormones estrogen and progesterone causes proliferation of epithelial and connective tissue elements. Dynamic changes seen in breast during the reproductive period of life in response to the hormones. Throughout first half of menstrual cycle the lobules are relatively dormant and after ovulation under the influence of hormones leads to increase in number of acini per lobule and the stromal edema. Up on menstruation hormone level fall offs and causes regression of the lobules. Estrogen induce the breast's ductal development and the progesterone primarily increases the epithelial cell differentiation and lobular development.[12,19] Progesterone also reduces the estrogen binding in mammary epithelium and limits the proliferation of tubular units. Hypothalamus secretes a GnRH, which stimulates the release of FSH and LH from the anterior pituitary. LH and FSH further stimulate the ovary to secrete estrogen and progesterone. Estrogen and progesterone, apart from the usual physiologic functions, also serve as negative feedback control for LH, FSH, and GnRH.[14,20]

RISK FACTORS AND ETIOLOGY [21,22]

Multifactorial causes are involved in the development of breast carcinoma. The main risk factors are the following:

1. Geographical factors- Breast cancers are more common in western population than India
2. Endogenous hormones- Early menarche and late menopause may cause increased risk of breast cancer. Late first pregnancy (age >35years), nulliparity, absence of breast feeding and postmenopausal obesity.
3. Family history- About 5-10% of breast cancers are inherited in autosomal dominant fashion.
4. Molecular genetics of breast cancer: 5-10% of all breast cancers arise from germ-line mutations in high-penetrance breast cancer susceptibility genes such as BRCA1, BRCA2, p53 and PTEN and causes a high individual risk for developing hereditary breast cancer.
5. Modifiable risk factors- Increased intake of fat, alcohol and smoking
6. Environmental factors- Ionising radiation

7. Benign breast disease- Ductal atypical hyperplasia are associated with increased risk
8. Exogenous hormones- Intake of oral contraceptive pills have showed an increased relative risk in developing breast cancer.
9. Hormone replacement therapy – In postmenopausal women, the possibility of breast cancer increases in patients receiving ART (Estrogen).
10. Physical inactivity

ETIOPATHOGENESIS OF BREAST CANCER[21,23]

Globally the rate of breast carcinoma is increasing especially in premenopausal stage of females. Breast cancers may be hereditary, arising in women with germline mutations in tumour suppressor genes or it can occur sporadic. However, environmental factors clearly affects the penetrance of hereditary forms of breast cancer. Both genetic and environmental factors can leads to sporadic form of breast cancer. Compared to the developing countries like India, developed countries have a six fold higher incidence of developing breast cancer. Patients with familial history of breast cancer, genetic mutations such as BRCA 1, BRCA 2, p53 and other rare mutations such as Ataxia telangiectasia gene and PTEN mutations have a higher chances of developing breast cancers. The studies shows that the trend towards increase in incidence of breast carcinoma than cervical cancer in India.[24] Breast cancer remains big challenge for the study of etiopathogenesis, early detection, diagnosis as well as therapeutic decision and its outcome. In order to know the pathogenesis of both familial and sporadic forms of breast cancer the identification of breast cancer susceptibility genes has a chief role. The factors influences the cause of breast cancer includes genetic changes, hormonal influences and environmental variables.[23]

MOLECULAR MECHANISMS OF CARCINOGENESIS AND TUMOUR PROGRESSION

Breast carcinoma is heterogenous group of disease and its histological appearance is diverse. The prognosis depends on several factors such as age of presentation, histological type, grade, lyphnode and marginal clearance especially in case of lumpectomy, status and response to treatment.

The causes of breast cancer are predisposed by mainly 3 factors such as genetic changes, hormonal influences and environmental variables. Genetic changes includes mutations in the proto- oncogenes and tumour suppressor genes.[21] The breast cancer genes identified to date contribute only ~30% of the familial risk.[25] Genetic factors involved in breast cancer comprises approximately 30 genes in which high penetrance group includes BRCA1 & BRCA2 also germline mutation in PTEN and TP53 results in early onset of breast carcinoma. Similarly STKI 1, CDH1, PALPB2 genes are also included in high penetrance since it leads to >4 fold increase risk. Genes considered as moderate risk of penetrance includes ATM and CHEK2 etc. Overexpression of the HER2/NEU proto-oncogene leads to the amplification in 30% of the invasive breast carcinoma, it's an epidermal growth factor and associated with poor prognosis. Similarly amplification of RAS and MYC genes also results in breast carcinoma.[26,27]

The hypermethylation of hormone receptor genes such as estrogen receptor adds the heterogeneity of the disease process. "ER expresses in less than 25% of luminal epithelial cells and has no expression in basal or stromal cells . More than 90% of mammary gland epithelial cell proliferation is contributed by luminal epithelial cells. Therefore, it was proposed that the ER positive cells actually promote neighbor cells proliferating by secreting paracrine growth factors."[28] In BRCA1 related breast cancer, around 70~80% of the cases are ER negative and only less than 20% of cases are ER positive, while ER positive cases are more prevalent in sporadic breast cancer compared with hormone receptor–negative BC, hormone receptor–positive BC is associated with less aggressive clinicopathological features and a better prognosis because of the benefits from endocrine therapy. The PR is encoded by an estrogen-regulated gene, and its synthesis requires estrogen and ER; therefore, ER-positive tumors are commonly PR positive, whereas ER-negative tumors are usually

PR negative. Literature shows that tumor growth associates with the increased ER positive mammary epithelial cells depending on the activation of MAPK/ERK pathway by the loss of BRCA1. ER-positive cancers are termed “luminal,” as these cancers most closely resemble normal breast luminal cells in terms of their mRNA expression pattern, which is dominated by genes that are regulated by estrogen.[28-30]

HER2-positive cancers arise through a pathway that is strongly associated with amplifications of the HER2 gene on chromosome 17q. They constitute approximately 20% of all breast cancers and may be either ER-positive or ER-negative. This is the most common subtype of breast cancer in patients with germline mutations in TP53 (Li-Fraumeni syndrome). These cancers have a distinct gene expression pattern that is dominated by genes related to proliferation that are regulated by signaling pathways lying downstream of the HER2 receptor tyrosine kinase.[31,32]

ER-negative, HER2-negative cancers arise through a distinct pathway that is independent of ER-mediated changes in gene expression and HER2 gene amplifications. Precursor lesions have yet to be described and as a result this is the least understood of the pathways. These tumours comprise about 15% of breast cancers overall, but are the most common tumour type observed in patients with germline BRCA1 mutations; they also occur with increased frequency in African American women. Sporadic tumours often have loss-of function mutations in TP53; mutations in BRCA1 are uncommon, but BRCA1 may be silenced in sporadic tumours through epigenetic mechanisms. These tumours have a “basal-like” pattern of mRNA expression that includes many genes that are expressed in normal myoepithelial cells.[33-35]

Breast carcinomas are heterogeneous tumor with distinct hormonal expression and characteristics. The gene expression patterns reflect the tumor phenotype, disease prognosis and systemic treatment planning. Based on the gene expression profile with the immunohistochemical panel of ER,PR, Her2neu,Ki67 index tumor classified in to four groups such as luminal A(ER+, PR+/-, Her 2 neu-, Ki67 <14%), luminal B(ER+,PR+/-, Her 2 neu+/-, Ki67>14%) , Her2+ (ER-, PR-, Her2neu +,

Ki67>14%), triple negative (ER-,PR-,Her2neu-, Ki67 >14%).[36,37]

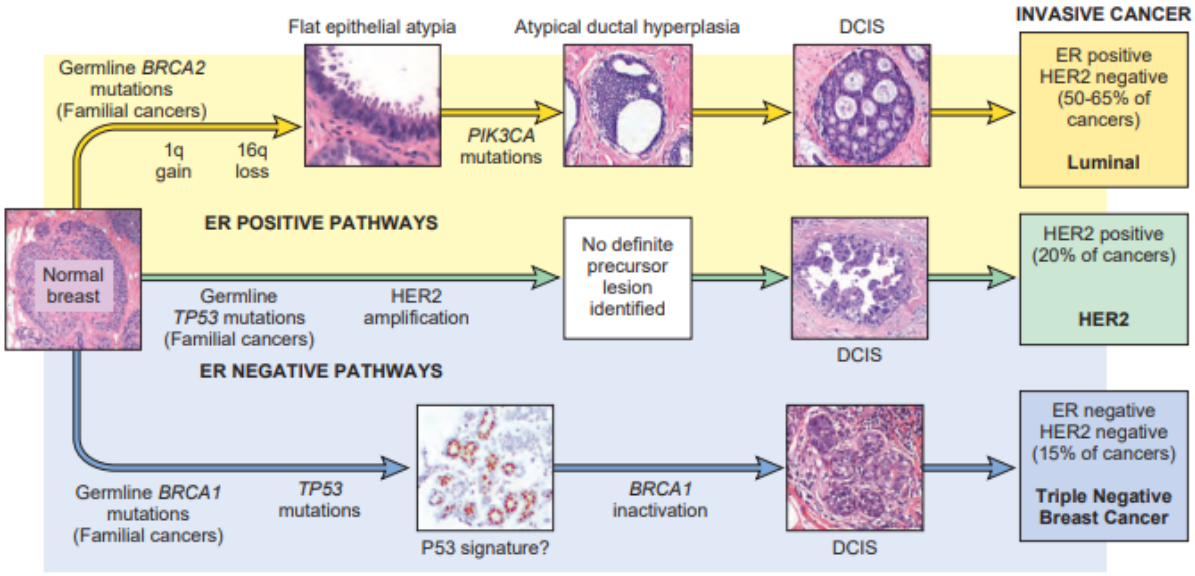

Figure3: Major pathways of breast cancer development [Source: Robbins 10th Edn][38]

Three main pathways have been identified. The most common pathway (yellow arrow) leads to ER-positive carcinomas. The precursor lesions include flat epithelial atypia and atypical hyperplasia. A less common pathway (blue arrow) leads to carcinomas that are negative for ER and HER2. The box with the question mark indicates that no precursor lesions have been identified—perhaps because lesions progress quickly to carcinoma. The third pathway (green arrow) consists of HER2-positive cancers, which may be ER-positive or ER-negative. Amplification of the HER2 gene is also seen in subset of atypical apocrine lesions, which may represent a precursor lesion. Each molecular subtype has a characteristic gene expression profile termed luminal, HER2 enriched and basal-like, respectively.[38]

When we look into the tumor progression we already know that neoplastic epithelial cells do not develop in isolation, but are dependent on interactions with stromal cells in the local microenvironment. Cancers occur in the areas of maximum mammographic density, suggesting that

increased amounts of fibrous stroma is both a marker of risk and biologically important for tumourigenesis. The role of stroma is not yet completely understood. The stroma is a complex mixture of fibroblasts, blood vessels, lymphatics, inflammatory cells, and extracellular matrix. Focal alterations in the stroma may play a direct role by creating a microenvironment conducive to tumour development and growth. Angiogenesis and tumour-associated inflammation are commonly associated with carcinoma, starting at the in situ stage. Nowadays therapies are targeting the stromal components which further helps in reduction of bulk and progression of tumor.[39,40]

Table 1: WHO classification of breast carcinoma[41]

Breast carcinoma	• Carcinoma with neuroendocrine features • Neuroendocrine tumour, well-differentiated • Neuroendocrine carcinoma, poorly differentiated (small cell carcinoma) • Carcinoma with neuroendocrine differentiation • Polymorphous carcinoma • Lipid-rich carcinoma • Glycogen-rich clear cell carcinoma • Sebaceous carcinoma • Clear cell hidradenoma
EPITHELIAL–MYOEPITHELIAL TUMOURS	• Pleomorphic adenoma • Adenomyoepithelioma • Adenomyoepithelioma with carcinoma • Adenoid cystic carcinoma

PRECURSOR LESIONS	• Ductal carcinoma in situ • Lobular neoplasia • Lobular carcinoma in situ • Classic lobular carcinoma in situ • Pleomorphic lobular carcinoma in situ • Atypical lobular hyperplasia
BENIGN EPITHELIAL PROLIFERATIONS	• Sclerosing adenosis • Apocrine adenosis • Microglandular adenosis • Radial scar/complex sclerosing lesion
ADENOMAS	• Tubular adenoma • Lactating adenoma • Apocrine adenoma • Ductal adenoma
MESENCHYMAL TUMOURS	• Nodular fasciitis • Myofibroblastoma • Desmoid-type fibromatosis • Inflammatory myofibroblastictumour • Benign vascular lesions • Haemangioma • Angiomatosis • Atypical vascular lesions • Pseudoangiomatous stromal hyperplasia • Granular cell tumour

	• Benign peripheral nerve-sheath tumours • Neurofibroma • Schwannoma • Lipoma • Angiolipoma • Liposarcoma • Angiosarcoma • Rhabdomyosarcoma • Osteosarcoma • Leiomyoma • Leiomyosarcoma
FIBROEPITHELIAL TUMOURS	• Fibroadenoma • Phyllodes tumour • Benign • Borderline • Malignant • Periductal stromal tumour, low grade • Hamartoma
TUMOURS OF THE NIPPLE	• Nipple adenoma • Syringomatoust umour • Paget disease of the nipple
MALIGNANT LYMPHOMA	• Diffuse large B-cell lymphoma • Burkitt lymphoma • T-cell lymphoma

	• Anaplastic large cell lymphoma, ALK-negative • Extranodal marginal-zone B-cell lymphoma of MALT type • Follicular lymphoma
METASTATIC TUMOURS	
TUMOURS OF THE MALE BREAST	• Gynaecomastia • Carcinoma • Invasive carcinoma • In situ carcinoma
CLINICAL PATTERNS	• Inflammatory carcinoma • Bilateral breast carcinoma

Invasive Ductal Carcinoma of Breast

Invasive ductal carcinoma (NOS) type largest group of infiltrating breast cancers. It represents the heterogeneous group of tumours that fail to exhibit sufficient characteristics to achieve classification as a specific histological type, such as lobular or tubular carcinoma. Also called as Invasive carcinoma of no specific type (ductal NST), invasive carcinoma not otherwise specified (ductal NOS), infiltrating ductal carcinoma. These cancers are heterogeneous group of malignancies characterized by invasion into the surrounding tissues and tendency to metastasize. Most of these tumors are derived from the mammary parenchymal epithelium particularly the cells of Terminal duct lobular unit (TDLU). They are also described as heterogeneous as they exhibit different morphological, immunohistochemical, prognostic and clinical characteristics.[23,42]

Gross: These tumours have no specific macroscopic features. There is a marked variation in size from < 10 mm to > 100 mm. They can have an irregular, stellate outline or nodular configuration.

The tumour edge is usually moderately or ill-defined and lacks sharp circumscription. Classically, invasive carcinoma NST is firm or hard on palpation and may have a “gritty” feel when cut with a knife. The cut surface is usually grey white with yellow streaks[38,41].

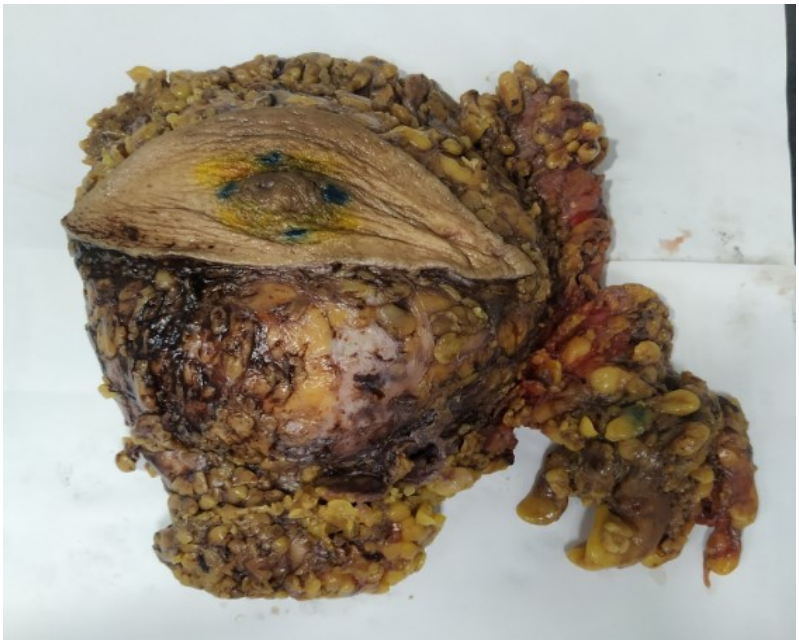

Figure 4: Gross image of mastectomy specimen with axillary clearance B/256/21

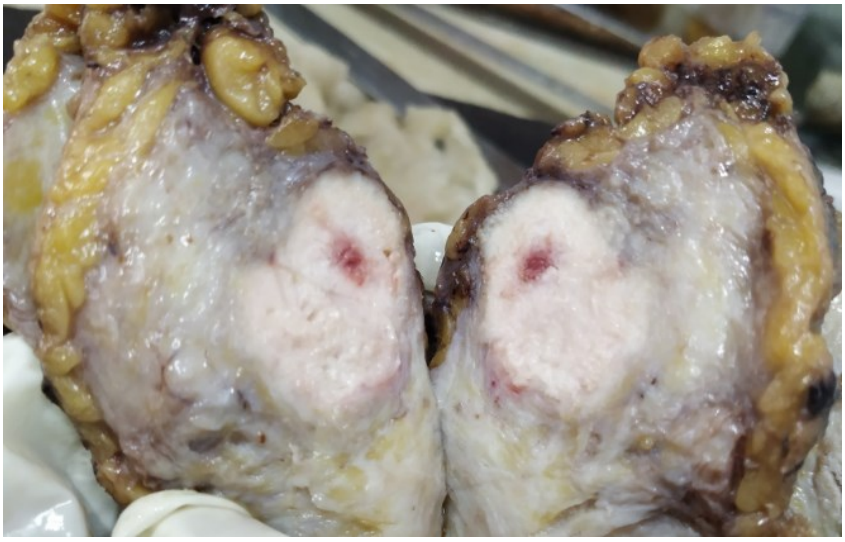

Figure 5: Cut surface showing grey white homogenous solid area with foci of haemorrhage B/256/21

Microscopy- Tumor cells are arranged in cords, clusters and trabecular. Few show solid or syncytial infiltrative pattern with little associated stroma. The cells have variable appearance. The cytoplasm is abundant and eosinophilic. Nuclei may be regular and uniform or highly pleomorphic with prominent, often multiple nucleoli. Mitotic activity may be virtually absent or extensive. In almost 80% of cases, foci of associated ductal carcinoma in situ (DCIS) will be present. There may be a highly cellular fibroblastic proliferation, a scanty element of connective tissue or marked hyalinization. There may be associated necrosis and periductal elastosis seen.

Grading is usually based on Bloom Richardson system of grading [38,42]

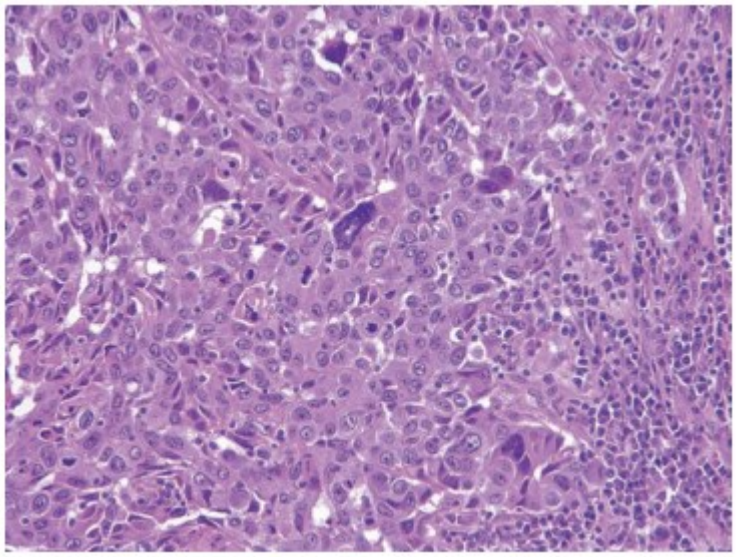

Figure 6: Microscopy (40X): showing tumorcells are arranged nests and in sheets, individual cells are round to oval with pleomorphic vesicular nuclei having prominent nucleoli. Periphery showing chronic lymphocytic infiltration. [Source WHO 5th Edition]

Lobular carcinoma : It comprises 5-15% of breast cancers. They are usually present with areas of in situ lobular carcinoma component and gross appearance is often irregular with poorly defined margins. The tumour cells are small to moderately sized cells usually non-cohesive with cells arranged in Indian file pattern[43].

Tubular carcinoma: Usually comprises 2% of breast cancers and are usually smaller in size (<2 cm). These tumors carry a better prognosis as they are less aggressive, increased use of mammography. Most lesions tend to be in T1 stage, and 90% of tumour express ER positivity.The most consistent microscopic features is the open Lumina lined by single layer of epithelial cells.[23,44]

Cribriform Carcinoma: It is the form of well differentiated infiltrating ductal carcinoma which has an excellent prognosis and shows cribriform pattern of growth and is often angulated with well-formed spaces giving a sieve like appearance. Tumour cells express apical snouts and show moderate degree of nuclear pleomorphism with occasional mitotic figures [23,42]

Medullary: Usually account for < 5% of breast malignancies. Due to presence of high amount of lymphoplasmacytic infiltrate in these tumors, they may mimic lymphoepithelial malignancies occurring in other sites.[45,46]

Few distinct histomorphological features are essential for diagnosis of medullary carcinoma. They are-[41,46]

- Symmetrical growth pattern (>75%)
- Absence of glandular structure.
- Diffuse lymphoplasmacytic infiltration.
- Nuclear pleomorphism.
- Complete circumscription.

MUCINOUS CARCINOMA – They are the slow growing tumours of breast consisting of tumour cells suspended or dispersed in pools of mucin. Their size may vary from 1 cm to 20 cm, usually circumscribed bosselated with glistening gelatinous appearance. Rarely cerebral infarction may occur due to mucin embolism and cause death. They carry a fairly good prognosis[41]

NEUROENDOCRINE TUMOURS (NET)– Represent 2-5% of malignant breast lesion usually present in 6th or 7th decade. NETs are group of neoplasms exhibiting features of neuroendocrine tumour of lung and gastrointestinal tract. There may be areas of de- differentiation in infiltrating ductal carcinoma but should show immune reactivity to neuroendocrine markers in >50% of cell population.[41,47]

INVASIVE PAPILLARY CARCINOMA – constitute 1-2% of breast malignancies and carry a fairly good prognosis. They are more common in post-menopausal women and have characteristic multiple nodular densities of mammography. Light microscopy shows delicate papillary structures with cells having moderate amount of amphophilic cytoplasm and may also exhibit apical snouting [23,41]

APOCRINE CARCINOMA – As mammary glands are highly modified sweat glands apocrine carcinoma can also occurs in breast with morphological and immunohisto profile of apocrine cells in >90% of cell population [41].

SECRETORY CARCINOMA – This is usually a low grade carcinoma that can occur in juvenile and in adults. It is comparatively a rare tumour with tumour cells having intra and extracellular secretory material[41].

INFLAMMATORY CARCINOMA – Incidence varies widely (1-10%). They are characterised by dermal lymphovascular infiltration and has been categorised under T4d due to its poor prognosis[42].

TNM CLASSIFICATION OF BREAST[41]

Table 2 - Primary tumor (pT)

pTX	Tumor cannot be assessed
pT0	No evidence of primary tumor
pTis	Ductal carcinoma in situ, Paget's disease ,encapsulated papillary carcinoma and solid papillary carcinoma
pTis (DCIS)	Ductal carcinoma in situ without invasive carcinoma
pTis(Paget's)	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 peaud'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)

Table 3 - Lymph nodes (pN)

pNX	Lymph nodes cannot be assessed
pN0	No regional lymph node metastasis histologically
pN0(i-)	No regional lymph node metastasis by histology or immunohistochemistry
pN0(+)	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

Table 4- Distant metastasis (M)

M0	No distant metastasis
pM1	Distant metastasis histologically proven > 0.2 mm

Prefixes

y: preoperative radiotherapy or chemotherapy

r: recurrent tumor stage

TABLE 5 - STAGE GROUPING 7th AJCC Staging[41]

Stage 0	Tis	N0	M0
Stage IA	T1	N0	M0
Stage IB	T0 , T1	N1mi	M0
Stage IIA	T0 , T1	N1	M0
	T2	N0	M0
Stage IIB	T2 T3	N1 N0	M0 M0
Stage IIIA	T0,T1,T2	N2	M0
	T3	N1,N2	M0
Stage IIIB	T4	N0, N1 , N2	M0
Stage IIIC	Any T	N3	M0
Stage IV	Any T	Any N	M1

MOLECULAR CLASSIFICATION [37,41]

Table 6 – Molecular classification of breast carcinoma

MOLECULAR SUBTYPE				
	LUMINAL A LIKE	LUMINAL B LIKE	HER2 ENRICHED	BASAL LIKE

Gene expression Pattern	Expression of luminal (low-molecular-weight) cytokeratins, and high expression of hormone receptors and associated genes	Expression of luminal (low-molecular-weight) cytokeratins and moderate to weak expression of progesterone receptor and associated genes	High expression of HER2 and other genes in amplicon on 17q12 Low expression of ER and associated genes	High expression of basal epithelial genes, basal cytokeratins Low expression of ER and associated genes Low expression of HER2 related genes
Clinical and biologic features	~60% of invasive breast cancers ER/PR positive HER2 negative Low proliferation rate	~10% of invasive breast cancers ER positive, PR low positive HER2 expression variable (positive or negative) Intermediate or high proliferation rate (Ki-67 high) Luminal B tends to be higher histologic grade	~15% of invasive breast cancers ER/PR negative HER2 positive (though not all HER2 enriched by molecular subtype are HER2+ by clinical definition) High proliferation rate TP53 mutation	~15% of invasive breast cancers Most ER/PR and HER2 negative ("triple negative") High proliferation rate TP53mutation common; BRCA1 dysfunction (germline, sporadic)

		than luminal A	common More likely to be high grade and nodepositive	Particularly common in African–American women
Histologic correlation	Tubular carcinoma Cribriform carcinoma Low grade invasive ductal carcinoma NSTClassic lobular carcinoma	Invasive ductal carcinoma NST Micropapillary carcinoma	High-grade invasive ductal carcinoma NST	High-grade invasive ductal carcinoma NST Metaplastic carcinoma Carcinoma with medullary features

Prognostic Factors [38,42]

1. Age: Younger than 50 years – best prognosis.
2. The risk of breast cancer increases with number of affected first degree relatives
3. Lymph node metastasis: Axilary lymph node status is the most important prognostic factor for invasive carcinoma in absence of distant metastasis.

4. Tumor size: It is one of the most powerful predictor of tumor behavior in breast cancer. The risk of axilary lymph node metastasis increased with the size of primary tumor, both lymph node metastasis and tumor size are independent prognostic factors.

5. Histopathological type: Morphological spectrum of invasive ductal carcinoma with an additional favourable prognosis are tubular, cribriform, medullary, pure mucinous, papillary, secretory carcinoma. A variant of lobular carcinoma associated with bad prognosis is signet ring carcinoma. Tumors which are aggressive than ordinary ductal carcinoma are squamous cell carcinoma, metaplastic carcinoma.

6. Histological grade: Most commonly used grading system is Nottingham Histological score (Scarff Bloom Richardson) .Survival for patients with well differentiated (Grade 1) carcinomas gradually declines to 70% at 24 years. Most deaths occur in poorly differentiated (Grade 3) carcinomas occur in first 10 years. Grade 2 (moderately differentiated) carcinomas have slightly better survival than grade 3.

7. Microvessel Density : Attempts have been made to quantitate the density of vessels and to correlate with various prognostic factors , few showed impressive results .Others failed to show significant correlation. There have been several reports of a direct association between density of tumor microvessels and risk of metastasis.

8. Lymphovascular invasion: Strongly associated with presence of lymph node metastasis. It is hazardous for local recurrence and poor prognostic factor for overall survival.

9. ER and PR receptors: 80% of carcinomas that are ER and PR positive respond to hormonal treatment.

10. Poor survival is associated with over expression Her 2 neu.

BRCA 1

Breast cancer gene (BRCA) are proteins normally expressed in the cells of breast and other tissues and is responsible for repairing DNA or destroys cells if not able to correct them. Every human being have both BRCA 1 and BRCA2 gene. These are unrelated proteins which playa a major role in preventing breast cancer. Mutation among these genes leads to higher life time threat of developing diseases. BRCA 1– Breast cancer type 1 protein is a tumour suppressor gene (also known as care taker gene). It is located on chromosome 17q21 was discovered by Dr.Mary - Claire King, professor of genome sciences and medicine in 1994. The first evidence for the existence of gene was done by Dr.MC King in laboratory at UC Berkeley in 1990. Four years later the gene was cloned in 1994 by the scientists at University of Utah, National Institute of Environmental Health Sciences (NIEHS) and Myriad Genetics.[48-50]

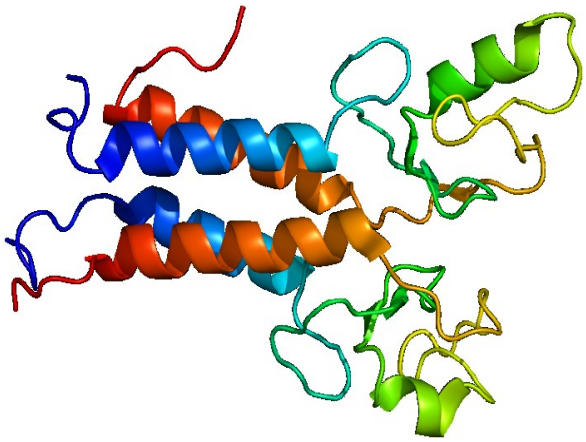

Figure 7: Structure of BRCA1 [51]

The BRCA1 gene is positioned on the long arm of chromosome 17, while BRCA2 is located on the long arm of chromosome 13. Gene mutated patients have an 80% risk of developing breast cancer particularly in the pre-menopausal age group. Many studies have proved that BRCA1 and BRCA2 prejudice a woman to breast cancer in only 5–10% of the total number of breast cancers and believe

that however family history may reflect common genes, it may also suggest shared environmental routine exposures.[50]

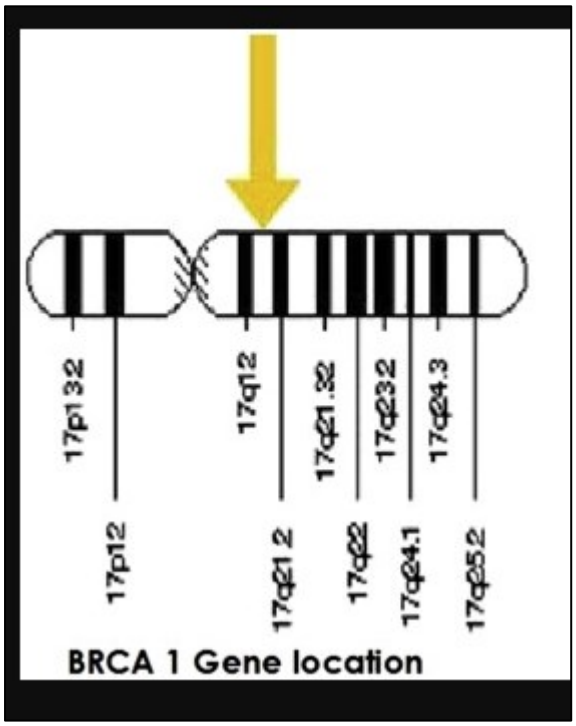

Figure 8: Location of BRCA1[52]

Other than its expression in epithelial cells of breast BRCA 1 is also expressed in endocrine tissues, neuroepithelial cells in their early stage of cell development. Similar to BRCA1, BRCA 2 expressed in variety of tissues but higher expression is in breast and thymus and lower rates in the lung, ovary and spleen.[53]

Only 25% of breast carcinoma cases have estimated familial susceptibility to breast carcinoma. Predisposition genes testing for germline mutations in high penetrance breast cancer has become standard practice. Studies shows that individuals with mutated BRCA genes are at significantly higher risk to develop cancer markedly breast and ovarian carcinoma compared to the general population. The frequency of breast carcinoma increases with advanced age. The data's shows that aggregate the risk of developing breast cancer by 70 years of age is approximately 37.9% for BRCA 1 carriers and 36.5% for BRCA 2 carriers.[54,55]

Functions of BRCA genes

BRCA 1 protein is expressed in the nuclei of normal epithelial and myoepithelial cells of the ductal and lobular region of breast. BRCA1 and BRCA2 genes are DNA repair genes that have cell cycle regulatory function. It is involved in repairing damaged DNA and plays a vital role in maintaining the stability of cells genetic information. It acts as a tumour suppressor gene and prevents cells from growing and dividing too rapidly. The BRCA 1 protein interacts with several other proteins to mend breaks in DNA. These breaks can also be due to radiation or some environmental exposures.[56,57]

Role of BRCA1 in breast carcinoma

Studies have been done to look in to the role of BRCA1 in breast carcinoma. In Indian population, among 40 sporadic breast cancer patients they observed that reduced expression of BRCA 1 in post-menopausal status and shows positive correlation with tumour grade as well. BRCA genes involved in the synthesis of multiprotein complexes that ensure transcriptional regulation of DNA synthesis, and the recognition and correction particularly of the double stranded breaks of certain DNA damages. Functional deficiencies due to the mutations in these DNA repair genes impair DNA repair and cause irregularities in the DNA synthesis. These mutations mostly (80%) occur as point mutations or deletion/insertion mutations. As a result of these mutations, the p53 dependent DNA breakdown is activated, which may cause cell cycle arrest and apoptosis.

BRCA genes play an important role not only in DNA repair, but also in transcriptional regulation, cell growth control and conservation of genomic integrity. It is very important to define morphological, immunochemistry, and molecular features of BRCA1 associated tumours to improve genetic testing and also gain further insight into biological characteristics of tumours.

Currently BRCA 1 screening is done based on the family history of breast, ovarian, prostate and pancreatic cancer among first degree relatives and other risk factors.[56-58]

Methods for detection of BRCA 1 mutation

Patients with BRCA1 mutation shows a good clinical outcome with poly ADP ribose polymerase

(PARP) inhibitor such as olaparib. Hence it is significant to test for BRCA mutation in tumor samples after routine histopathological assessment and diagnosis.[59]

Direct nucleotide sequencing is considered as the gold standard technique for BRCA mutation detection. The mutations detected in these genes include frameshift mutations, nonsense mutations or missense mutations.[60]

The studies shows that when DNA sequencing done in 5000 breast cancer patients they found 92 carriers whereas only 35 had been identified by clinical screening. 60% (57/92) of cancer causing BRCA variants had not been detected by clinical screening. We have to screen all the patients irrespective of risk factors. Recent study done in Chinese population shows higher risk of contralateral breast cancer for BRCA mutation carriers. BRCA1 which is a tumor suppressor gene, its mutation results in shortening BRCA1 protein leads in loss of its physiologic function. Studies reported that aggressive triple-negative breast cancer (TNBC) associated with sporadic mutations in BRCA1 [61,62]

Only 0.1-0.2% of general population are carriers of BRCA1 and BRCA2 mutations. BRCA1 and BRCA2 mutations are detected in 2-3% of all breast cancer cases. Families with frequent BRCA mutations are those with early-age breast cancer cases and ovarian cancers occurring at any age. The penetrance of pathogenic BRCA mutations and age of cancer diagnosis appear to vary both within and among family members. Some populations like Ashkenazi Jews, carry these gene mutations with higher rates. For the detection of presence of BRCA1 mutation in family Manchester scoring system (MSS) can be used. MSS incorporates the family history of breast carcinoma in first degree relatives, age of diagnosis, male breast cancer, ovarian, prostate and pancreatic cancer. [63-66]

The diagnostic accuracy for the detection of BRCA mutation by direct sequencing is time consuming and expensive hence there is an alternative established screening methods with comparable accuracy. These includes the single-strand conformation polymorphism (SSCP), restriction endonuclease fingerprinting (REF)-SSCP, conformation-sensitive gel electrophoresis

(CSGE), fluorescence-based conformation-sensitive gel electrophoresis (F-CSGE), two-dimensional gene scanning (TDGS), protein truncation test (PTT), and denaturing high-performance liquid chromatography (DHPLC).Recently by using sequence specific probes real time PCR, the array-based chip technology using SNP-specific oligonucleotides, and MALDI-TOF mass spectrometry has been used. As a part of routine standard histopathology procedure the tumor samples are processed and stored as formalin fixed paraffin embedded blocks (FFPE). DNA extracted from FFPE have some limitations such as formalin crosslinking and deamination of cytosine nucleotides may cause artefactual sequence alterations. [67,68]

BRCA1 expression altered by variety of mechanisms including germline or somatic mutation and promotor hypermethylation. Immunohistochemistry (IHC) method can be used in retrospective study of FFPE samples which is relatively cost effective and specificity.[69,70]

BRCA1-related breast cancers can be suspected in routine histopathology with higher histologic grade, proliferative rate and show a predominance of triple negative pathology compared with sporadic tumors. This triple negative phenotype of BRCA1-related breast cancers tend to progress directly to invasive disease without the development of a precancerous ductal carcinoma in situ component. Therefore, it is less likely to detect the breast cancer early, even by mammographic imaging.[71,72]

Risk of breast cancer in a woman with a BRCA1 mutation is 20% after 40 years of age, 51% after 50 years, and 85% after 70 years. The risk of ovarian cancer development is 40-50% after 70 years of age. The risk of breast carcinoma development in the carriers of BRCA2 mutations is 28% after 50 years of age and 84% after 70 years and the risk in ovarian cancers is 4% after 50 years of age and 27% after 70 years.[73-75]

BRCA1 is associated with other tumors such as prostate cancer and colon cancer. The risk for developing prostatic and pancreatic tumor increased in the carriers of BRCA1 mutations compared to general population. And the carriers of BRCA2 mutations are exposed to a higher chance of

ovarian cancer, male breast cancer, pancreatic cancer and prostate cancer. Although rare, the carriers of BRCA2 mutations may also develop malignant melanomas, carcinomas of the fallopian tubes, as well as gallbladder and biliary tract tumors. Studies have indicated that BRCA1/2 gene mutations do not cause a predisposition to the progress of borderline neoplasms and are not associated with stromal tumors or malignant germ cell tumors [48,64].

Studies[53] shows that the following characteristics that should trigger testing for germline BRCA1/2 mutation in patients already diagnosed with breast cancer ,

- Family history of breast, ovarian/tubal/peritoneal cancer, pancreatic, or aggressive prostate cancer
- Young age at diagnosis (<50 years)
- Triple Negative Breast Cancer
- Breast cancer in male
- Ashkenazi Jewish heritage
- Personal history of ovarian or pancreatic cancer
- Detection of somatic *BRCA*1/2 mutation
- Patients with metastatic Her2 – negative breast cancer who is eligible for treatment with PARPi

The value of BRCA1/2m testing for cancer risk reduction in the breast and ovarian cancer is well-established. Recent studies have found evidence that BRCA1/2mcarriers with BC have high rates of response to platinum salts in the metastatic and neoadjuvant settings; currently, most of the data were derived from BRCA1/2m carriers with TNBC.[76] For BRCA1/2m carriers with metastatic TNBC, platinum chemotherapy has been revealed to be superior to docetaxel in the first-line setting. For BRCA1/2m carriers in the early setting, it is not yet clear whether platinum agents are superior to, or provide additional benefit to conventional anthracycline-based or taxane-based chemotherapy.[77] However a platinum-based BRCA1/2 testing NM Tung and JE Garber 148regimen can be chosen for BRCA1/2m carriers with newly diagnosed TNBC for whom an anthracycline is contraindicated. For BRCA1/2m carriers with hormone receptor-positive BC, standard chemotherapy should likely be

used until more data regarding the efficacy of platinum chemotherapy are available. [78]

Although the prevalence of BRCA1 and BRCA2 mutations may vary according to geographical location, whereas BRCA2 is not frequently mutated in sporadic breast cancers in comparison with BRCA1.[69]

Loss of heterozygosity were found more in association with familial breast carcinoma with BRCA mutation. Hypermethylation and reduced expression of BRCA 1 indicates the sporadic carcinogenesis. 80% of triple negative breast carcinoma cases shows BRCA1 mutation. Gene microarray expression profiling shown similar phenotype between BRCA1-mutated tumors and basal tumors hence termed as "BRCAness." Studies has been done to assess the role of BRCA1 in sporadic breast carcinoma and shows that 21.9% of LOH of BRCA1. Inaddition they also found that BRCA1 mutation associated with negative ER expression and poor prognosis.[79-81]

Materials And Methods

STUDY DESIGN – Laboratory based exploratory study.

SOURCE OF DATA: All breast cancer specimens received in the Department of Pathology from R.L.Jalappa Hospital and Research Center attached to Sri Devaraj Urs Medical College, Tamaka, and Kolar from Oct 2019 to July 2021

DURATION OF STUDY : Two Years.

Inclusion Criteria:

- Women with invasive ductal carcinoma - irrespective of age.

Exclusion Criteria:

- Women subjected to neoadjuvant radiotherapy / chemotherapy before modified radical mastectomy.
- Women who underwent chemotherapy for other cancer over the past 5 years.

Sample size was estimated based on the down regulation or absent expression of BRCA 1 in tumor tissues. It was reported to be 30% in a study done by Hedau et al[82] in 2015 with 95% level of confidence with absolute error of 12%

- Estimated sample size is **56**.
- Equation sample size is = $\frac{Z_{1-\alpha}{}^2 p(1-p)}{d^2}$
- Here $Z_{1-\alpha}$ = Standard normal variant
- p = Expected proportion in population based on previous studies
- d = Absolute error of 12%

Collection of Data

Fifty six patient who had diagnosed with Invasive ductal Carcinoma of breast in the Department of Surgery at R L Jalappa Hospital and Research Centre.

- ***Prospective study***: The paraffin blocks were collected after taking written consent from the patients and analysed. All the slides were re-screened for further analysis.
- ***Retrospective cases:*** paraffin blocks and slides will be retrieved from the archives of department of Pathology. Clinical information, tumor size, and axillary lymphnode status will be obtained from medical datas and pathology reports. All the hematoxylin and eosin slides will be screened for histological type, tumor grade and nodal metastasis.

Statistical Analysis

Data entered in MS excel and analysis will be done using SPSS 22 version software.

Qualitative data will be obtainable in the form of proportions and pie diagrams,bar charts

Quantitative data will be represented as mean, standard deviation (If it is following normal distribution) Median and interquartile range (if it is not following standard deviation)

To assess the association between histopathological parameters and BRCA1 with the ER, PR and Her 2 neu status by using pearson correlation test

p value <0.01 is considered as statistically significant.

IMMUNOHISTOCHEMICAL EXAMINATION

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

Table 7: Details of IHC marker

Antigen	Clone	Species	Producer	Control	Stain
BRCA1	Polyclonal	Rabbit	Biogenex	Control tissue-Biogenex FB-345 P	Nucleus

THE IHC PROCEDURE INCLUDES FOLLOWING STEPS

1. Sections are 3-5µm thickness, floated on to organosialine coated slide and left on hot plate at 60^{o} over night

2. **Deparaffinization** using Xylene I and II—15 min each

3. **Dexylinisation** using absolute alcohol I and II—1 min each

4. **Dealcoholisation** using 90% and 70% alcohol—1 min each

5. Washing with distilled water.

6. **Antigen Retrieval technique:** Microwave power 10 for 6 minutes in TRIS EDTA buffer of pH-9.0 for 2 cycles.

7. Distilled water rinsing for 5 minutes. Transfer to TBS (Tris buffer solution pH- 7.6) - 5minutes x 3 times-wash.

8. **Peroxidase block**- Thirty (30) minutes to block endogenous peroxidase enzyme.

9. TBS buffer for 5 minutes washing for 3 times.

10. Power block for 15 min to block the non-specific reaction with other tissue antigen.

11. Cover sections with primary antibody for 45min

12. Rinse with TBS (Tris buffer solution pH- 7.6) for 5 min x 3 times wash with gentle agitation.

13. Super enhancer added for 20 min to enhance the reaction between primary and secondary antibodys

14. TBS wash 5min for 3 times to wash unbounded antibodys

15. Super sensitive poly HRP added for 30 min to elongate chain and added DAB results in chromogen development within 5 mins.

16. TBS wash for 5 min x 3 times.

17. Tap water wash x 5 min then Counterstain with Hematoxylin

18. Dehydrate , clear and mount with DPX

DOCUMENTATION AND INTERPRETATION OF DATA

All slides were revived and histopathological data such as tumor size, grade of the tumor, lymph node metastasis was interpreted and documented. These slides were stained with IHC marker BRCA1 and scoring was done

GRADE OF THE TUMOR[41]

Table 8 - NBR histologic grading system in breast cancer

Criteria	**Score 1**	**Score 2**	**Score 3**
Tubule formation	More than 75%	10 to 75%	Less than 10%
Nuclear pleomorphism	Minimal variation in nuclear size and shape	Moderate variation in nuclear size and shape	Marked variation in nuclear size and shape
Mitotic counts per 10 HPF	0-5	5-10	More than 11

Overall Grade

- Grade 1(scores of 3, 4, or 5)
- Grade 2 (scores of 6 or 7)
- Grade 3 (scores of 8 or 9)

Scoring of BRCA 1 done based on the scoring system given by Yoshikawa et al[7].

Table 9: IHC of BRCA1 Scoring in breast carcinoma

Score 0	**0% nuclear staining (absent staining)**
Score 1	**<20% nuclear staining (reduced staining)**
Score 2	**20%–80% nuclear staining**
Score 3	**>80% nuclear staining**

Score 0 and 1 considered as altered expression (mutated) and score 2 and 3 considered as positive staining.

Table 10:IHC of ER,PR – Allred Scoring in breast carcinoma[82]

Score for percentage of positive tumor cells (PS)		**Score for average intensity of staining (IS)**	
Score	Interpretation	Score	Interpretation
0	No staining	0	None
1	<1%	1	Weak
2	1-10%	2	Average
3	11-33%	3	Strong
4	34-66%		
5	>66%		

Allred Score = PS+IS

HER-2/neu membrane staining in tumor cells will be scored from 0 to 3 according to 2018 **ASCO guidelines**[83]

Table 11 : IHC Scoring of Her2neu in breast carcinoma

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

Ki 67 was scored as per Kanyılmaz G et al[84] and as follows

Table12: IHC scoring of Ki67 in breast carcinoma

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

NOTTINGHAM PROGNOSTIC INDEX.[85]

NPI = (0.2 X S) + N +G

- **Lymph nodes = number of lymph nodes, 0=1, 1-3 = 2, >3 = 3**

NPI	Score	5 Year survival	Prognosis
I	≤ 2.4	96%	Excellent
II	>2.4 - ≤ 3.4	93%	Good
III	>3.4 - ≤5.4	78%	Moderate
IV	>5.4	44%	Poor

Table 13 – Nottingham Prognostic Index Scoring in breast carcinoma

Results

The study duration from January 2019 to July 2021. Total 56 cases were collected and IHC with BRCA1 and scoring was done.

Microscopic Images

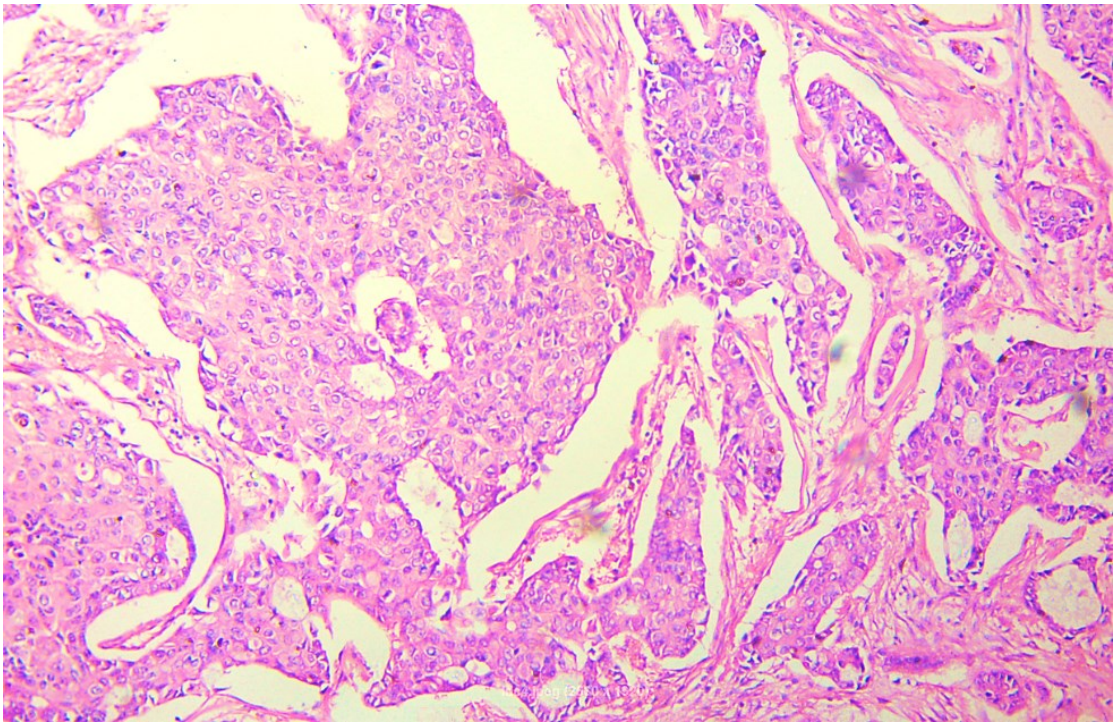

Figure 9: Invasive ductal carcinoma Breast (Not otherwise specified) – H & E – 40x

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

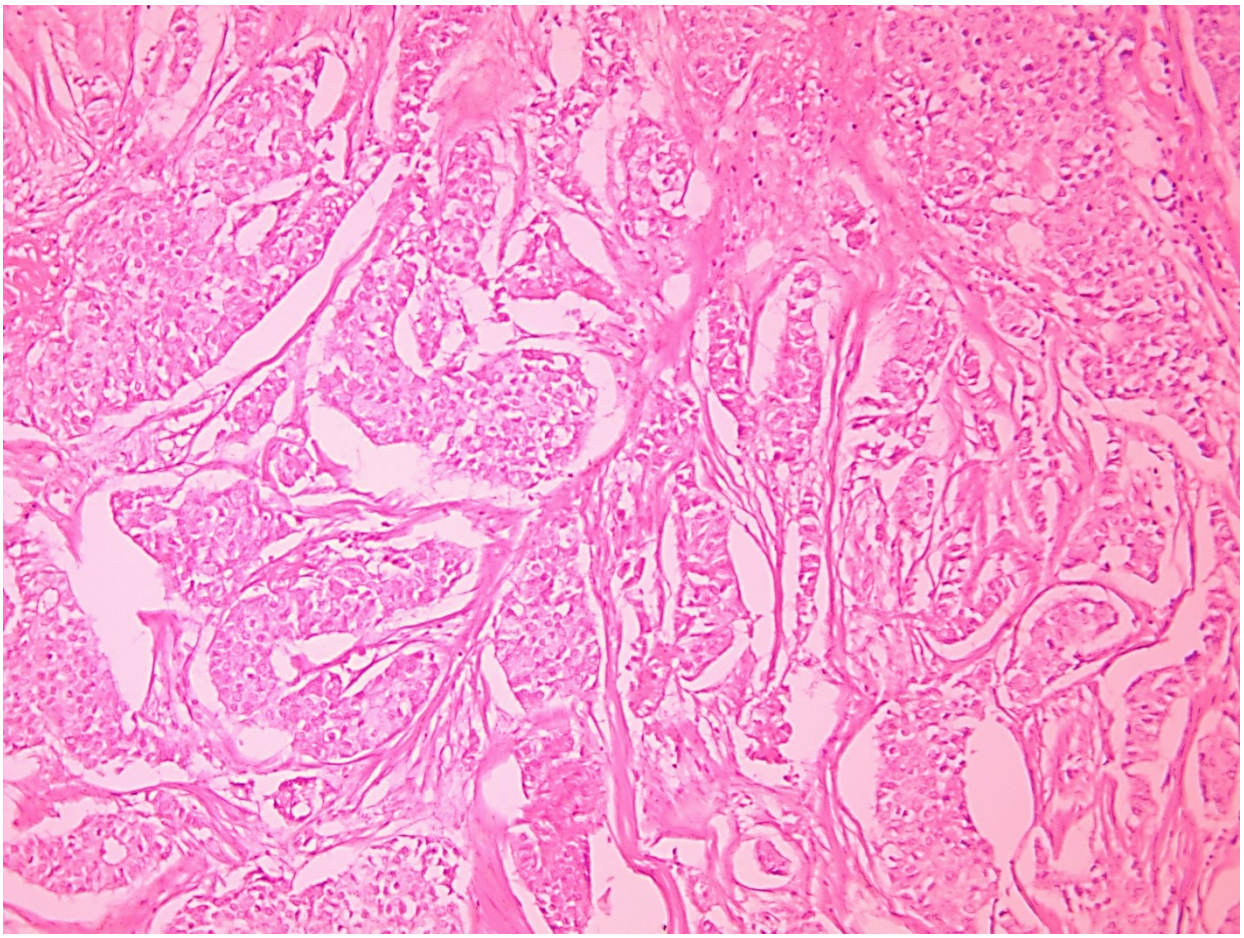

Figure 10– Invasive ductal carcinoma Breast – Grade 1 (H & E) – 40x

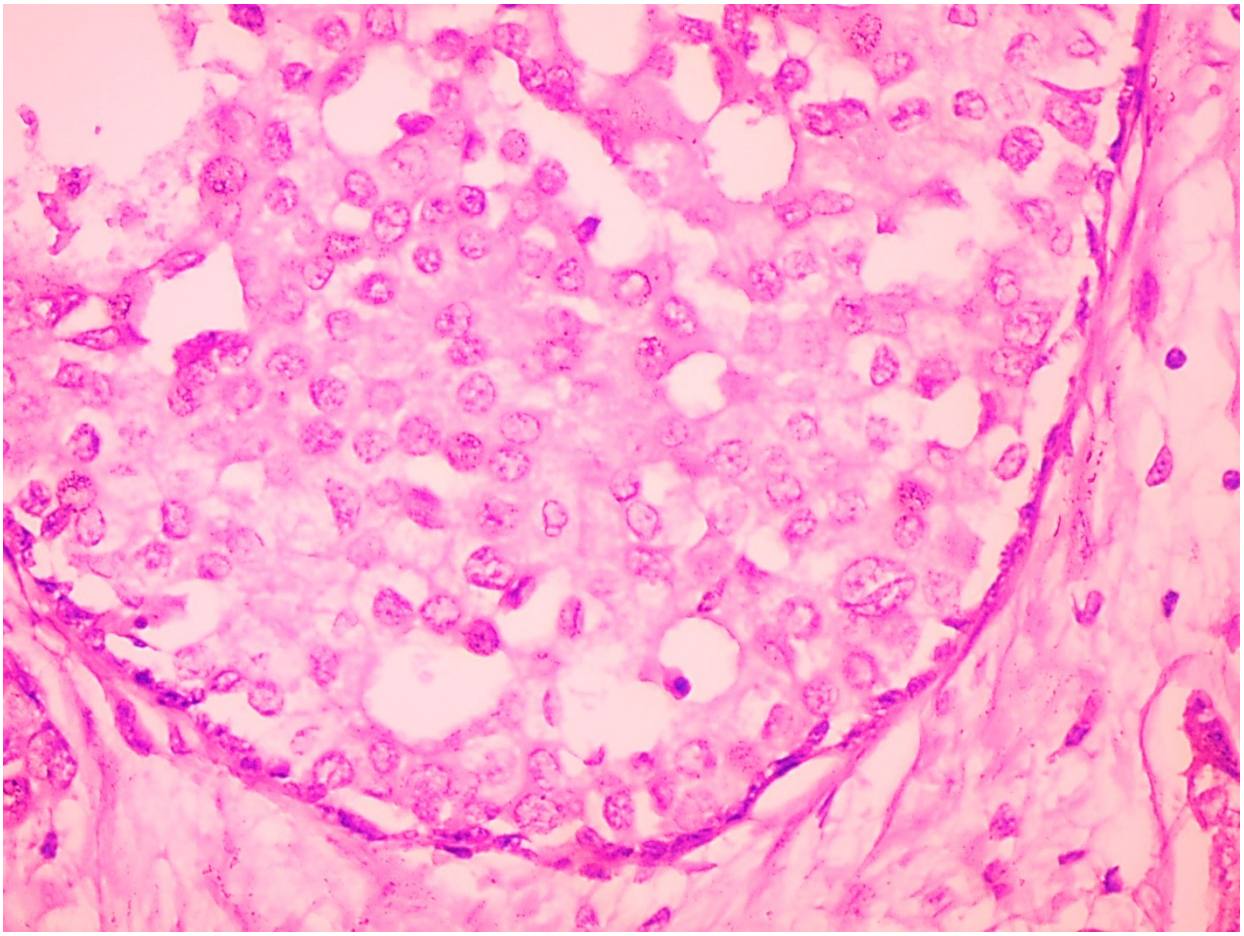

Figure 11 – Invasive ductal carcinoma Breast – Grade 2 (H & E) – 100x

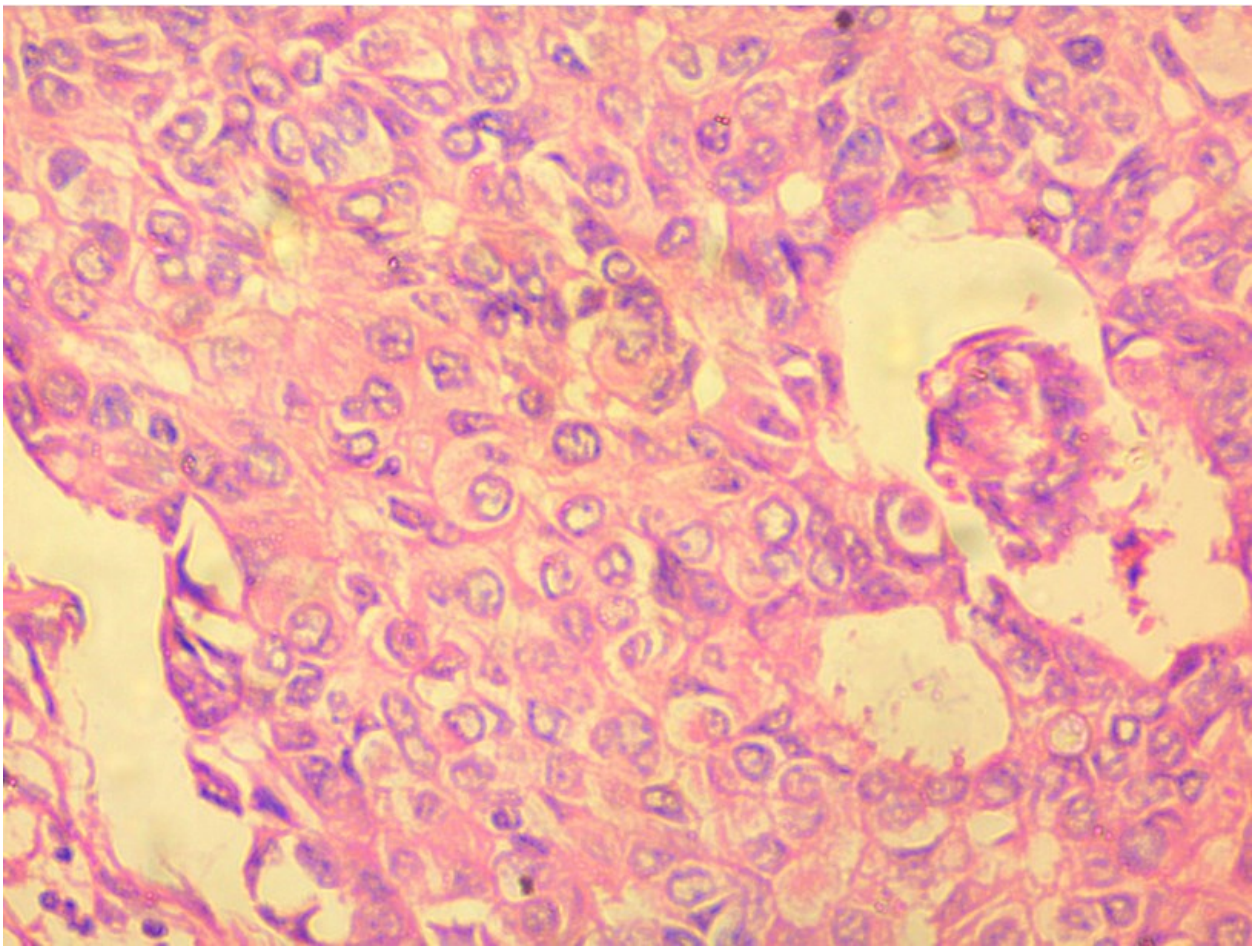

Figure 12 – Invasive ductal carcinoma Grade 3 – 100x

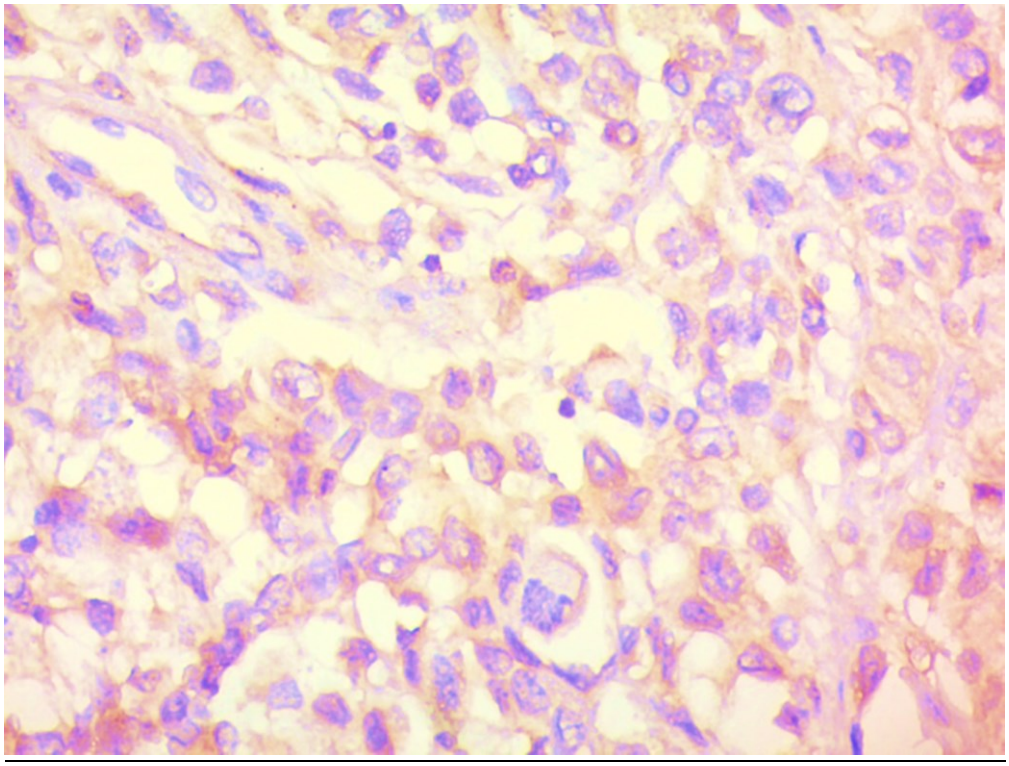

Fig13: IDC- BRCA1 IHC – 100x (intensity of nuclear positivity is absent Score 0)

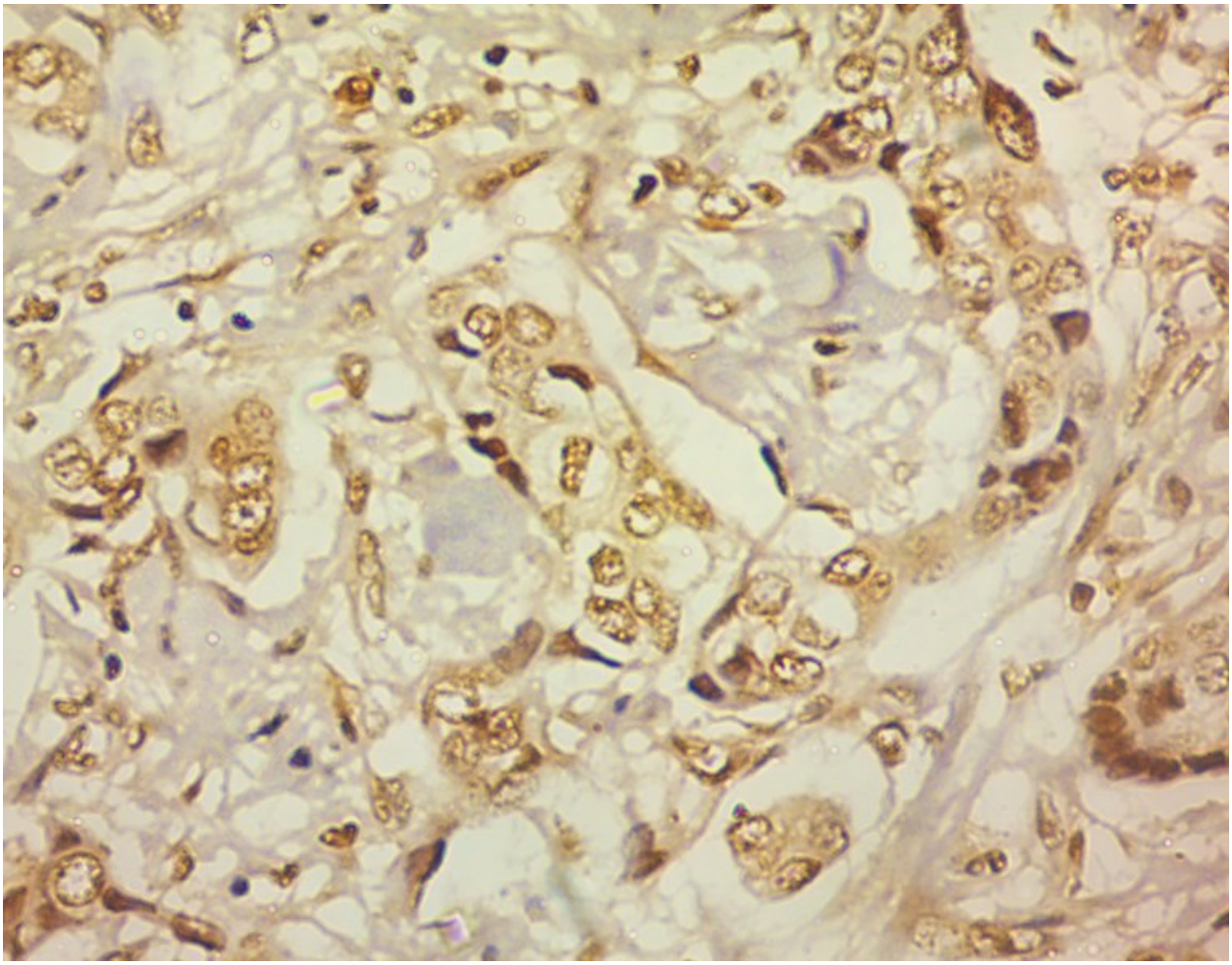

Fig 14: IDC- BRCA1 IHC – 100x (intensity of nuclear positivity is <20% Score 1)

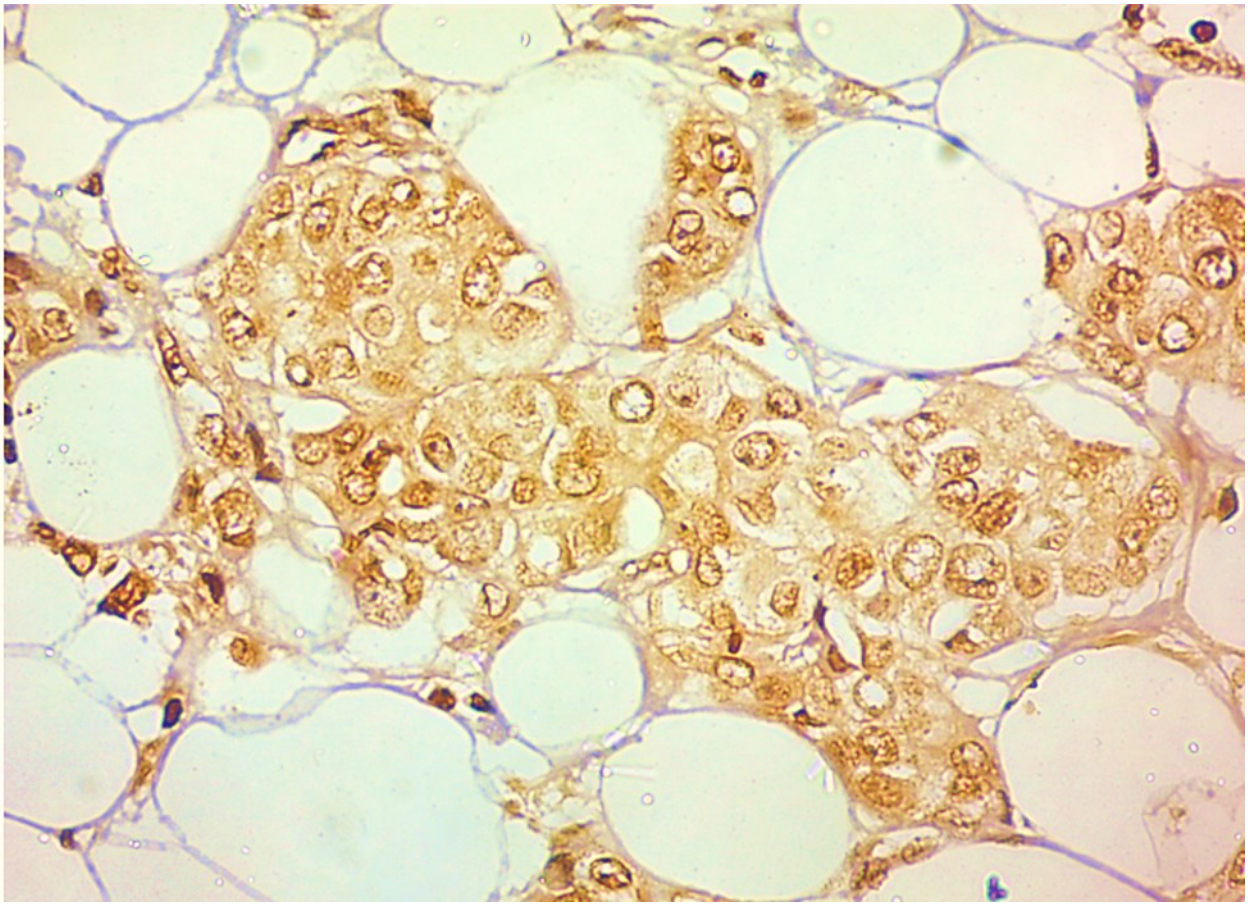

Fig15: IDC- BRCA1 IHC – 100x (intensity of nuclear positivity is 20-80% Score 2)

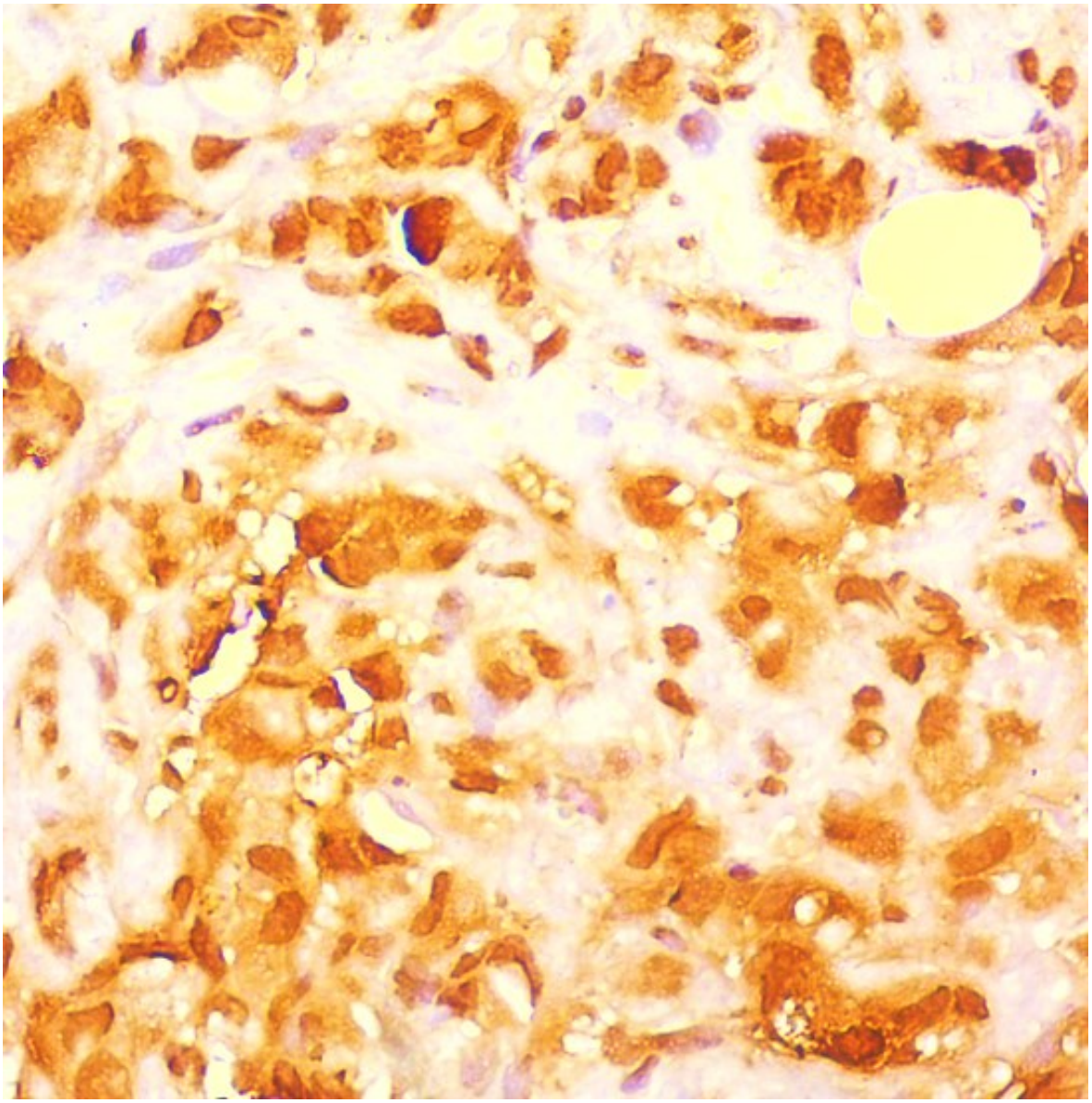

Fig16: IDC- BRCA1 IHC – 100x (intensity of nuclear positivity is >80% Score 3)

AGE DISTRIBUTION

Out of total 56 cases, the youngest age was 31 years and oldest age was 82 years. The average age of presentation is 52 years. Majority of the patient belonged to 50- 59 years which constituted 17 cases (30.1%) , followed by 40-49 years constituting 12 cases (21.4%), 09 cases (16%) belonged to 31 -39 years, 60-69 and >70 years each age group.

Table: 14 Distribution of subjects according to age

Age	Frequency	Percentage
31- 39	9	16.0
40 -49	12	21.4
50 -59	17	30.1
60-69	9	16.0
>70	9	16.0
Total	56	100

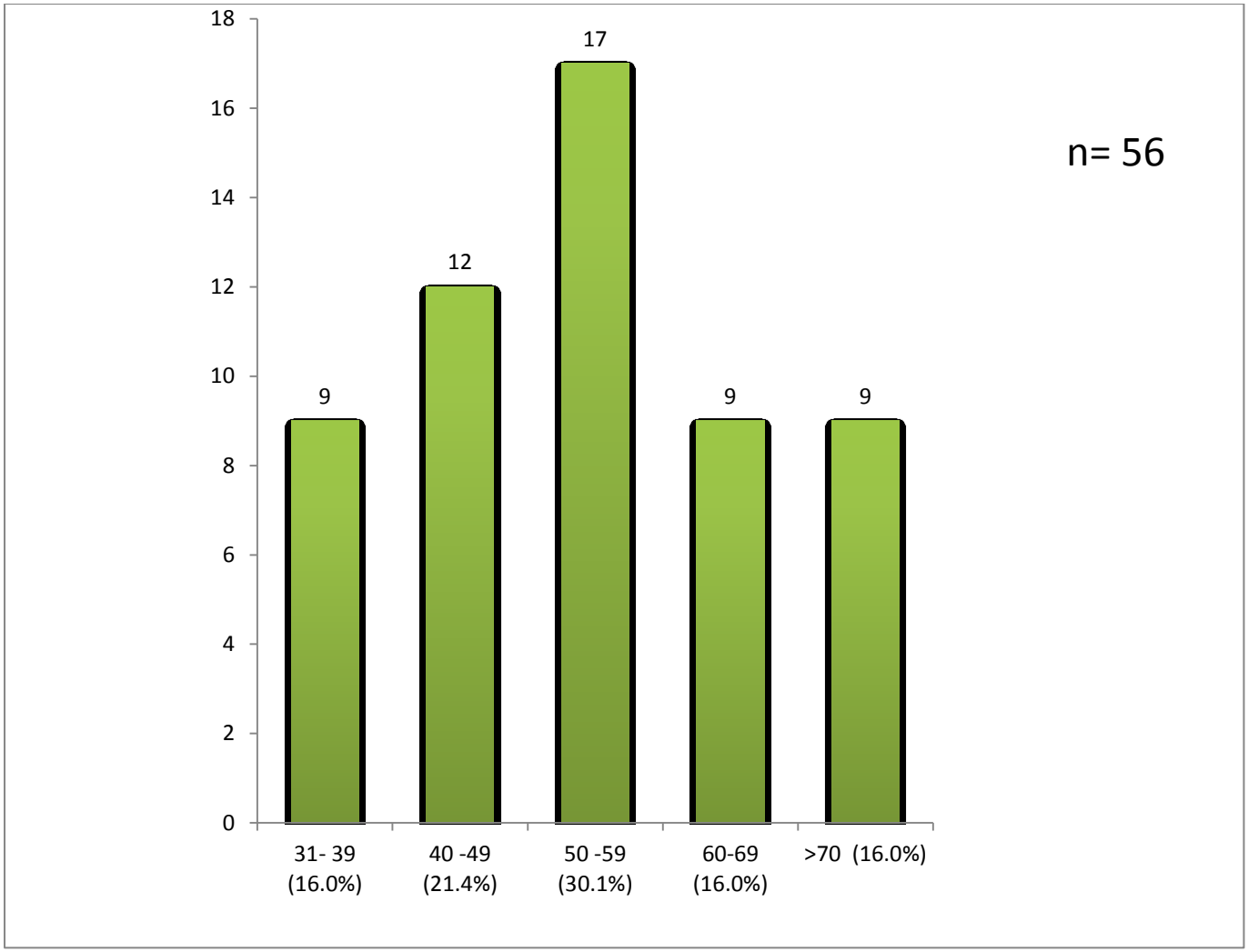

Chart1: Bar diagram showing the age distribution of subjects in the study.

Family History

Among 56 cases 36 patients responded to our query on family history of carcinoma of breast/ovarian carcinoma among first degree relatives. No positive history was found in any of the cases.

TUMOR SIZE

Table 15 – Distribution of subjects based on Tumor size

Tumor size	Cases	Percentage
T1 (<2CMS)	9	16.1
T2 (2- 5 CMS)	32	57.1
T 3 (>5CMS)	15	26.8
Total	56	100.0

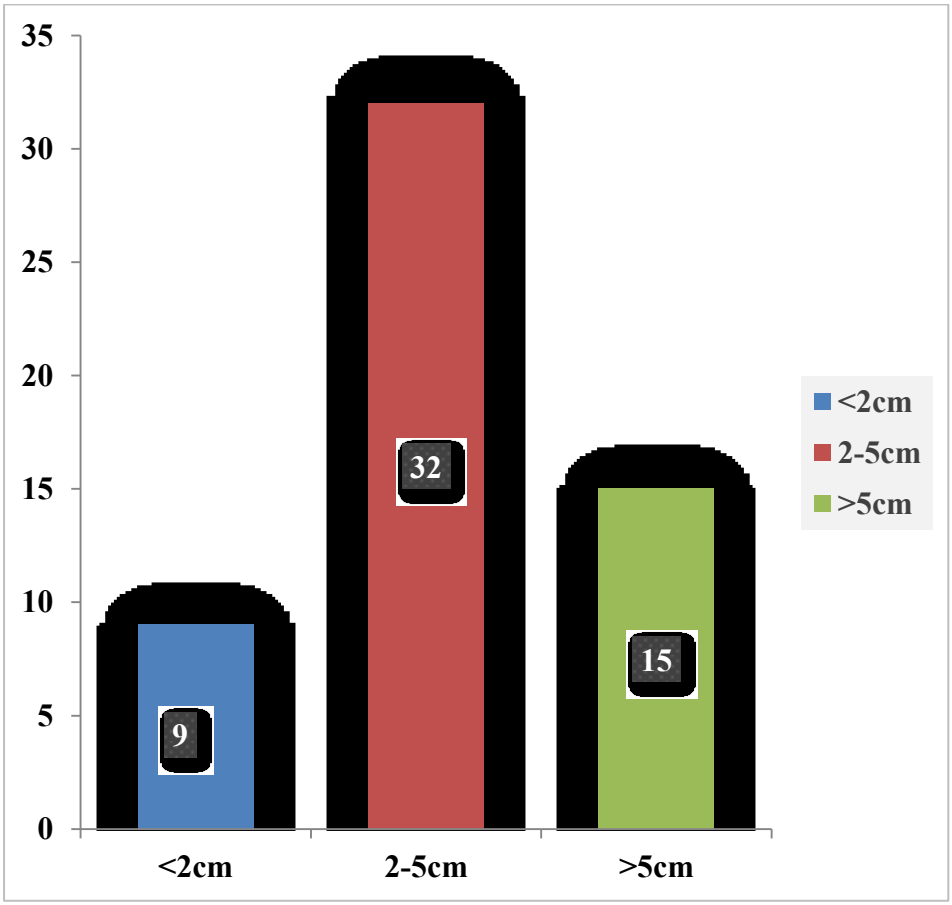

Chart 2: Bar diagram showing the tumor size distribution of subjects in the study.

Majority of the case belongs to T2.

DISTRIBUTION OF THE CASES INTO GRADE OF TUMOR

Tumor grading was done with Modified Scarff – Bloom Richardson Grading

Table 16 : Distribution of subjects based on tumor grade

Grade	Frequency	Percent
1	26	46.4
2	21	37.5
3	9	16.1
Total	56	100.0

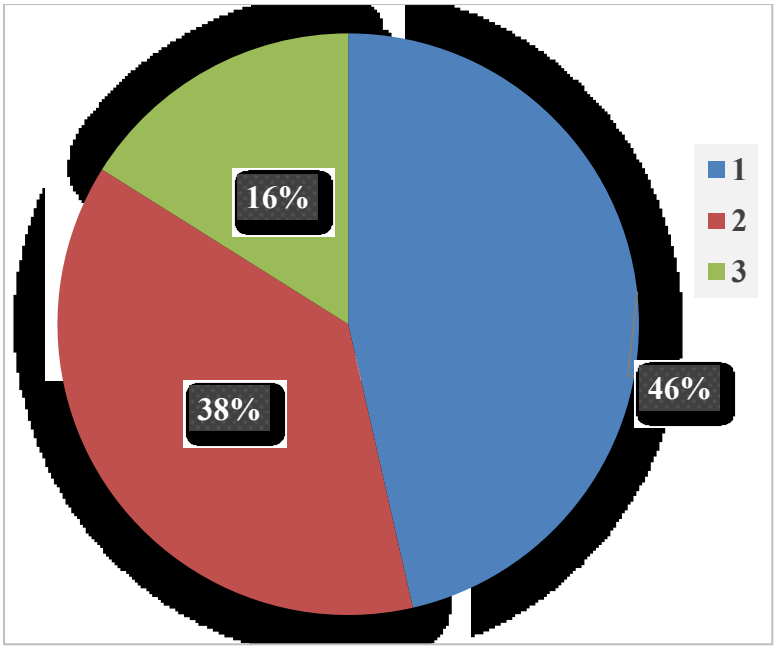

Chart 3: Pie chart showing the frequency of tumor grade distribution in the study group.

Distribution of subjects based on 7th AJCC TNM Staging

Table 17: Distribution of subjects based on 7th AJCC TNM Staging

7th AJCC TNM Staging	Frequency	Percent
I	6	10.7
II	30	53.6
III	20	35.7
Total	56	100.0

53.6% cases were in Stage II

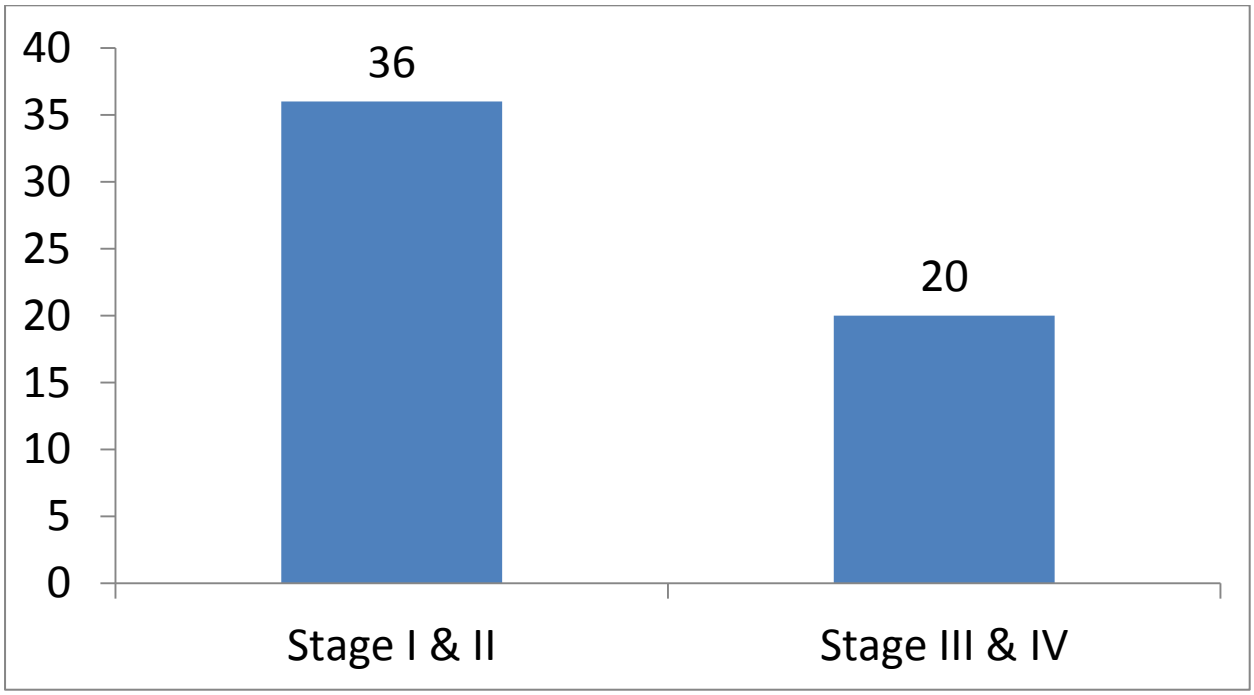

Chart4: Bar diagram showing the frequency of T staging in the study group

Distribution of subjects based on Nottingham prognostic index

Table 18: Distribution of subjects based on NPI in breast carcinoma

	Frequency	**Percent**
Excellent	9	16.1
Good	13	23.2
Moderate	21	37.5
Poor	13	23.2
Total	56	100

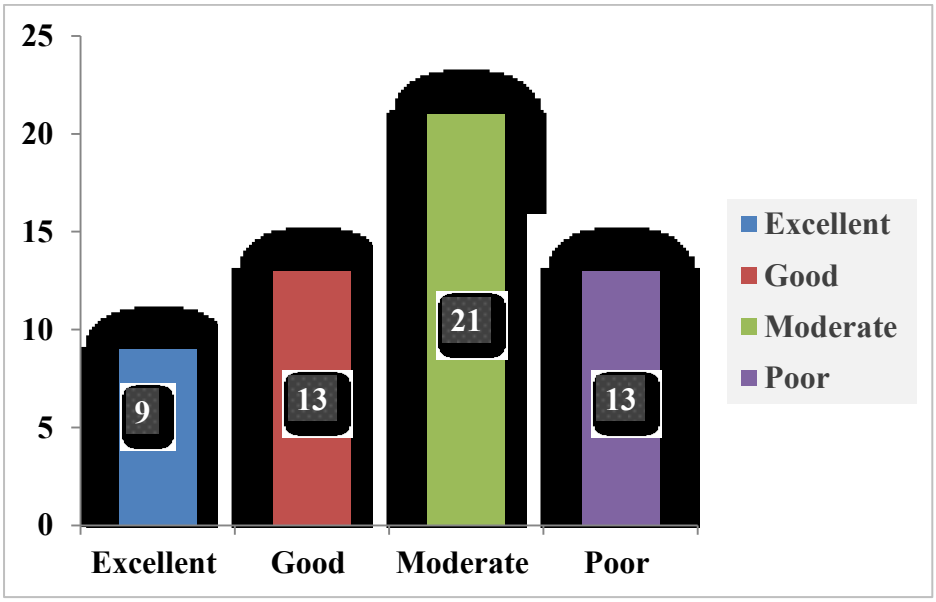

Chart 5: Bar diagram showing the nottingham prognostic index among the study subjects.

Hormonal expression in IDC Breast carcinoma

ER and PR scoring with Allred Scoring

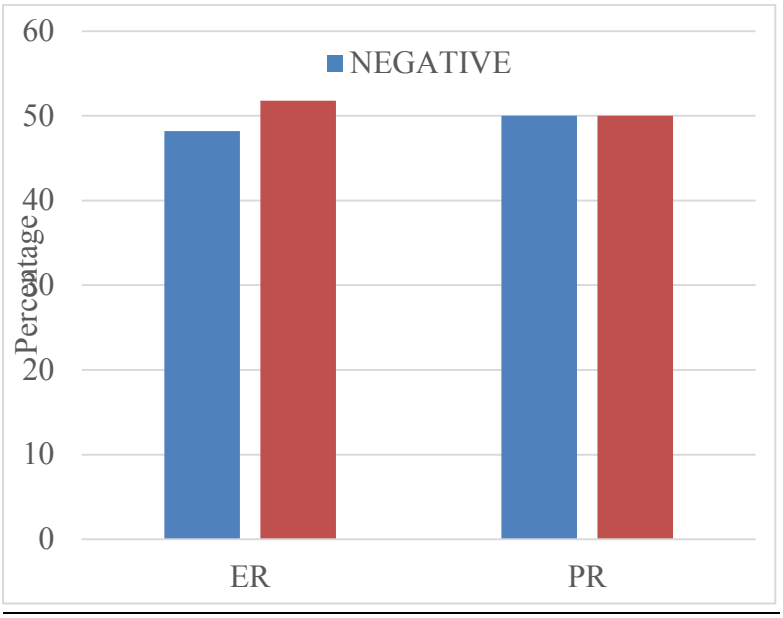

Chart 6: Bar diagram showing the ER,PR expression in the study group

Her 2 Neu Scoring – 2018 ASCO guidelines

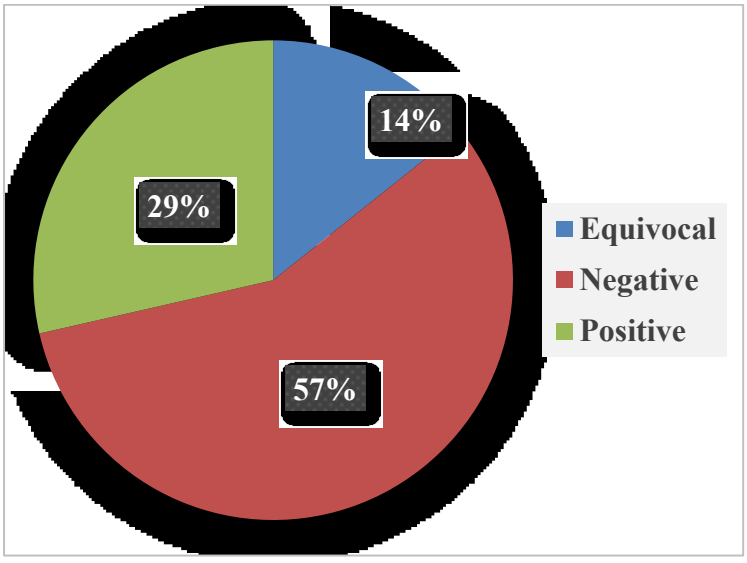

Chart 7: Pie chart showing the frequency of Her2 neu expression among the study group

Ki67 proliferation Index

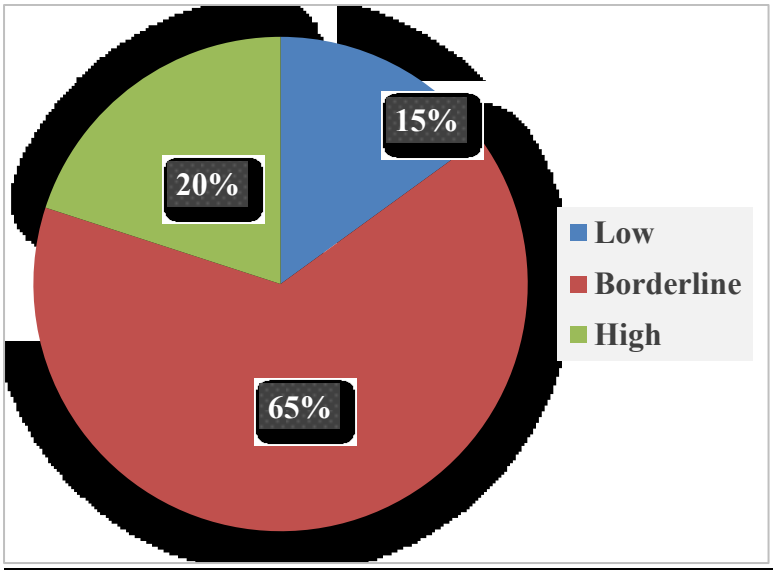

Chart 8: Pie chart showing the frequency of Ki67 proliferation index among the study group

Molecular subtyping

Table 19: Distribution of subjects based on molecular subtyping in breast carcinoma

Molecular subtyping	**Frequency**	**Percent**
Luminal A	23	41.1
Luminal B	7	12.5
Her 2+	11	19.6
TNC	15	26.8
Total	56	100

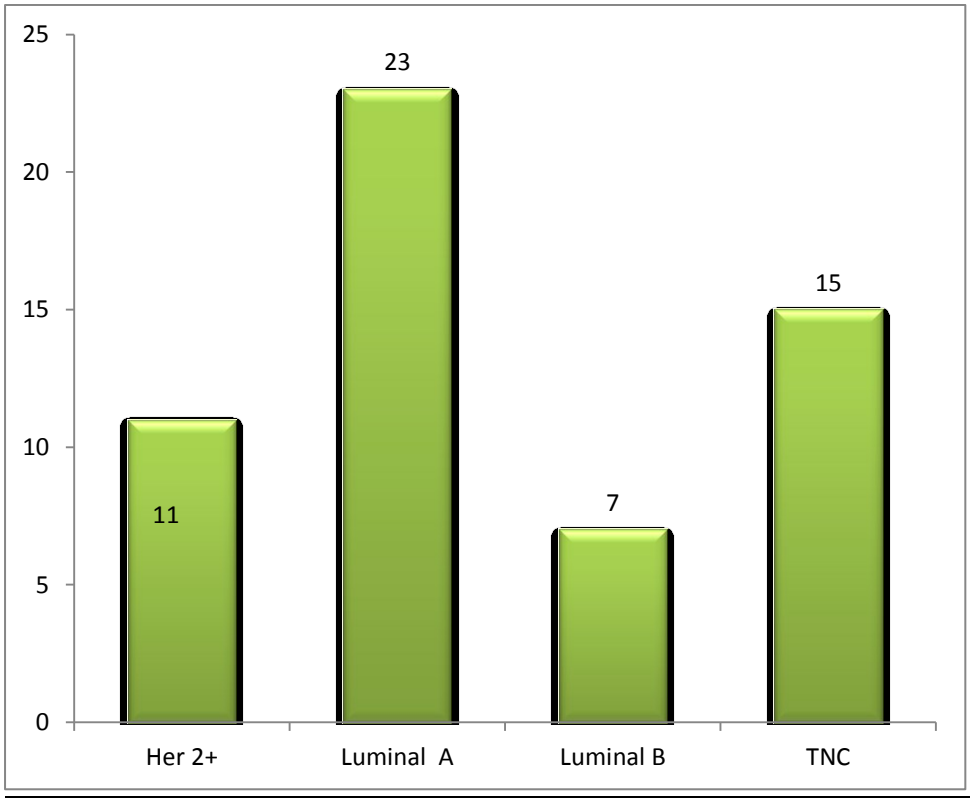

Chart 9: Bar diagram showing the molecular subtyping in the study group

Expression of BRCA 1 in IDC

Table 20: Distribution of subjects based on expression of BRCA1 in IDC

Positive	38	67.9
Altered	18	32.1
Total	56	100.0

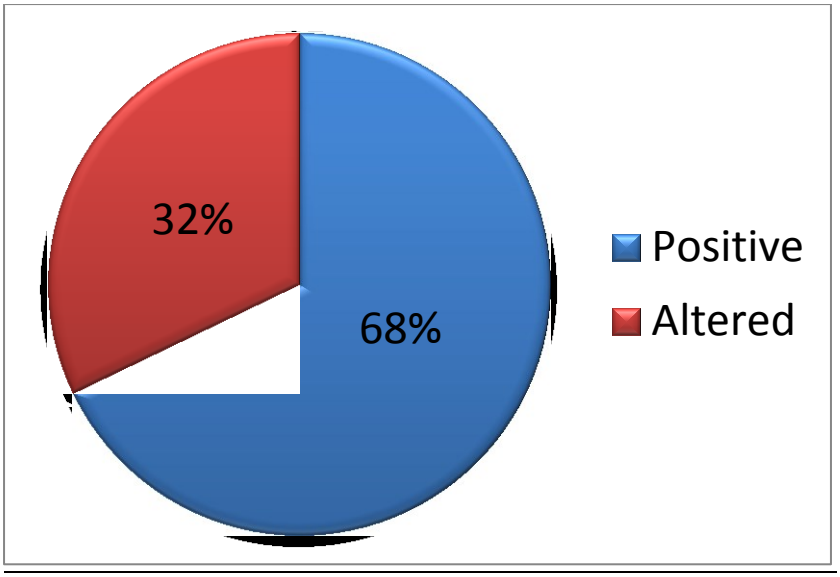

Chart 10: Pie chart showing the frequency of altered BRCA1 expression in the study group

CORRELATION between BRCA1 EXPRESSION WITH CLINICOPATHOLOGICAL PARAMETERS

Association between age and BRCA 1 expression

Table 21 – Association of age and BRCA1 expression in IDC cases

Age group	Altered Expression n=18	Positive Expression n= 38	Total n=56
31-39	3 (16.6%)	6 (15.7%)	9 (16)
40-49	5 (27.8%)	7 (18.4%)	12(21.4)
50-59	7 (38.8%)	10 (26.3%)	17 (30.1)
60-69	2 (11.1%)	7 (18.4%)	9 (16.0)
>70	1 (5.6%)	8 (21.05%)	9 (16.0)

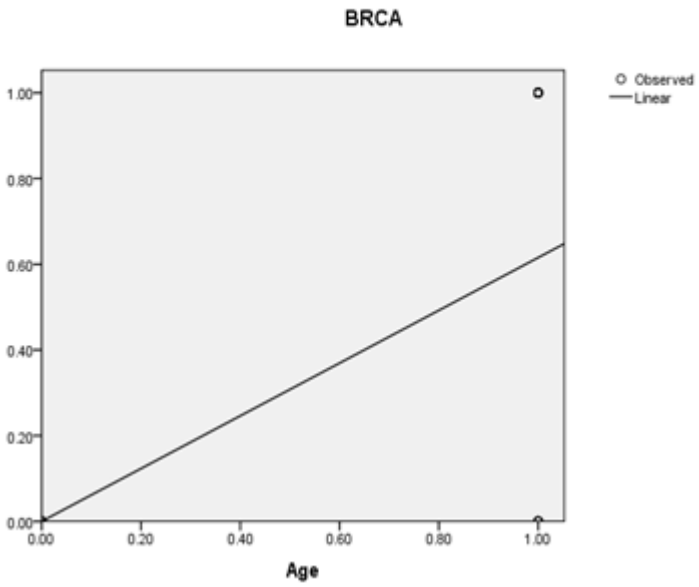

Chart 11: Pearson correlation graph showing the positive association of age and altered BRCA1expression

Present study shows BRCA 1 expression in 18/56 cases. BRCA1 mutation associate with postmenopausal status in 72%.

Table 22: Association between Tumor Size and BRCA 1 expression in IDC cases

Tumor size	**Altered Expression n = 18**	**Positive Expression n = 38**	**Total n = 56**
<3cms	7 (38.8%)	13 (34.2%)	20 (35.7%)
>3cms	11 (61.1%)	25 (65.7%)	36 (64.2%)

BRCA1 expression was significantly correlating with size of tumor with $p<0.01$ ($r=.714$)

Table 23: Association between Lymphnode status and BRCA 1 expression in IDC cases

Lymphnode status	**Altered Expression**	**Positive Expression**	**Total**
Positive	9 (50%)	19 (50%)	28 (50%)
Negative	9 (50%)	19 (50%)	28 (50%)

No correlation found between lymphnode status and BRCA1 expression.

Table 24 : Association between Tumor Grade and BRCA1 expression in IDC cases

Tumor Grade	**Altered Expression**	**Positive Expression**	**Total**
Low Grade	12 (66.6%)	15 (39.4%)	27 (48.2%)
High Grade	6 (33.3%)	23 (60.5%)	29 (51.7%)

Tumor grade was negatively correlating with BRCA1 expression with $p<0.01$ ($r = -.395$)

Table 25 : Association between Tumor Staging and BRCA 1 expression in IDC cases

Tumor Staging	**Altered Expression**	**Positive Expression**	**Total**
Lower Stage (I and II)	9 (50%)	24 (63.15%)	33(58.9%)
Higher Staging (III, IV)	9 (50%)	14 (36.8%)	23(41.07%)

Table 26 : Association of NPI and BRCA1 expression in IDC cases

NPI	**Altered Expression**	**Positive Expression**	**Total**
Excellent (2.0 -2.4)	-	9 (23.68%)	9 (16.1%)
Good (2.4 - 3.4)	8(44.4%)	5 (13.15%)	13 (23.2%)
Moderate (3.4 - 5.4)	8(44.2%)	13 (34.2%)	21 (37.5%)
Poor (>5.4)	2 (11.1%)	11 (28.9%)	13 (23.2%)

Table 27: Association of ER and BRCA1 expression in IDC cases

ER	Altered Expression	Positive Expression	Total
Positive	9 (50%)	18 (47.3%)	29 (51.8%)
Negative	9 (50%)	20 (52.6%)	27 (48.2%)

Table 28: Association of PR and BRCA1 expression in IDC cases

PR	Altered Expression	Positive Expression	Total
Positive	**8 (44.4%)**	**20 (52.7%)**	**28 (50%)**
Negative	**10 (55.6%)**	**18 (47.3%)**	**28 (50%)**

Table29: Association of Her2neu and BRCA1 expression in IDC cases

Her 2 neu	**Altered Expression**	**Positive Expression**	**Total**
Positive	6 (33.3%)	10 (26.3%)	16(28.6%)
Equivocal	2 (11.1%)	6 (15.7%)	8(14.3%)
Negative	10 (55.6%)	22 (57.8%)	32 (57.1%)

Table 30: Association of Ki67 and BRCA1 expression in IDC cases

Ki67	**Altered Expression**	**Positive Expression**	**Total**
High	5 (27.7%)	3 (7.8%)	8 (20%)
Borderline	2 (11.1%)	24 (63.15%)	26 (65.0%)
Low	5 (27.7%)	1 (2.63%)	6 (15%)

Table 31: Association of molecular subtyping and BRCA1 expression in IDC cases

Molecular classification	Altered Expression	Positive Expression	Total
Luminal A	8 (44.2%)	15 (39.4%)	23 (41.1%)
Luminal B	3 (16.6%)	4 (10.5%)	7 (12.5%)
Her2+	4 (22.2%)	7 (18.4%)	11 (19.6%)
TNBC	3 (16.6%)	12 (31.5%)	15 (26.8%)

Among 56 cases 44.2% in Luminal A which shows a significant correlation with BRCA1 expression with $p<0.01$ ($r=.054$)

Table 32: Association of BRCA1 with Clinicopathological parameter

	Age	Size	Grade	Lvm phn ode	Stagi ng	NPI	ER	PR	Her2 neu	Ki67	Mole cular Subty ping
BRCA 1	.879**	.923**	.739**	.407**	.513**	.801**	-.308*	-.395**	-.657**	.675**	.054
	.000	.000	.000	.002	.000	.000	.021	.003	.000	.000	.690

**. Correlation is significant at the 0.01 level
*. Correlation is significant at the 0.05 level

Discussion

BRCA1 which is a tumor suppressor gene displays an autosomal dominant pattern of inheritance with variable penetrance. Eventhough it is involved in familial cancer it may be involved during the evolution of breast carcinoma even in sporadic cases.[81,86]

AGE DISTRIBUTION

In the present study, the age group ranged from 31 years to 82 years with mean age of 52 years, which is similar to the study done by Verma et al [88] with 26.7% cases in premenopausal and 73.2% cases in post menopausal age group.

In our study BRCA1 expression is altered in 18/56 cases (32.1%), among those 18 cases 5 cases (28%)belongs to premenopausal age group and 13 cases (72%)belongs to postmenopausal age group.

Table 33: Comparison of age distribution with other studies.

Studies	Age (post-menopausal status)	Premenopausal status
Varma et al[88]	42.4%	57.6%
Hedau et al[82]	57.5%	42.5%
Kumar et al[89]	43.9%	56.1%
Present Study	72%	28%

Compared to other studies our study showed most of the patients with BRCA1 mutation belonged to postmenopausal group. This predilection may be because number of cases in postmenopausal age group were more(73.2%).

Size

Among 18 cases BRCA1 mutation associated with larger tumor size. In the present study 11(61.1%) of BRCA1 mutation found to be with larger tumor size (>2cms) which is in concordance with the study done by Verma et al[88] (64.8%), Rekha et al[58] (38.7%) and Sharma et al (81.5%). It is discordance with the study done by Fang et al showed 60% in lower tumor size (<2cms = 60%).

BRCA1 mutation associated with larger tumor size may be because of the aggressive behavior of the tumor.

LYMPHNODE STATUS

In our study we found that 50% of the BRCA1 mutation cases shows lymphnode involvement.

Table 34: Comparison of lymphnode status with other studies.

Lymphnode status	Verma et al[88]	Sharma et al[90]	Fang et al[91]	Ye et al[92]	Present Study
Positive	13	27	5	14	9
Negative	11	43	10	48	9

Our study shows no substantial correlation between the lymphnode involvement and BRCA1 expression. Similar results seen in study done by Verma et al and Yoshikawa et al. But the study done by Rakha et al[58] and Yang et al[93] showed a negative association between lymphnode involvement and BRCA1 mutation.

Tumor Grade

Comparison of distribution of the cases of general tumor grade with other studies

Table 35: Comparison of tumor grade distribution with other studies.

Grade	Utnal et al [94]	Rakha et al [58]	Verma et al [88]	Hussein et al[95]	Present study
1	33(57.9%)	260(17.6%)	16(29.6%)	13(15.6%)	**26(46.4%)**
2	17(29.8%)	483(32.7%)	27(50%)	29(34.9%)	**21(37.5%)**
3	7(12.3%)	732(49.7%)	11(20.4%)	41(49.4%)	**9(16.1%)**
Total	57	1475	54	83	**56**

In current study the highest number of cases belonged to grade 1 i.e 26 cases (46.4%) and least were of grade 3 i.e 9 cases (16.1%) which did not correlate with the studies done by Rakha et al[58],Verma et al [87]and Hussein et al[95]. This may be because of the pathogenesis or the tumor biology may be different in our area compared to other countries. Our findings are concordance with the study done by Utnal et al[94] which shows 57.9% cases were of grade 1.

Table 36: Comparison of altered BRCA1 expression with tumor grade and other studies.

Tumor grade (Histological grade)	**Altered BRCA 1 expression**									
	Present study		**Verma et al**[88]		**Hussein et al**[95]		**Rakha et al**[58]		**Sharma et al**[90]	
1	11/26	**42.3%**	3/16	18.7%	9/13	69.2%	24/260	9.2%	0/27	0
2 & 3	7/30	**23.3%**	21/38	55.2%	57/70	81.4%%	328/1215	26.9%	27/27	100%
Total (n)	18/56	32.1%	24/54	44.4%	66/83	79.5%	352/1475	23.8%	27/70	38.5%

When we analyse the altered BRCA1 expression in breast carcinoma our study showed 32.1% of BRCA 1 mutation which is concordance with the study done by Verma et al, Rakha et al and Hussein et al.

In present study the frequency of altered BRCA 1 expression was saw in 42.3% of grade 1 cases, which correlated with the study done by Hussein et al. It is contradicting with the study done by Sharma et al which showed no BRCA 1 mutation in grade 1. But Verma et al , and Rakha et al found that BRCA1 mutation associated with higher grade.

In our study grade1 tumor showed more BRCA1 positivity as compared to other studies. This may be due to varied behavior of these genes in our geographic area.

TUMOR STAGING

Tumor stage is an important prognostic factor in breast cancer. Higher stage of the tumor is related with higher number of lymph node metastasis as it is a dependent variable.

Table 37: Comparison of altered BRCA1 expression with tumor staging and other studies.

Staging	Hussein et al[95]	Present Study
I	2(3%)	6(10.7)
II	31(47%)	30(53.6)
III	33(50%)	20(35.7)

We found a significant association between the tumor staging and BRCA1 expression. BRCA1 mutation seen more with higher the tumor stage. This finding supported by the earlier study done by Ashraf et al[96] and Bugrein et al[97].

Nottingham Prognostic Index

Table 38: Comparison of altered BRCA1 expression with NPI and other studies.

NPI	Rakha et al	**Present Study**
Good	173(11.8%)	**8(44.4%)**
Moderate	453(31%)	**8(44.2%)**
Poor	172(11.7%)	**2 (11.1%)**
Total	1461	**18**

When we analysed the NPI with the BRCA1 mutation 44.4% of cases were in Category I and II each. Our findings were concordance with the study done by Rakha et al[58].

We have done follow-up via telecommunication, 8/56 expired. When we look in to the survival rate, 8 cases expired and 1 among them died because of complications associated with angina. Among 8

cases only 3(37.5%) cases showed BRCA1 mutation and all were in postmenopausal age group. It may be because of uneven distribution of cases in two age groups. However due to small size of sample number no logical conclusion could be arrived at in understanding the survival status of these patients.

BIOMARKERS IN BREAST CARCINOMA

Table 39: Comparison of altered BRCA1 expression with biomarkers and other studies.

Biomarkers	ER		PR		Her 2 neu			Ki67	
	Positive	Negative	Positive	Negative	Positive	Borderline	Negative	<14	≥14
Verma et al[88]	9(36)	15(51.7)	5(20)	19(65.5)	5(50)	3(37.5)	16(44.4)		
Ye et al[92]	14(22.22)	49(77.78)	14(22.22)	49(77.78)	3(4.76)	-	60(95.24)	4(6.35)	50(79.37)
Fang et al[91]	2(22.2)	7(77.8)	2(22.2)	7(77.8)	1(11.1)	-	8(88.9)		
Sharma et al[90]	5(18.5)	22(81.5)	5(18.5)	22(81.5)	-	-	-	8(29)	19(71)
Hussein et al[95]	19(34)	37(66)	23(42)	33(58)	8(36.3)	-	14(63.6)	-	-
Present Study	9 (50%)	9 (50%)	8 (44.4%)	10 (55.6%)	6 (33.3%)	2 (11.1%)	10 (55.6%)	3(25%)	9(75%)

Study done by Hussein et al showed negative association of ER PR with BRCA1 mutation which was seen in 52.72%. Similar findings was also seen with Her2neu negative(63.63%) with BRCA1 mutation. This is useful in molecular profiling and BRCA1 can be implemented as worthwhile prognostic marker in breast carcinoma patient. Study done by Verma et al[87] showed ER negative

(62.5%) associated with altered BRCA1 expression. Similar findings seen in study done by Yang et al, Yoshikawa et al but there was no significant association. In addition study done by Rakha et al[58] noticed a substantial association between ER negativity and BRCA1 expression.

Sharma et al[90] also supported our findings that the frequency of ER PR negative expression seen with BRCA1 mutation.

Our findings are correlating with study in Chinese population by Fang et al showed 77.8% of BRCA1 mutated cases are ER/PR negative and 88.9% showed Her2 neu negative. Its also supported by findings of Ye et al and Mohit Kumar et al.

Earlier studies proved that "BRCA1 acts as the inhibitor of E-ER signaling by interacting and inhibiting ER or inhibiting downstream effectors of ER. The functional interaction between E-ER and BRCA1 ensures the quality of replicated genome DNA when the cells experience proliferation under mitogenic effect of E-ER. When BRCA1 is in absence or insufficient, the balance is break down and the cells start to accumulate genomic mutations, contributing to the oncogenic transformation of mammary epithelial cells." Study done by Foulkes et[98] al showed that 70- 80 % of breast carcinoma cases were ER negative. In addition they found that ER positivity associated with sporadic breast cancer. Another mechanism of inhibition of transcriptional activity of ER by BRCA1 protein.[99]

Ki67 proliferation index frequency is high (71%) in BRCA 1mutated cases as found by Sharma et al[90]. Similar findings seen in study done by Ye et al[92] (79.37%). This may be due to the aggressive behavior of the tumor.

MOLECULAR SUBTYPING IN INFILTRATVE DUCTAL CARCINOMA

BRCA 1 mutation were not analysed with the molecular subtyping. Our study shows among 18 cases of BRCA1 mutation 8 (44.2%) were in luminal A, 3 (16.6%) were in luminalB, 4 (22.2%) were Her 2 enriched type and 3 (16.6%) composed of triple negative cases.

BRCA1 mutation mostly associated with triple negative cases, however in our study only 3 cases of triple negative subtype showed BRCA1 mutation.[98]

Patients with BRCA1 mutations are known to be benefited by PARP inhibitor therapy selection of patients for this treatment depending on the first degree relatives having BRCA1 mutation would be futile in a resource deficit countries were genetic analysis for BRCA1 mutation takes a lot of time and money. More studies has to be done to look into the benefit of PARP therapy in BRCA1 breast carcinoma cases irrespective of familial history. In addition they may get better outcome with anthracycline-taxane containing regimens. Hence triple negative cases even though have high grade BRCA1 targeted therapy may gave better prognosis.[59,60] The role of BRCA1 in the routine breast carcinoma workup panel is debatable, further multicentric studies with larger population are needed.

SUMMARY

Summary

- The present study was undertaken in Department of pathology, Sri Devaraj Urs Medical College, Tamaka, Kolar from October 2019– July 2021.
- Retrospective cases were also collected from January 2019 to september2019.
- Total of 56 cases were collected out of which majority belonged to the age group of 50 – 59 years (30.1%)
- Majority cases were of Grade I tumor (46.4%)
- Majority of the cases (57.1%) had tumor size between 2 – 5 cms
- Majority of cases were Nottingham Prognosis Index category III having moderate prognosis (37.5%)
- Out of 56 cases the altered BRCA1 expression was seen in 18 cases (32.1%). Among the 56 cases we could do the follow up in 36 cases.
- Present study shows 72% cases associated with postmenopausal status.
- BRCA1 expression is altered with higher the tumor size (61.1%)
- No correlation found between lymphnode status and BRCA1 expression.
- There was statistically a significant correlation between BRCA1expression and higher tumor stage.(r value - 0.513).Out of 18 BRCA1 mutated cases, 83.3% belongs to higher staging.
- Out of 56 cases we found negative correlation with altered BRCA1 expression ER and PR with $p<0.01$ ($r= -0.308$) and ($r= -0.395$) respectively.
- Out of 56 cases, 13 cases was in Nottingham Prognostic Index category II among that 8cases (44.2%)showed altered BRCA 1 expression. 21 cases were of NPI category III out of which only 8 cases (44.2%) showed altered BRCA 1 expression. 13 cases were of NPI category IV , out of which 2 cases (11.1%) showed altered BRCA 1 expression. The altered BRCA1expression and NPI category was statistically significant (r value - 0.801)

- Out of these 36 cases 8 patients expired during the follow up and 1 case was of age 82 and her death was not related to breast carcinoma.
- With respect to the molecular subtyping in IDC breast carcinoma total 41.1% distribution seen in luminal A type. Among the BRCA1 mutated cases 44.2% were in luminal A type. When we look in to the triple negative breast carcinoma cases 3/15 (16.6%) showed BRCA1 mutation.

CONCLUSION

Conclusion

In this study to look into altered expression of BRCA1 in infiltrating ductal carcinoma, 32.1% cases showed mutated BRCA1 expression. Though BRCA1 expression correlated with postmenopausal age, larger tumor size, higher tumor grade and stage and negative hormonal status, positive family history was not present in all cases.

In view of availability of PARP inhibitor therapy in BRCA1 mutated patients, feasibility of using this marker routinely in all cases of infiltrating ductal carcinoma should be looked in to.

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ANNEXURE

Annexure I

INFORMED CONSENT FORM

STUDY TITLE: EXPRESSION OF BRCA 1 BY IMMUNOHISTOCHEMISTRY

AND IT'S ASSOCIATION WITH ER, PR, HER 2 NEU STATUS IN INFILTRATIVE DUCTAL CARCINOMA OF BREAST.

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

I have had my opportunity to ask my questions regarding various aspects of the study and my questions are answered to my satisfaction.

I, the undersigned, agree to participate in this study and authorize the collection and disclosure of my personal information for the dissertation.

Name and signature / thumb impression Date:

(Subject) Place:

Name and signature / thumb impression Date:

Place:

(Witness/Parent/ Guardian/ Husband)

ANNEXURE II

PATIENT INFORMATION SHEET

STUDY TITLE: EXPRESSION OF BRCA 1 BY IMMUNOHISTOCHEMISTRY

AND IT'S ASSOCIATION WITH ER, PR, and HER 2 NEU STATUS IN INFILTRATIVE DUCTAL CARCINOMA OF BREAST.

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

The main aim of the study is to check for the presence of BRCA1 protein expression in tumor cells in breast carcinoma and its association with ER, PR, and Her2 neu status in Infiltrative Ductal Carcinoma.

You are requested to participate in a study conducted by the department of pathology as a part of dissertation. This study will be done on breast carcinoma specimens of the patients. The specimens will be collected from the department of pathology, SDUMC,Kolar.

This study will be approved by the institutional ethics committee. The information collected will be used only for dissertation and publication. There is no compulsion to agree to participate. You are requested to sign / provide thumb impression only if you voluntarily agree to participate in the study.

All information collected from you will be kept confidential and will not be disclosed to any outsider. Your identity will not be revealed. You will not receive any monetary benefits to participate in this research.

This informed consent document is intended to give you a general background of study. Please read the following information carefully and discuss with your family members. You can ask your queries related to study at any time during the study.

If you are willing to participate in the study you will be asked to sign an informed consent form by which you are acknowledging that you wish to participate in the study and entire procedure will be explained to you by the study doctor. You are free to withdraw your consent to participate in the study any time without explanation and this will not change your future care.

For any clarification you are free to contact the investigator.

PRINCIPAL INVESTIGATOR: Dr. PRINCY.S.SOMAN

Contact number: 08129988373

ANNEXURE III

PATIENT PROFORMA

Name :	
Age:	Hospital Number:

Anonymised Sample No:

Chief complaint :

History of presenting illness :

Past history :

Family History:

Personal history :

Local examination:

Clinical TNM staging :

Biopsy Number:

Gross :

Microscopy :

Histopathological diagnosis :

Grading:

IHC Scoring

BRCA 1 _____ ER _____,

PR scoring _____ Her 2 neu scoring _____ Ki67 _____

Keys to Master Chart

T – T staging according to 7th TNM Staging of breast carcinoma

N – N staging according to 7th TNM Staging of breast carcinoma

M – M staging according to 7th TNM Staging of breast carcinoma

P – Positive lymphnode status

N –Negative lymphnode status

NPI – Nottingham Prognostic Index

IDC – Infiltrating Ductal Carcinoma of breast

ER – Estrogen Receptor protein

PR -Progesterone Receptor protein

Her2 neu – Human Epidermal Growth Factor Receptor 2 neu protein

BRCA1- Breast carcinoma gene 1

Neg –Negative

TNC – Triple Negative Breast carcinoma

Her2+ - Her 2 enriched

Hosp N – Hospital Number

MASTER CHART

Biopsy no	Age	Hosp N	Tumor size(cm)	Tumor Grade	Lymph node	T	N	M	Stage	NPI	Diagnosis	ER	PR	Her 2 neu	Ki67	Molecular typing	BRCA 1
31	48	664790	8	2	P	3	3	x	III	6.6	IDC	4	4	Neg		Luminal A	1
347	55	678651	4	1	P	2	2	x	III	4.8	IDC	Neg	Neg	Neg		TNC	0
369	82	681338	2	1	N	1	0	x	II	2.5	IDC	3+	3+	Neg		Luminal A	0
371	58	681638	4	3	P	2	1	x	II	4.8	IDC	Neg	Neg	Neg		TNC	2
386	80	682643	2	2	P	1	3	x	III	5.4	IDC	5+	7+	Neg		Luminal A	2
550	49	694955	12	3	N	3	0	x	III	4	IDC	Neg	Neg	3+		Her 2+	0
641	45	690144	10	1	N	3	0	x	II	4	IDC	5+	5+	Neg		Luminal A	3
921	44	706401	2.5	1	N	2	0	x	II	2.5	IDC	8+	7+	Neg		Luminal A	3
984	53	708238	3.2	1	N	2	0	x	II	2.64	IDC	6+	Neg	Neg		Luminal B	0
1108	52	407606	3.5	1	N	2	0	x	II	2.7	IDC	7+	6+	Neg		Luminal A	2

1252	52	717035	2.2	2	P	2	1	x	II	3.44	IDC	3+	3+	Neg		LuminalA	1
1373	75	726027	4	1	P	2	1	x	II	2.8	IDC	7+	6+	Neg		LuminalA	2
1392	40	728557	4	3	P	2	1	x	II	4.8	IDC	Neg	Neg	3+		Her 2+	3
1410	75	726902	6	3	N	3	0	x	II	5.2	IDC	10+	7+	3+		Luminal A	2
1454	50	713964	3.5	1	N	2	0	x	II	2.7	IDC	Neg	Neg	Neg		TNC	3
1490	55	730817	3	2	P	2	3	x	III	5.6	IDC	Neg	Neg	Neg		TNC	3
1599	80	736905	9	3	P	3	1	x	III	6.8	IDC	6+	6+	Neg	0.1	Luminal A	3
1643	53	735987	2.5	3	P	2	1	x	II	5.5	IDC	6+	5+	Neg	0.25	Luminal A	3
1744	40	734649	9	2	N	3	0	x	II	4.8	IDC	Neg	Neg	Neg	>45%	TNC	2
1745	60	740099	4	2	N	2	0	x	II	3.8	IDC	Neg	Neg	3+	>25%	Her2+	1
2257	41	761094	2.2	3	P	2	2	x	III	6.4	IDC	5+	4+	3+	0.05	Luminal A	2
2275	55	684653	1	1	N	1	0	x	I	2.3	IDC	8+	4+	3+	>15%	Luminal A	3
2390	54	767363	7	2	P	3	3	x	III	6.4	IDC	Neg	Neg	Neg	>25	TNC	0
2476	36	742083	10	2	P	4	2	x	III	8	IDC	Neg	Neg	Neg	10	TNC	2
2523	33	757437	4	2	P	2	2	x	III	6.8	IDC	3	2	Neg	>15	Luminal A	3

2549	55	746844	10	3	P	3	2	x	III	8.2	IDC	Neg	Neg	2	<15	Her 2+	3
2726	38	759213	2	1	P	1	1	x	II	2.4	IDC	Neg	Neg	3	25	Her 2+	0
2830	42	786531	5	1	P	2	2	x	I	4	IDC	6	6	2	0.2	Luminal A	0
2905	60	732092	1.2	2	N	1	1	x	III	3.2	IDC	Neg	Neg	3	10	Her2+	2
2972	42	797271	3	1	N	2	0	x	I	2.6	IDC	4	4	neg	<10%	Luminal A	1
182	53	815423	4	2	P	2	2	x	II	5.8	IDC	Neg	Neg	3	>15	Her2+	2
228	33	812928	7	1	P	3	0	x	III	5.4	IDC	neg	neg	neg	>15%	TNC	3
480	65	825541	2.4	2	N	2	0	x	II	3.4	IDC	4	6	2	15	Luminal A	2
524	65	834087	2	1	N	1	0	x	I	2.4	IDC	Neg	Neg	Neg	>15	TNC	3
621	53	815423	4	2	P	7	1	2	II	5.8	IDC	Neg	Neg	3	15	Her2+	2
889	60	845877	6.5	2	P	4	3	x	III	6.3	IDC	Neg	Neg	Neg	30	TNC	3
910	70	844590	2.5	1	P	2	3	x	II	4.5	IDC	8	5	Neg	20	Luminal B	3
914	65	837500	4.5	1	N	2	0	x	II	3.9	IDC	Neg	Neg	Neg	10	TNC	3
935	61	845622	4	1	P	7	4	2	III	4.8	IDC	6	4	2	15	Luminal B	3
1002	39	820441	4.5	1	N	0	2	0	II	1.9	IDC	4	Neg	2	10	LuminalA	2

1044	56	839107	2	1	P	1	1	x	II	3.4	IDC	Neg	Neg	Neg	30	TNC	1
1354	59	857344	3.5	1	N	2	0	x	II	2.7	IDC	6	7	Neg	10	Luminal A	0
1407	72	863435	3.5	2	N	2	0	x	II	2.7	IDC	Neg	Neg	Neg	5	TNC	2
1445	31	852475	2.5	2	N	2	1	x	II	3.5	IDC	Neg	7	3	20	Luminal B	3
1462	73	865603	3	1	N	2	0	x	II	2.6	IDC	6	5	Neg	10	Luminal A	3
1476	60	865757	3.3	1	P	2	1	x	III	4.6	IDC	6	5	Neg	10	Luminal A	1
1648	45	873038	2.5	2	N	2	0	x	II	3.5	IDC	4	6	2	15	Luminal B	1
1878	36	878950	1.5	1	N	2	0	x	I	2.3	IDC	Neg	Neg	3	15	Her 2+	1
1913	67	879823	6.3	2	P	3	1	x	III	5.2	IDC	Neg	Neg	Neg	15	TNC	3
1920	45	882814	4.5	2	N	2	1	x	III	3.9	IDC	6	7	2	25	Luminal A	3
2017	50	876387	4.5	2	N	3	0	x	II	2.89	IDC	6	7	5	30	Luminal B	1
33	76	885577	6	2	N	3	0	x	II	4.2	IDC	Neg	Neg	3	20	Her2+	3
256	48	892991	7	3	P	4	1	x	III	6.6	IDC	6	4	2	15	Luminal B	2
410	35	897214	4	1	P	3	2	x	III	4.8	IDC	4	6	3	10	Luminal A	0
544	37	905154	9	1	N	3	0	x	II	3.8	IDC	Neg	Neg	3+	20	Her 2+	3

893	50	923478	1.7	1	N	1	0	x	I	2.3	IDC	Neg	Neg	Neg	20	TNC	2