“EXPRESSION OF CD133 IN INVASIVE DUCTAL CARCINOMA OF BREAST”

BY

DR. PREETI ASHOK UTNAL, MBBS

DISSERTATION SUBMITTED TO

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH

TAMAKA, KOLAR, KARNATAKA

IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF MEDICINE

IN

PATHOLOGY

UNDER THE GUIDANCE OF

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Dear Dr. Preeti Ashok Utnal,

This is with reference to your application seeking financial assistance from the ICMR for MD/MS/DM/MCh dissertation thesis entitled **"Expression of CD133 in invasive ductal carcinoma of breast"**.

I am glad to inform you that Director General, ICMR, based on the recommendation of Expert Committee, has sanctioned a sum of **Rs.50, 000/- (Fifty thousand only)** to you for providing an electronic and hard copy of your dissertation thesis to the ICMR. Mandatory requirement to avail this opportunity is to provide us with an undertaking duly forwarded through the guide, to the undersigned, enabling us to release the grant.

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Date: *Signature of the Candidate*

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LIST OF ABBREVATIONS

DCIS – Ductal Carcinoma Insitu

DFS – Disease Free Survival

ER – Estrogen Receptor

Her 2 – Human Epidermal Growth Factor Receptor 2

IDC - Infiltrating Duct Carcinoma

NPI – Nottingham Prognostic Index

NSBR - Nottingham modification of the Scarff-Bloom Richardson grading scheme

PR – Progesterone Receptor

TDLU – Terminal Duct Lobular Unit

Tis – Insitu Carcinoma

ABSTRACT

BACKGROUND:

Mammary gland is an important organ of the body consisting of stromal and epithelial components. There has been a recent increasing trend in malignant and non-neoplastic lesion of breast in western as well as in Indian population. A large variety of risk factors have been implicated in the development of breast cancer and are hence considered multifactorial rather than a single entity. A wide range of potential prognostic features have been studied in breast cancer and are mainly divided into two groups i.e. Histopathological and Molecular. Among many theories to explain the relapse and resistance to treatment in breast carcinoma's, cancer stem cell model suggests that in breast cancer tumour initiation and propagation is driven by a population of self-renewing tumour cells known as cancer stem cell. Numerous stem cell markers such as CD133, ALDH1, CD44, CD166, and CD34 are available for identification and localisation of cancer stem cells.

AIMS AND OBJECTIVES:

- To study the expression and localisation of CD133 in Invasive Ductal Carcinoma (Not Otherwise Specified).
- To correlate expression of CD133 with tumour size, node metastasis, tumour grade, stage, Nottingham prognostic index.

MATERIALS AND METHODS:

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, Kolar from December 2017 to September 2019.

Also cases of breast cancers were retrieved from archives of pathology from January 2015 to November 2018. The slides were stained using IHC marker CD 133. The expression of CD 133 was correlated with following histopathological parameters Tumor size, Grade, lymph node metastasis, tumor stage , Nottingham prognostic index and Disease free survival and results were analyzed.

RESULTS:

A total of 57 cases were studied and majority of the patients were in-between age of 41 – 60 years. Highest number of cases were in Grade I (57.9%), T2 stage (40.35 %) , N 3 lymph node stage (33.33%).Cd 133 expression was 77.08%.

On further analysis, there was a statistically significant association between the CD 133 expression and nodal metastasis, tumor stage and Nottingham prognostic index.

CONCLUSION:

CD 133 expression is associated with histopathological parameters higher tumor grade , higher tumor size , lymphnode metastasis, higher tumor stage and poor

Nottingham prognostic index and worse DFS. CD133 markers may potentially serve as prognostic markers and novel potential therapeutic targets in breast cancer.

Key words- Breast cancer, CD 133, Histopathological parameters , Prognosis

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INTRODUCTION

INTRODUCTION

Breast, is an important organ of the body consisting of stromal and epithelial components. There has been an increasing trend in non-neoplastic and malignant lesion of breast in western as well as in Indian population.

Breast cancer is the most common malignant tumour in women 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 data from International Agency for Research on Cancer (IARC) information system, invasive breast carcinoma is 55.4% of all breast cancer cases diagnosed and occupying the highest prevalence of all types of invasive breast carcinoma in International agency for research on Cancer during 2017 worldwide. It is the second most frequent cause of cancer death next to lung carcinomas with (626,679 deaths, 6.6% of total) in females.[1]

In Indian females incidence ranges from 19.3 to 89.7 per 100,000 population. In 2012, 144,937 women were newly detected with breast carcinoma and a total of 70,218 succumbed to it (cancer registry data).[2] In Karnataka, Breast cancer constitutes 27.5% of all cancers and is the most common cancer in women.[2]

Numerous markers have been described in breast carcinoma for both prognostic and therapeutic purpose, antibodies against Estrogen Receptor, Progesterone Receptor, Human Epidermal growth Receptor2/ neu are to name few.

Among many theories to explain the relapse and resistance to treatment in breast carcinoma's, cancer stem cell model suggests that in breast cancer tumour initiation and propagation is driven by a population of self-renewing tumour cells known as cancer stem cell. Numerous stem cell markers such as CD133, ALDH1, CD44, CD166, and CD34 are available for identification and

localisation of cancer stem cells.[3] Recently a few studies have been done on role of CD 133 in breast carcinomas showing varied findings. A meta-analysis study done by Zhan Li et al[4] suggested that CD133 can be a predictor of clinical outcomes , prognosis and it can be used as a potentially new gene therapy target for breast cancer patients. However, the prognostic role of CD133 expression in breast cancer is still controversial. Kim et al[5] suggested that CD133 high-expression patients had shorter Overall Survival and Disease Free Survival than CD133 low-expression or negative cases. Conversely, Margaret et al[6] found no significant difference between CD133 high expression and CD133 low expression in breast cancer patients regarding survival time.

Schmohl et al[7] said that the incidence of malignancy in solid or hematopoietic tumors and recurrence after perfect remission was caused by the presence of a small but very influential population of cancer stem cells, so that appropriate treatment was needed, one of which was to make CD133 as therapeutic target stem cells but were still in the early stages of the clinical phase.

Very few studies have been done to look into the expression and localisation of cancer stem cell marker CD133 in breast carcinoma. And there are varied findings regarding the expression of CD133 and its correlation with grade, lymph node metastasis, tumour size, Nottingham prognostic index.[8,9,10,11]

AIMS & OBJECTIVES

OBJECTIVES OF THE STUDY

- To study the expression and localisation of CD133 in Invasive Ductal Carcinoma (Not Otherwise Specified).
- To correlate the expression of CD133 with tumour size, node metastasis, tumour grade, stage and Nottingham prognostic index.

REVIEW OF LITERATURE

REVIEW OF LITERATURE:

History and Background

"The female breast has been a symbol of beauty, fertility and femininity. The written records of breast cancer date back to antiquity since the location of the organ permitted easy identification. The Edwin Smith Surgical Papyrus dating back to 3,000–2,500 B.C and possibly attributable to Imhotep (the Egyptian physician-architect), provides authentic accounts of breast cancer". A case was said to be incurable if the disease was "cool to touch, bulging and spread all over the breast".[12] Carcinoma (karkinoma), Scirrhous (hard, Greek skirros) and Cacoethes (malignant disease, Greek kakoethes) in the medical lexicon owe their origins to Hellenistic writings. Hippocrates theory in 400 B.C. on imbalance of humours (blood, phlegm, and yellow and black bile) as a cause of disease and his classic descriptions of the progressive stages of breast cancer, represent early hypotheses on the cause of cancer.[9]

The ancient Greek and Egyptian physicians postulated that the cessation of menstruation was somehow linked to cancer, it probably had to do with the association of cancer with old age.[13]

DEVELOPMENT OF BREAST[14]

The breasts develop from the mammary ridges or milk lines, which are thickenings of the epidermis that first appear on the ventral surface of the 5 week fetus. These ridges extend from the axilla to the upper medial region of the thigh. In humans, most of the ridge do not develop further and disappears during fetal development. Few studies show Molecular mechanisms guiding embryonic mammary gland development and the potential role of stem cells in normal mammary development and maintenance.[15,16] Mesenchymal condensation occurs around an epithelial stalk, the breast bud, at the site of mammary development on the chest wall in the 15th week of gestation. Growth of cords of epithelium into the mesenchyme produces a group of solid epithelial columns, each of which gives rise to a lobe in the mammary gland. The papillary layer of the fetal dermis continues to encase these growing epithelial cords and it ultimately evolves into the vascularized fibrous tissue surrounding individual ducts and their branches of ducts that form lobules. Myoepithelial cells appear to arise from basal cells between weeks 23 and 28 of gestation.[17] They play an important role in the branching morphogenesis of the mammary gland through the synthesis of basement membrane.

Less cellular, more collagenized stroma that originates in the reticular dermis extends into the breast to encompass lobes and subdivisions of lobes, forming the suspensory ligaments of Cooper that attach the breast parenchyma to the skin.[17] Coincidentally, differentiation of the mesenchyme into fat within the collagenous stroma occurs between weeks

20 and 32. In the last 2 months of gestation, canalization of the epithelial cords occurs, followed by the development of branching lobuloalveolar glandular structures. The mammary pit is a depression in the epidermis where the lactiferous duct converge. Near birth, the nipple *is* formed by evagination of the mammary pit. The earliest stages of fetal mammary gland formation

appear to be independent of steroid hormones, whereas the actual development of the breast structure after the 15th week is influenced largely by testosterone. In the last weeks of gestation, the fetal breast is responsive to maternal and placental steroid hormones and prolactin, which induce secretory activity. This is manifested after birth by the secretion of colostrum and palpable enlargement of the breast bud.

GROSS ANATOMY OF BREAST[15]

The mammary gland forms the secondary sexual characteristics of females and breast milk is the only source of nutrition for the neonates until 6months of age. In young adult females, each breast is a rounded eminence largely lying within the superficial fascia anterior to the upper thorax but spreading laterally to a variable extent. In the adult female the base of the breast i.e its attached surface, extends vertically from the second to sixth rib, and in transverse plane from sternal edge medially to the mid axillary line laterally. The superolateral quadrant is prolonged towards the axilla along the inferolateral edge of pectoralis major from which it projects a little and may extend through the deep fascia up to the apex of the axilla. The breast lies on the deep pectoral fascia, which overlies pectoralis major and serratus anterior superiorly and external oblique and its aponeurosis inferiorly.

The nipple and areola

The nipple projects from the center of the breast anteriorly. The level of the nipple varies dependent on the size and shape of the breast. The areola is the disc of skin that circles the base of the nipple.

HISTOLOGY[15]

Extending posteriorly from the nipple, the large and medium sized ducts, glandular structures and surrounding stroma form approximately 20 interconnected lobes. Within a single lobe the small ducts branch and terminate in glands known as Terminal Duct lobular units (TDLUs). The 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 surface of the nipple.

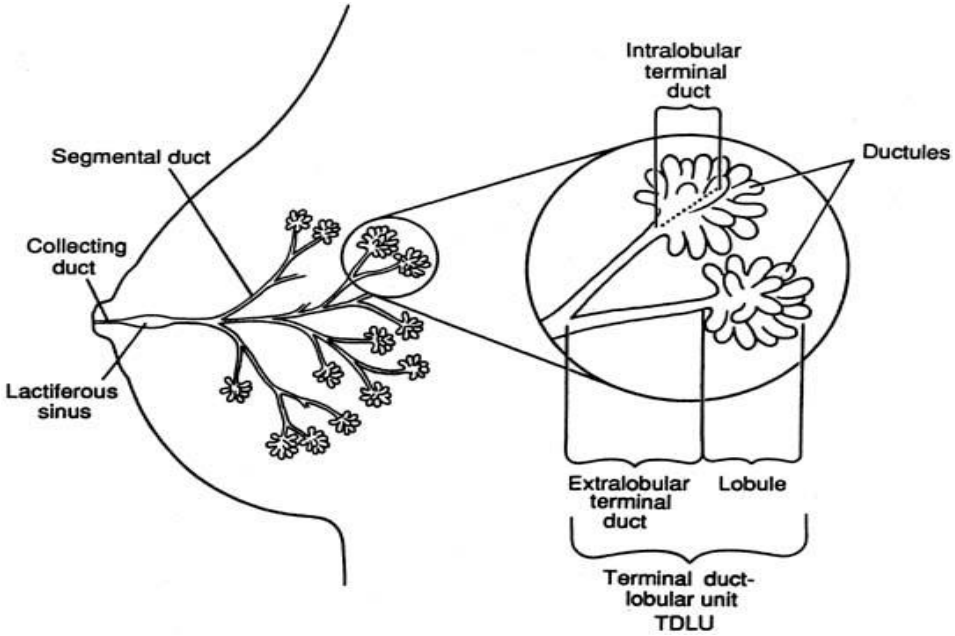

Figure 1- Diagram of a breast lobe and a terminal duct lobular unit[19]

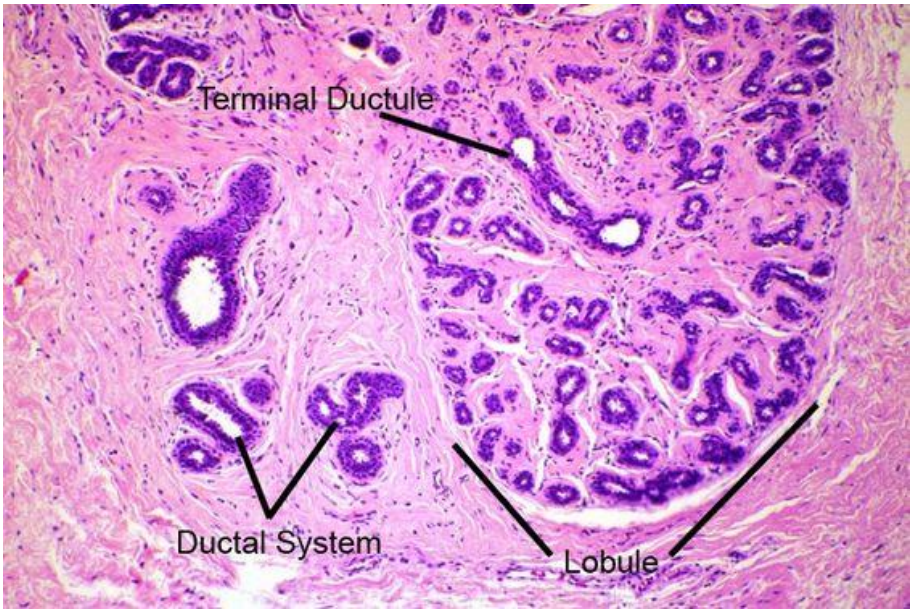

Figure 2 - Low power magnification of a section of normal breast tissue. This shows the lobule made up of terminal duct lobular units. Within the lobule it is possible to see a terminal ductile, also noted is the structures of the ductal system[20]

The cellular lining throughout the ductal lobular system is bilayer. It consists of a luminal epithelial cell layer and a basal myoepithelial cell layer. The importance of this double cell layer cannot be overemphasized, because it is one of the guides used to distinguish benign from the malignant lesions.

The parenchyma of the female breast consists of the ducts and lobules, intralobular fibrous tissue and abundant adipose tissue. The amount of adipose tissue varies depending on the age and general habitus of the women.

"The nipple predominantly consists of dense fibrous tissue mixed with fascicles of smooth muscles. The later component given the nipple its erectile capability and contributes to expression of milk. The areola is more heavily pigmented than the surrounding skin of the

breast and becomes even more so during the pregnancy. In this area the skin has pilosebaceous units and it is one of the few areas of the body that contains apocrine and eccrine sweat glands."

SOFT TISSUE[18]

The breasts are composed of lobes that contain a network of glandular tissue consisting of branching ducts and terminal secretory lobules in a connective tissue stroma. The lobes interwine in three dimensions and merge at their edges. The connective tissue stroma that surrounds the lobules is dense and fibrocollagenous. The intralobular connective tissue has a loose texture that allows the rapid expansion of secretory tissue during pregnancy. Fibrous strands extend between the layer of deep fascia that covers the muscles of the anterior chest wall and the dermis. These suspensory ligaments support the breast.

LYMPHATIC DRAINAGE OF BREAST[21]

The carcinomas of the breast commonly tend to invade and spread through the lymphatic channel to the regional lymphnodes. Hence it is of great importance to the operating surgeon and the reporting pathologist. The following are the important groups of lymph node draining the breast tissue

1. Axillary group of lymphnodes
2. Intra mammary group of lymph nodes
3. Other groups such as Supraclavicular, Sub diaphragmatic, Posterior intercostal and Cephalic group of lymph nodes

RISK FACTORS AND ETIOLOGY [21& 22]

The origin of breast cancer is multifactorial and involves geography, age, gender, genetic factors, diet and alcohol obesity, lifestyle, Endocrine factors and molecular genetics

1. Diet: High-calorie diet rich in animal fat and proteins, combined with a lack of physical exercise.
2. Specific environmental exposures operative in the development of breast cancer (e.g. radiation, alcohol, exogenous hormones) have been identified, but are associated with a lower risk.

- Reproductive lifestyle: Women with early menarche, nulliparous, parous with late age at first delivery, Infertility and lack of breast feeding, late age at menopause.

3. Endogenous Hormone: Sex steroids (androgens, estrogens, progestogens) have an important role in the development of breast carcinomas.
4. Molecular genetics of breast cancer: Five to ten percent of all breast cancers arise from germ-line mutations in high-penetrance breast cancer susceptibility genes such as BRCA1, BRCA2, p53 and PTEN and confer a high individual risk for developing hereditary breast cancer. The BRCA1 gene is located on the long arm of chromosome 17, while BRCA2 is located on the long arm of chromosome 13. Gene-positive patients have an 80% risk of developing breast cancer especially in the pre-menopausal age group. Many studies have proved that BRCA1 and BRCA2 predispose a woman to breast cancer in only 5–10% of the total number of breast cancers and believe that even though family history may reflect shared genes, it may also suggest shared environmental lifestyle exposures.

5. Role of HER2-NEU antigen: It's a growth factor protein, which is over-expressed in 20–30% of invasive breast cancers and also shown to be associated with poorer outcome and shortened survival. In addition, HER-2/neu-positivity is thought to predict the likelihood of resistance or sensitivity to some conventional hormonal therapies like tamoxifen. Herceptin (transtuzumab) a recombinant humanized anti-HER-2/neu monoclonal antibody has been shown to improve outcomes for women with metastatic breast cancer, either alone or in combination with chemotherapy.
6. Triple negative breast cancer (TNBC): Breast cancer negative for estrogen, progesterone and HER-2/neu receptors. It makes up to 20% of all breast cancers and currently has no standard treatment. This type of breast cancer (TNBC) has also been associated with higher recurrence rates, faster growth and poorer prognosis, compared with other conventional breast cancers and it is only sensitive to chemotherapy.

ETIO PATHOGENESIS OF BREAST CANCER[23]

Like other cancers, breast cancers are clonal proliferations that arise from cells with multiple genetic aberrations, acquisition of which is influenced by hormonal exposures and inherited susceptibility genes. Breast cancers may be hereditary, arising in women with germline mutations in tumor suppressor genes or sporadic. However, environmental factors clearly influence the penetrance of hereditary forms of breast cancer and both genetic and environmental factors contribute to sporadic forms of breast cancer. The identification of breast cancer susceptibility genes has provided important insights into the pathogenesis of both familial and sporadic forms of breast cancer.[24]

FAMILIAL BREAST CANCER

Approximately 12% of breast cancers occur due to inheritance of an identifiable susceptibility gene or genes. The probability of a hereditary etiology increases when there are multiple affected first-degree relatives, early onset cancers, multiple cancers or family members with other specific cancers. In some instances cancer risk is an autosomal dominant trait that is conferred by inheritance of a defective copy of a tumor suppressor gene. In such instances, a single sporadic mutation in the remaining normal allele is all that is required to completely lose tumor suppressor function, which is likely to be the initiating driver mutation in these forms of breast cancer. The major known susceptibility genes for familial breast cancer— BRCA1, BRCA2, TP53, and CHEK2—are all tumor suppressor genes that have normal roles in DNA repair and maintenance of genomic integrity. It is likely that complete loss-of-function of these proteins creates a "mutator" phenotype, an increased propensity to accumulate genetic damage that speeds cancer development. Mutations in BRCA1 and BRCA2 are responsible for 80% to 90% of "single gene" familial breast cancers and about 3% of all breast cancers. Penetrance varies from 30% to 90% depending on the specific mutation present.[24,25]

BRCA1 (on chromosome 17q21) and BRCA2 (on chromosome 13q12.3) are both large genes, and hundreds of different mutations distributed throughout their coding regions have been associated with familial breast cancers. The frequency of mutations that increase breast cancer risk is only about 1 in 400 persons in the general population and inconsequential polymorphisms are common. BRCA1-associated breast cancers are commonly poorly differentiated, have "medullary features" (a syncytial growth pattern with pushing margins and a lymphocytic response) and are biologically very similar to ER-negative/HER2-negative breast cancers identified as "basal-like" by gene expression profiling. BRCA2-associated breast

carcinomas also tend to be relatively poorly differentiated, but are more often ER-positive than BRCA1 cancers. The remaining known susceptibility genes accounts for fewer than 10% of hereditary breast carcinomas. Germline mutations in TP53 (Li-Fraumeni syndrome)

and mutations in CHEK2 together account for about 8% of breast cancers caused by single genes. Three other tumor suppressor genes-PTEN (Cowden syndrome), STK11 (Peutz-Jeghers syndrome), and ATM (ataxia telangiectasia)- are mutated in less than 1% of all familial breast cancers. Most of these genes play complex and interrelated roles in maintaining genomic integrity. After a cell sustains DNA damage, it must undergo cell cycle arrest and either repair its DNA or die by apoptosis. ATM senses DNA damage and with p53 and CHEK2 induces cell cycle arrest. BRCA1, BRCA2, and CHEK2 all have important functions in repair of double stranded DNA breaks. If any of these functions are impaired, the likelihood that cells with permanent DNA damage will survive is increased and the mutation will be propagated. BRCA1 and BRCA2 are part of a large complex of proteins that are required to repair double stranded DNA breaks through a process called homologous recombination, in which a normal sister chromatid is used as a template for repairing the broken stretch of DNA. BRCA1 and BRCA2 are expressed ubiquitously, so the link to breast cancer is not obviously explained by tissue-specific patterns of gene expression. An alternative possibility is that breast epithelial cells may be particularly prone to suffer the type of DNA damage that BRCA1 and BRCA2 are required to repair. BRCA1 also interacts with protein complexes that regulate chromatin structure and it remains possible that its tumor suppressive role involves functions that are independent of DNA repair.[26,27]

SPORADIC BREAST CANCER

The major risk factors for sporadic breast cancer are related to hormone exposure: gender, age at menarche and menopause, reproductive history, breastfeeding and exogenous estrogens. Other environmental risk factors, proven or suspected, include radiation exposure and exposure to chemicals with estrogen-like effects. Estrogen clearly functions as a promoter of breast cancers, probably through several different effects on the breast. Hormonal exposure stimulates breast growth during puberty, menstrual cycles and pregnancy, thereby increasing the number of cells that can potentially give rise to a cancer. The proliferation of breast epithelium during the menstrual cycle is also conducive to the accumulation of DNA damage and the temporary lull in cell division that occurs during the latter part of the menstrual cycle may allow time for defective DNA repair to occur and for mutations to become "fixed" in the genome. Repeated rounds of this process during each cycle may underlie the association between the cumulative numbers of menstrual cycles a woman experiences and her risk of developing breast cancer. Once premalignant or malignant cells are present, hormones can stimulate their growth as well as the growth of normal stromal cells that may aid and abet tumor development.[28]

MOLECULAR MECHANISMS OF CARCINOGENESIS AND TUMOR PROGRESSION

The diverse histologic appearances of breast carcinomas and putative precursor lesions are the outward manifestations of the complex genetic and epigenetic changes that drive carcinogenesis. As with other cancers, resident breast tissue stem cells have been hypothesized

to be the cell of origin for all breast cancers. Once the process is initiated in such cells by a driver mutation, there appear to be three major genetic pathways of carcinogenesis **ER positive, HER2-negative cancers arise via the dominant pathway of breast cancer development, constituting 50% to 65% of cases.** This is the most common subtype of breast cancer in individuals who inherit germline mutations in BRCA2. They are often associated with gains of chromosome 1q, losses of chromosome 16q and activating mutations in *PIK3CA*, a gene that encodes phosphoinositide-3 kinase (PI3K) which is an important component of signaling pathways downstream of growth factor receptors. These same genetic lesions are often found in flat epithelial atypia and atypical ductal hyperplasia, which are hypothesized to be precursor lesions for this subtype of breast cancer. 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. As discussed later, tumors arising through this pathway include at least two major molecular subtypes that differ in their proliferation rate and response to therapy.[29]

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.

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 tumors comprise about 15% of breast cancers overall, but are the most common tumor type observed in patients with germline *BRCA1* mutations; they also occur with increased frequency in African American women. Sporadic tumors of this type often have loss-of function mutations in *TP53*; mutations in *BRCA1* are uncommon, but *BRCA1* may be silenced in sporadic tumors through epigenetic mechanisms. These tumors have a "basal-like" pattern of mRNA expression that includes many genes that are expressed in normal myoepithelial cells.

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 greatest mammographic density, suggesting that increased amounts of fibrous stroma is both a marker of risk and biologically important for tumorigenesis. 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 tumor development and growth. Angiogenesis and tumor-associated inflammation are commonly associated with carcinoma, starting at the in situ stage. With better understanding of the role played by stroma, it may be possible to develop therapies that target stromal components. The final step of carcinogenesis, the transition of carcinoma in situ to invasive carcinoma, is both the most important and the least understood. The majority of genetic changes observed in invasive carcinomas are already present in the associated carcinoma in situ. It is possible that the same molecular events that allow for the normal formation of new ductal branch points and lobules during pregnancy—abrogation of the basement membrane, increased proliferation, escape from growth inhibition,

angiogenesis, and invasion of stroma—may be replicated during invasion. Remodeling of the breast during post-pregnancy involution, which involves inflammatory and "wound healing-like" tissue reactions and is known to increase the risk of tumor invasion, may also facilitate the transition of carcinoma in situ to invasive carcinoma. As can be surmised from this discussion, breast cancer is not one disease, but many, each with its own clinical characteristics and optimal prevention and treatment strategies. This recognition has led to the introduction of new molecular classification systems.[29]

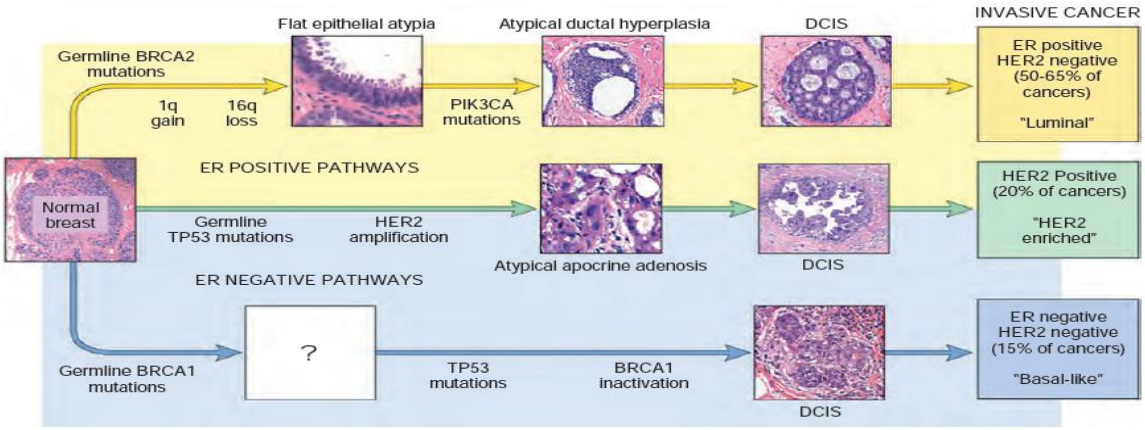

Figure 3 - Pathogenesis of breast carcinoma. Three main pathways have been identified. The most common pathway (yellow arrow) leads to ER-positive carcinomas. The precursor lesions include flat epithelial atypia and atypical hyperplasia. A less common pathway (blue arrow) leads to carcinomas that are 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. [23]

WHO CLASSIFICATION OF BREAST CARCINOMA[22]

Table 1 – WHO classification

EPITHELIAL TUMOURS	• Microinvasive carcinoma
INVASIVE BREAST CARCINOMA	• Invasive carcinoma of no special type (NST) • Pleomorphic carcinoma • Carcinoma with osteoclast-like stromal giant cells • Carcinoma with choriocarcinomatous features • Carcinoma with melanotic features • Invasive lobular carcinoma • Classic lobular carcinoma • Solid lobular carcinoma • Alveolar lobular carcinoma • Pleomorphic lobular carcinoma • Tubulolobular carcinoma • Mixed lobular carcinoma • Tubular carcinoma • Cribriform carcinoma • Mucinous carcinoma • Carcinoma with medullary features • Medullary carcinoma • Atypical medullary carcinoma • Invasive carcinoma NST with medullary features • Carcinoma with apocrine differentiation • Carcinoma with signet-ring-cell differentiation • Invasive micropapillary carcinoma • Metaplastic carcinoma of no special type

	• Low-grade adenosquamous carcinoma • Fibromatosis-like metaplastic carcinoma • Squamous cell carcinoma • Spindle cell carcinoma • Metaplastic carcinoma with mesenchymal differentiation • Chondroid differentiation • Osseous differentiation • Other types of mesenchymal differentiation • Mixed metaplastic carcinoma • Myoepithelial carcinoma
RARE TYPES	• Carcinoma with neuroendocrine features • Neuroendocrine tumour, well-differentiated • Neuroendocrine carcinoma, poorly differentiated (small cell carcinoma) • Carcinoma with neuroendocrine differentiation • Secretory carcinoma • Invasive papillary carcinoma • Acinic cell carcinoma • Mucoepidermoid carcinoma • Polymorphous carcinoma • Oncocytic carcinoma • Lipid-rich carcinoma • Glycogen-rich clear cell carcinoma • Sebaceous carcinoma • Salivary gland/skin adnexal type tumours - Cylindroma • 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
INTRADUCTAL PROLIFERATIVE LESIONS	• Usual ductal hyperplasia • Columnar cell lesions including flat epithelial atypia • Atypical ductal hyperplasia
PAPILLARY LESIONS	• Intraductal papilloma • Intraductal papilloma with atypical hyperplasia • Intraductal papilloma with ductal carcinoma in situ • Intraductal papilloma with lobular carcinoma in situ • Intraductal papillary carcinoma • Encapsulated papillary carcinoma • Encapsulated papillary carcinoma with invasion • Solid papillary carcinoma In situ • Invasive
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

"MORPHOLOGICAL TYPES OF BREAST CANCER"

INVASIVE DUCATL CARCINOMA (NO SPECIFIC TYPE)[22]

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.[30,31]

Macroscopy / 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.

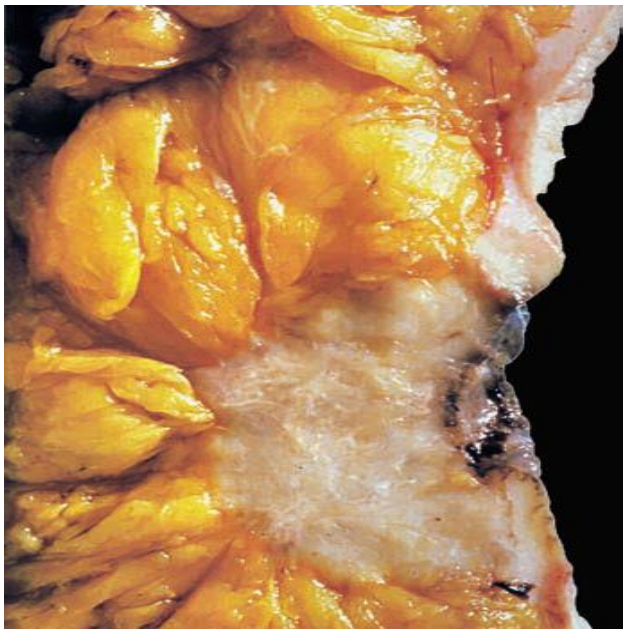

Figure 4 - Gross image showing grey white areas on cut section. WHO breast [22]

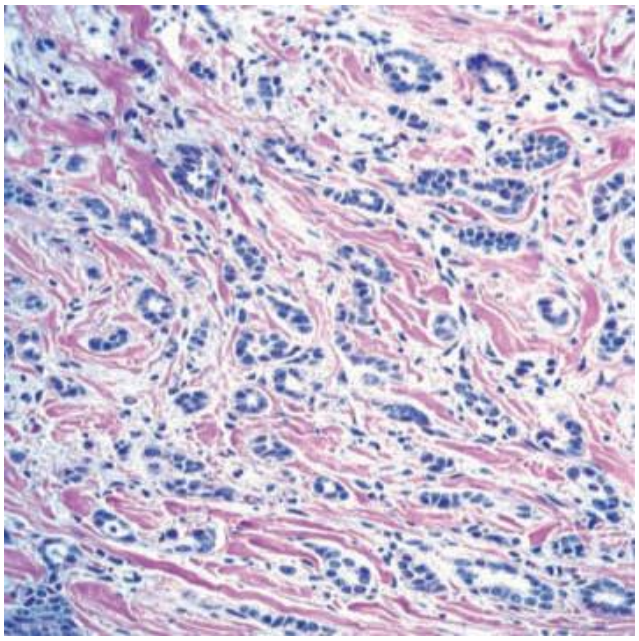

Figure 5 –Microscopy showing tumor cells arranged in tubules and in cords .WHO breast [22]

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.[30,31]

LOBULAR CARCINOMA – This entity comprises of 5-15% of all breast cancers with increasing trend in recent years.[32,33] They are usually present with focal in situ lobular carcinomas and gross appearance is often irregular with poorly defined margins. The classic pattern of ILC is characterized by a proliferation of small cells, which lack cohesion and appear

individually dispersed through a fibrous connective tissue or arranged in singlefile linear cords that invade the stroma called as India File pattern.[34,35]

TUBULAR CARCINOMA – Usually comprises 2% of all 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**.**[36]

The most consistent microscopic features is the open Lumina lined by single layer of epithelial cells.[37]

CRIBRIFORM CARCINOMA – The mean tumor size is 3.1cms. It is the form of well differentiated invasive duct carcinoma with excellent prognosis and shows cribriform growth pattern and are 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.[38,39]

MEDULLARY CARCINOMA – Usually account form < 1% of all breast malignancies. A very high lymphoplasmacytic reaction in these tumours may mimic lymphoepithelial malignancies occurring in other sites.[40]

Few distinct histomorphological features are essential for diagnosis of medullary carcinoma.[41,42] They are-

1. Symmetrical growth pattern (>75%)
2. Absence of glandular structure.
3. Diffuse lymphoplasmacytic infiltration.
4. Nuclear pleomorphism.
5. Complete circumscription

MUCINOUS CARCINOMA – They are the slow growing tumors consisting of tumor cells surrounded by pools of mucin. Their size may vary from 1 cm to 20 cm, usually circumscribed bossylated with glistening gelatinous appearance. Rarely cerebral infarction may occurs due to mucin embolism and cause death. They carry a fairly good prognosis.[43,44]

NEUROENDOCRINE TUMOURS – Represented 2-5% of malignant breast lesion usually present in 6^{th} or 7^{th} decade. They are a group of neoplasms exhibiting features of neuroendocrine tumour of lung and gastrointestinal tract. There may be areas of de-differentiation in infiltratory ductal carcinoma but should show immune reactivity to neuroendocrine markers in >50% of cell population.[45]

INVASIVE PAPILLARY CARCINOMA – constitute 1-2% of breast cancers 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 papillae with cells having amphophilic cytoplasm and can also exhibit apical snouting.[22]

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.[46,47]

SECRETORY CARCINOMA – This is usually a low grade carcinoma that can occur in juvenile and in adults.These account for <0.15%. It is a relatively rare tumor with tumor cells having intra and extracellular secretory material.[48,49]

INFLAMMATORY CARCINOMA – Incidence varies widely (1-10%). They are characterised by dermal lymphovascular infiltration and has been categorized under T4d due to its poor prognosis.[50]

TNM CLASSIFICATION OF BREAST[22]

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[22]

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 GENETICS AND MOLECULAR CLASSIFICATION OF BREAST CARCINOMA[5]

MOLECULAR GENETICS

The development of invasive breast carcinoma involves multiple genetic alterations, similar to other carcinomas of various anatomic sites. The common molecular alterations include

1. Growth receptor overexpression (such as HER2/neu amplification, EGFR overexpression, FGFR1 or FGFR2 overexpression)
2. Growth factor overexpression (FGF1/FGF4)
3. Intracellular signaling molecule alterations (HRAS mutation)
4. Cell cycle regulator alterations (TP53 mutation, *RB* inactivation, CCND1 gene amplification).
5. Adhesion molecule alterations (reduced expression of E-cadherin, reduced expression of P-cadherin, overexpression of cathepsin D).

In addition, some types of breast carcinoma, such as secretory carcinoma, lobular carcinoma, and adenoid cystic carcinoma, exhibit distinctive genetic changes, as described in the respective sections

MOLECULAR CLASSIFICATION[5]

Table 6 – Molecular classification

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	~15% of invasive breast cancers ER/PR negative HER2 positive (though not all HER2 enriched by molecular subtype are HER2+ by	~15% of invasive breast cancers Most ER/PR and HER2 negative ("triple negative") High proliferation rate TP53mutation

		high proliferation rate (Ki-67 high) Luminal B tends to be higher histologic grade than luminal A	clinical definition) High proliferation rate TP53 mutation common More likely to be high grade and nodepositive	common; BRCA1 dysfunction (germline, sporadic) 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[52 &53]

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: Axillary 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 axillary 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 variants of invasive ductal carcinoma with a more favorable 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 a risk factor 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. Her 2 neu over expression is associated with poor survival. Some of the markers which are still under research and can double up as prognostic marker. One such marker is the stem cell marker.

STEM CELL MARKERS

The term was first coined by eminent German biologist Ernst Haeckel. The stem cells are defined as having the capacity to both self-renew and give rise to differentiated cells .[54 & 55] Stem cells provide an opportunity to investigate the mechanisms that regulate embryonic development, cellular differentiation and organ maintenance. Their proliferation and differentiation capacities, stem cells have great potential for the development of novel cell-based therapies .[56 & 57] In addition, recent studies suggest that dysregulation of stem cell properties may be the cause of certain types of cancer.[58 & 59]

The concept of stem cells has extended from embryonic stem cells (ESCs) and adult stem cells to cancer stem cells (CSCs) and induced pluripotent stem (IPS) cells. By self-renewal, more stem cells are generated which maintain an undifferentiated status. Through differentiation, stem cells give rise to a mature cell type. Embryonic stem cells are capable of

differentiating into all tissues during embryonic development. Adult stem cells play important roles in replenishing and repairing adult tissues.

The first modern evidence for a role of stem cells in cancer came in 1994 with a study of Human acute myeloid Leukaemia, in which an AML-initiating cell population was identified from AML patients by transplantation into severe combined immune-deficient (SCID) mice.[59] The leukemia-initiating cells were enriched on the basis of cell surface marker expression (CD34+/CD38−). In 2003, human Cancer Stem Cells were identified in solid tumors, including breast and brain cancer.[60 &61] The subsequent reports identified Cancer Stem Cells in a variety of tumors, including colon, pancreas,lung, prostate, melanoma and glioblastoma. Notably, as few as 100 cancer stem cells wereable to form tumors in non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice. [60]

Expression of cell surface markers such as CD44, CD24, CD29, CD90, CD133, epithelial specific antigen (ESA) and aldehyde dehydrogenase1 (ALDH1) have been used to isolate and enrich CSCs from different tumors. [60 & 61] The expression of CSC surface markers is tissue type-specific, even tumour subtype-specific. For example, CD44+CD24−/low Lineage and ALDH+ were characterized for breast CSCs; CD133+ for colon, breast , brain and lung; CD34+CD8− for leukaemia, CD44+ for head and neck, CD90+ for liver, CD44+/CD24+/ESA+ for pancreas Cancer Stem Cells .[62]

In the last two decades, breast cancer research has majorly focused on the identification, isolation and characterization of breast cancer stem cells (BCSCs). In order to do so, some genes with stem cell properties were studied and their corresponding proteins were subsequently validated as markers of breast cancer stem cells.[63 & 64] As a consequence, many studies have been published describing the impact of BCSCs identified by these established markers, such as

hyaluronan receptor (CD44), signal transducer CD24, CD 133 and aldehyde dehydrogenase- 1 (ALDH1), as tumor-initiating cells in breast cancer progression with high propensity to metastasize and to be resistant to therapeutic treatments .[65] Due to increasing evidence for such ability many researches have been done to demonstrate, which altered genes or dysregulated gene signaling pathways potentially contribute for the tumorigenic potential of BCSCs. In fact, NOTCH, WNT/β-catenin, or Hedgehog signaling pathways were shown to be deregulated in subpopulations of these cells .[65] With the evidence and forthcoming regarding the effects of the stroma and the microenvironment in breast tumor progression, several genes have also been reported to be associated with BCSCs. The phenomenon of epithelial–mesenchymal transition (EMT) and mesenchymal–epithelial transition in breast cancer cells during tumor progression is an important discovery.[65]

With this knowledge, targeting Breast Cancer Stem Cells for breast cancer treatment was researched and some important inhibitors targeting subpopulations of BCSCs or gene signaling pathways that regulate these subpopulations are reported to be strongly effective. Due to the heterogeneity of Breast carcinoma different BCSC markers and different combinations of these markers are seen to be associated with aggressive forms of breast carcinoma .[65]

As a consequence, different BCSC phenotypes have been described and characterized. Beyond the tenets of the CSC model, it is important to define which BCSC phenotypes have high tumorigenic potential and also great ability to resist therapeutic agents. Moreover, it is also crucial to determine which oncogenes or tumor-suppressor genes, other than those already described, are consistently mutated within these phenotypes being able to drive tumorigenesis. In Invasive Breast Cancer (IBC), several markers have been

immunohistochemically characterized showing that the prevalence of stem cell-like and more differentiated markers varies according to tumor subtype and histological stage. For this reason, a concise review is presented here regarding the implications of the most studied markers of BCSCs and phenotypes in breast cancer progression and treatment, as well as a description of promising inhibitors able to target these cells.

CD133 (Prominin-1)

CD133 or Prominin- 1 has been recently included in CSC research. It is named as prominin-1 for its prominent location on the protrusion of cell membranes and was the first gene identified in those for a class of novel pentaspan transmembrane glycoproteins. It was initially considered to be a marker of hematopoietic stem cells and CD133mRNA transcript is also found in normal non lymphoid hematopoietic tissue and has been shown to play a role in stem cell migration and asymmetric division.[65]

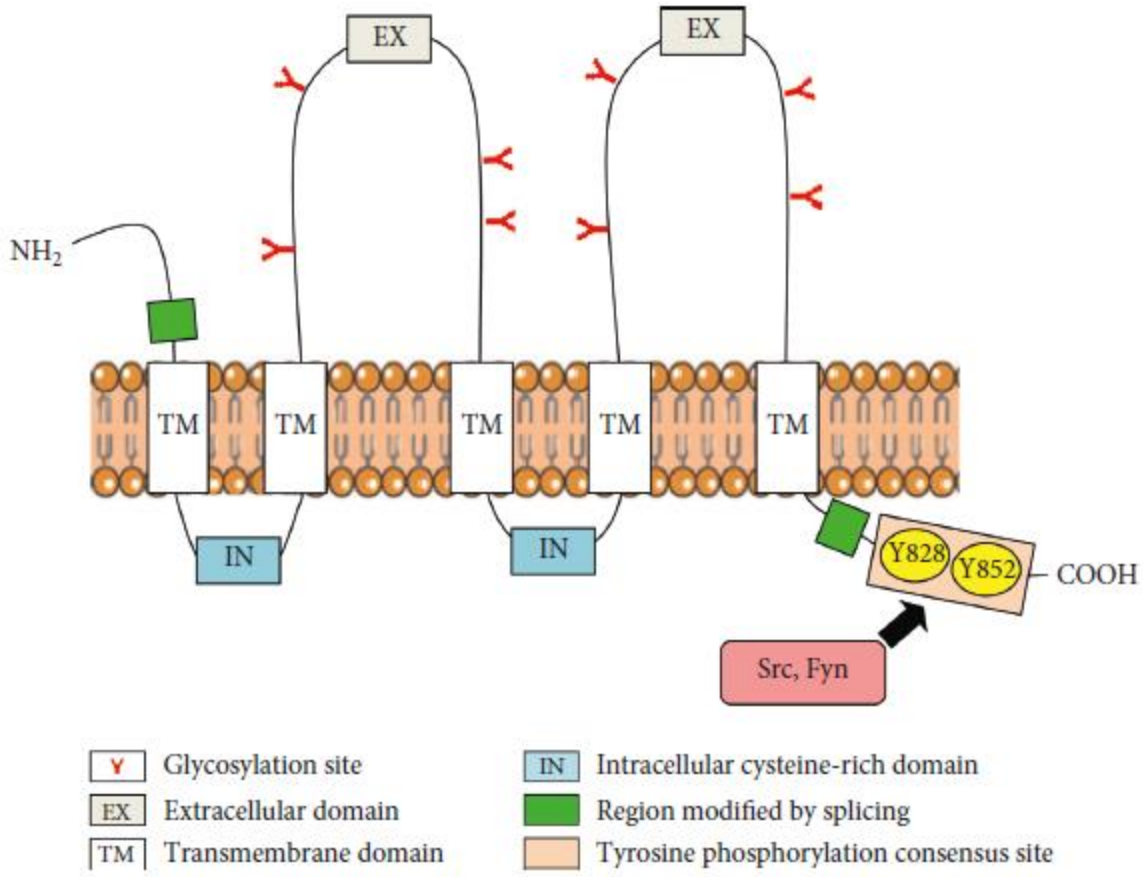

Figure 6 : Structure and regulation of CD133 (a) CD133 protein structure in which the C-terminal tyrosine-phosphorylation consensus site, which comprises 5 tyrosine residues including Y828 and Y852, and the splice variants regions are indicated.[65]

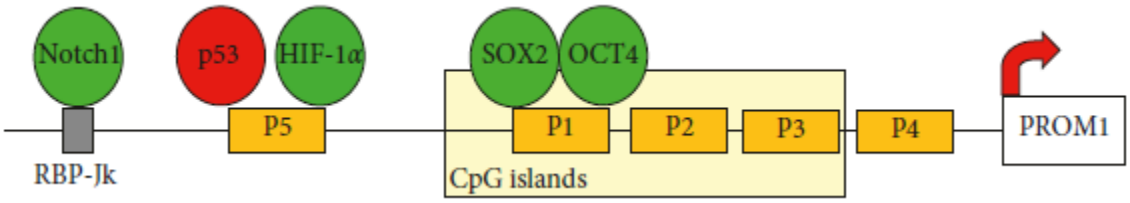

Figure 7 - Schematic representation of the 5untranslated region of the CD133 gene. Transcription factors that positively (green circles) or negatively (red circles) regulate CD133 expression by direct binding to the different promoters are reported. Direct binding of Notch1 to the site for RBP-Jk located upstream P1–P5 promoters is also indicated.[65]

CD133 is reported to be overexpressed in several tumors including cancers of the brain, colon, liver, pancreas, kidney, lung, endometrium, ovary and bone. Liu et al [66] demonstrated expression CD 133 in Invasive breast carcinomas, where they assumed that its expression could be of help in a accurate prediction of breast cancer aggressiveness and determination of the most suitable treatment. Actually, in BRCA1-associated breast cancer cell lines, CD133+ sorted cells were shown to have CSC properties, including a greater colony-forming efficiency, higher proliferative output and greater capability to form tumors in NOD/SCID mice.[67]

CD133 is also proved to be suitable in the identification of CSCs in triple negative breast cancers through several in vitro[68,69] and in vivostudies.[70] In addition, the recent use of CD133 to detect circulating tumor cells in patients with triple-negative breast cancer has increased the attention on this marker, emphasizing its role in prognosis in this breast cancer subtype.[71,72] Expression of CD133 was also recently reported in 22 out of 25 cases of inflammatory breast cancer.[73] These results indicate the need for more advanced research to understand the role of CD133 in Breast Cancer Stem Cells. Expression of SC-associated genes, such as NOTCH1, ALDH1, fibroblast growth factor receptor 1 and SRY-box1, and was shown to be increased not only in CD44+/CD24−/low but also in CD133+ breast cancer cells. Xenograft-initiating breast cancer cells enriched in CD44+/CD49fhigh/ CD133/2high cells were also shown to have elevated expression of Nanoghomeobox (NANOG), SRY-box 2 and polycomb complex protein BMI-1.[74] Further extensive CD133 studies in breast cancer needs to be done to confirm CD133+ breast cancer cells as tumor initiating cells. Due to the increasing importance of CD133 expression in breast cancer progression, attempts have been made to correlate its expression with tumor relapse and resistance to chemotherapeutic agents. In fact, CD133 expression is reported to correlate with tumor recurrence in patients with breast cancer .[75] In

drug-sensitive MCF-7 cells, only a small fraction of cells was found to be CD133-positive .[76] In another study, polymeric nanoparticles loaded with paclitaxel and surface functionalized with antibody to CD133 demonstrated efficient elimination of tumor-initiating cells in vitro and significant inhibition of tumor regrowth in vivo.[77] With such results, CD133 is regarded as a potential target for anticancer therapeutics, being possible to reduce tumor recurrence in breast cancer through the elimination of CD133+ cells. Thus, additional studies investigating specific drugs that efficiently target this protein are required.

MATERIALS & METHODS

MATERIALS AND METHODS

Duration of Study: Two Years (June 2017 – November 2019)

Place of Study: Department of Pathology, Sri Devaraj Urs Medical College, Kolar

Study design: Laboratory based exploratory study.

SAMPLE SIZE:

- Sample size was estimated based on the expression of CD133 in cancer cells. It was reported to be 53.3% in a study done by Sahara et al[9] in 2015 with 80% level of confidence with absolute error of 10%, the estimated sample size was 41. Sample size increased to 57 while doing Immunohistochemical stain.

- Equation, sample size = $\frac{Zx^2 \times p \times Q}{d^2}$

  Zx – Standard normal variant @99% = 2.57

  P - 53.3%

  Q – 46.7

  d – Absolute error of 10%.

COLLECTION OF DATA

Fifty seven patients who underwent Modified radical mastectomy for treatment of invasive ductal carcinoma at R L Jalappa Hospital and Research Centre from 2015-2018 were

included in this study. Paraffin blocks and slides were retrieved from the archives of the department of Pathology. Clinical information, tumor size and axillary lymph node status were obtained from medical records and the pathology reports. All the Hematoxylin and eosin stained slides were screened for histological type, tumour grade and nodal metastasis.

INCLUSION CRITERIA:

- Women with Infiltrating Ductal carcinoma (NOS) type who underwent Modified radical mastectomy.

EXCLUSION CRITERIA:

- Women subjected to neoadjuvant radiotherapy / chemotherapy before Modified radical mastectomy.
- Recurrent tumors
- Women who received chemotherapy for other cancer over past 5 years.

STATISTICAL ANALYSIS:

Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. **Chi-square test** was used as test of significance for qualitative data. Continuous data was represented as mean and standard deviation. **Independent t test** was used as test of significance to identify the mean difference between two quantitative variables.

Graphical representation of data: MS Excel and MS word was used to obtain various types of graphs such as bar diagram, Pie diagram.

p value (Probability that the result is true) of <0.05 was considered as statistically significant after assuming all the rules of statistical tests.

Statistical software: MS Excel**,** SPSS version 22 **(**IBM SPSS Statistics, Somers NY, USA) was used to analyze data.

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 .

Table 7 – Details of IHC marker

Antigen	Clone	Species	Producer	Dilution	Control	Stain
CD 133	EPR16508	Rabbit	abcam	1 : 500	Kidney	Cytoplasm and Cytoplasmic membrane

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 15 minutes in TRIS EDTA buffer of pH-9.0 for 3 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. TBS buffer for 5 minutes washing for 3 times.
9. Drain slides for a few seconds (do not rinse) and wipe around the sections with tissue paper.
10. Cover sections with primary antibody diluted in TBS (1: 500) with 1% BSA
11. Then Incubate overnight at 4^{0}C.
12. Rinse with TBS (Tris buffer solution pH- 7.6) for 5 min x 3 times wash with gentle agitation.
13. Apply enzyme-conjugated secondary antibody to the slide, diluted in TBS with 1% BSA, and incubate for 1 h at room temperature.
14. Develop with chromogen for 10 min at room temperature.
15. Rinse in running tap water for 5 min.
16. Counterstain with Hematoxylin
17. Dehydrate , clear and mount
18. 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 CD133 and scoring was done

GRADE OF THE TUMOR [51]

Table 8 - NSBR 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 CD 133.[78]

Table9 - The intensity of CD 133positivity

INTENSITY OF CD133	SCORE
Negative	**0**
Weak	**1**
Moderate	**2**
Strong	**3**

Table 10 - The extent of positivity according to percentage %

PERCENTAGE OF CELLS SHOWING POSITIVITY	SCORE
<10%	**1**
11% - 50%	**2**
51% - 75%	**3**
>75%	**4**

FINAL SCORE = Intensity of positivity x extent of positive score = 0 to 12%

$\geq$ 3 = considered positive.

NOTTINGHAM PROGNOSTIC INDEX.[79]

Table 11 – Nottingham Prognostic Index

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

NPI = (0.2 X S) + N +G

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

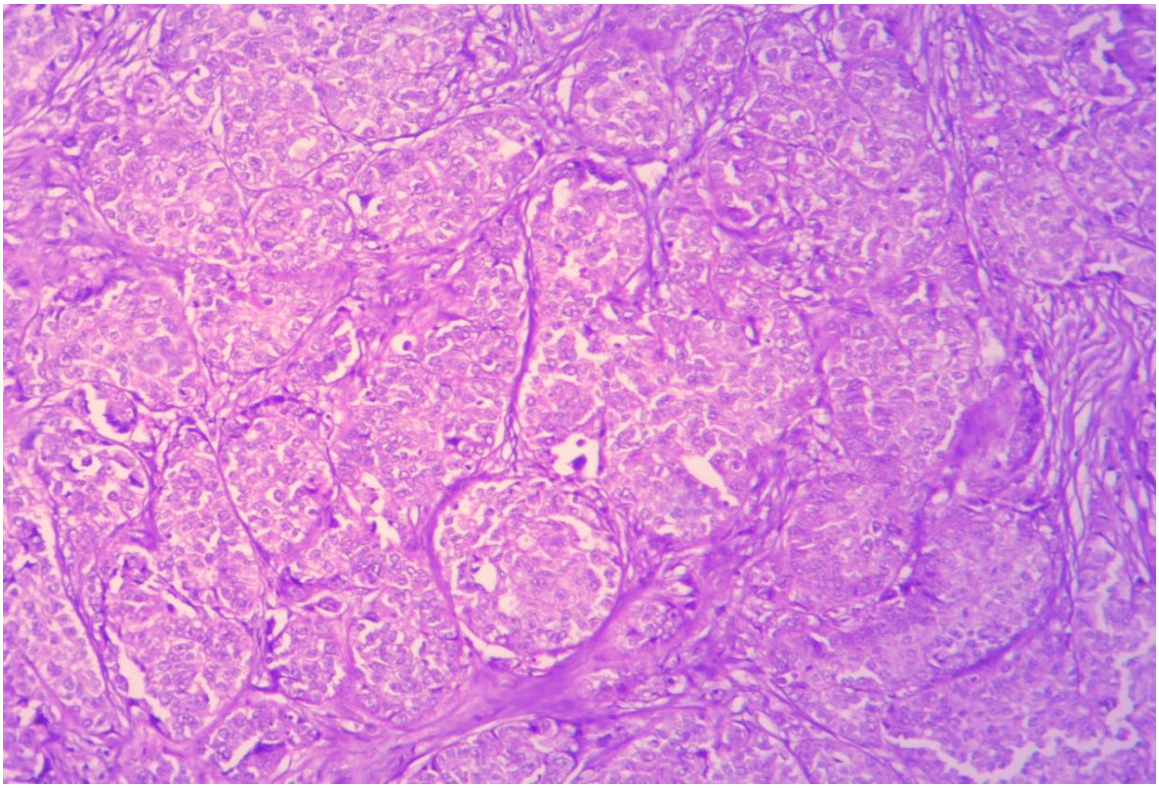

Figure 8 –Invasive ductal carcinoma Breast (Not otherwise specified) – H & E – 100x

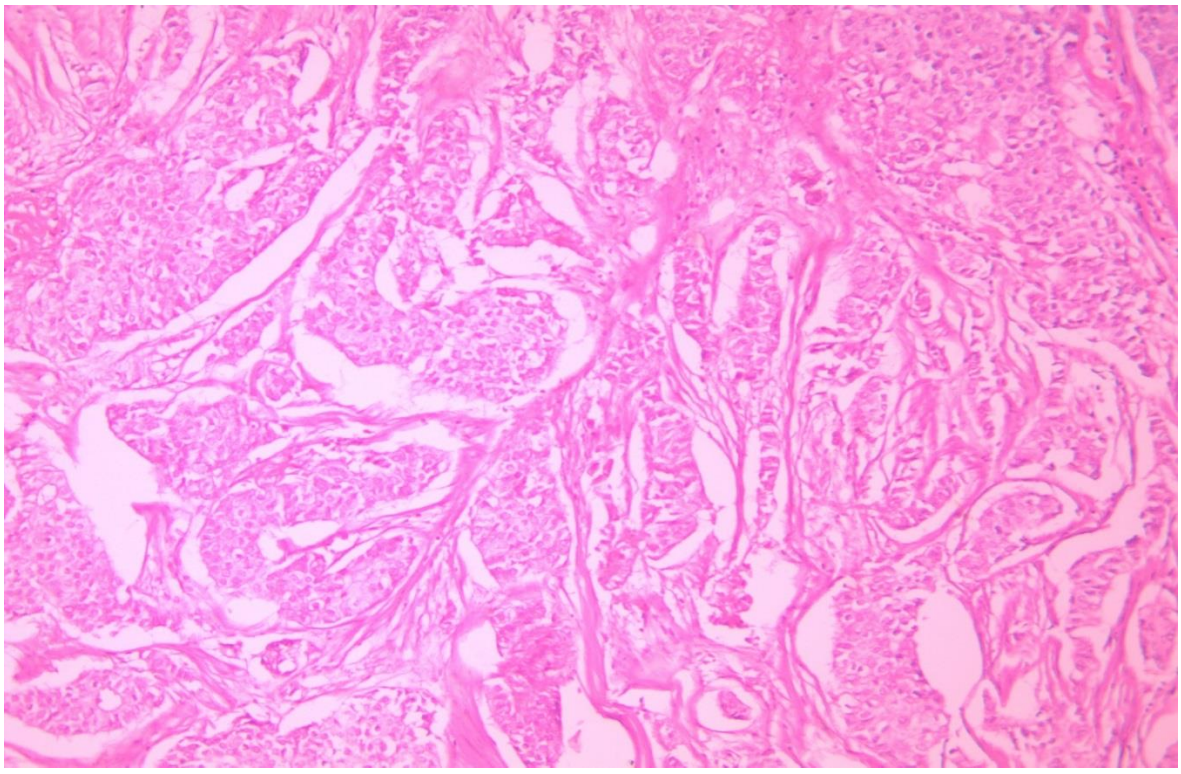

Figure 9 – Invasive ductal carcinoma Breast – Grade 1 (H & E) – 100x

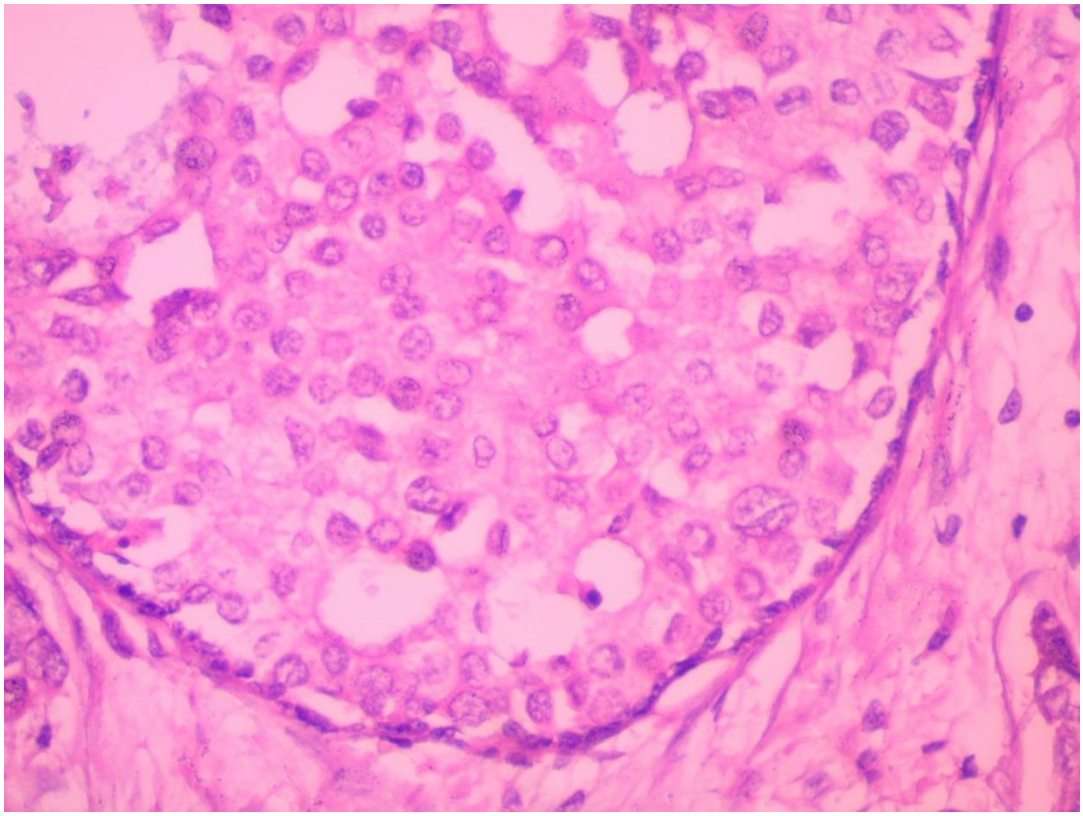

Figure 10 – Invasive ductal carcinoma Breast – Grade 2 (H & E) – 100x

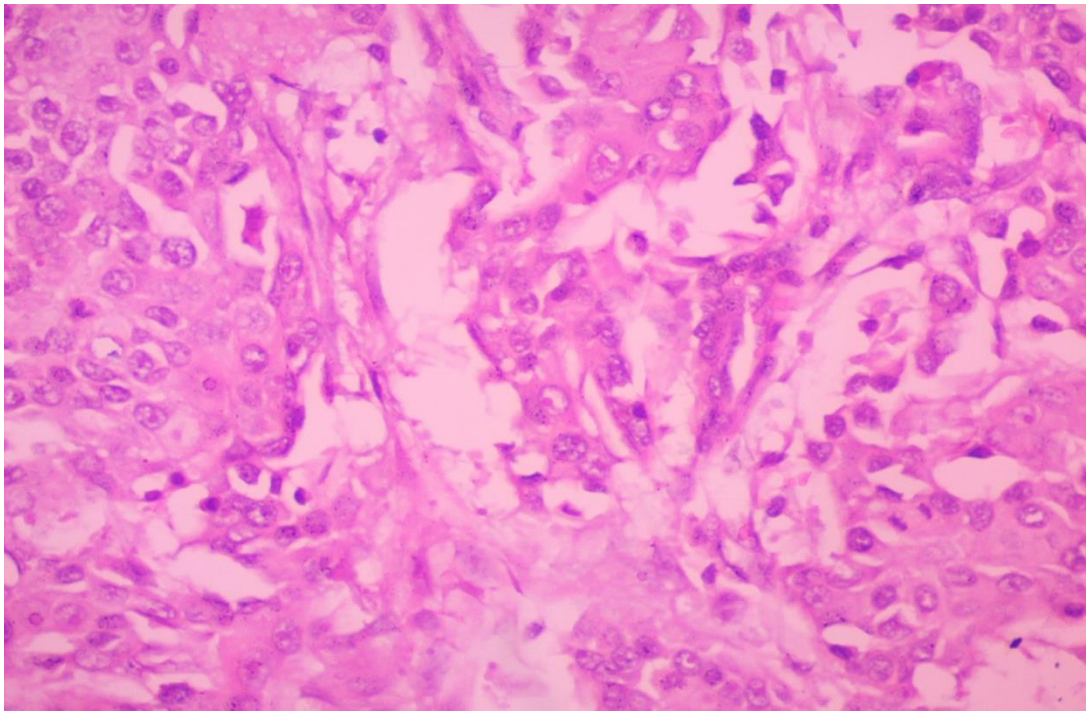

Figure 11 – Invasive ductal carcinoma Grade 3 – 100x

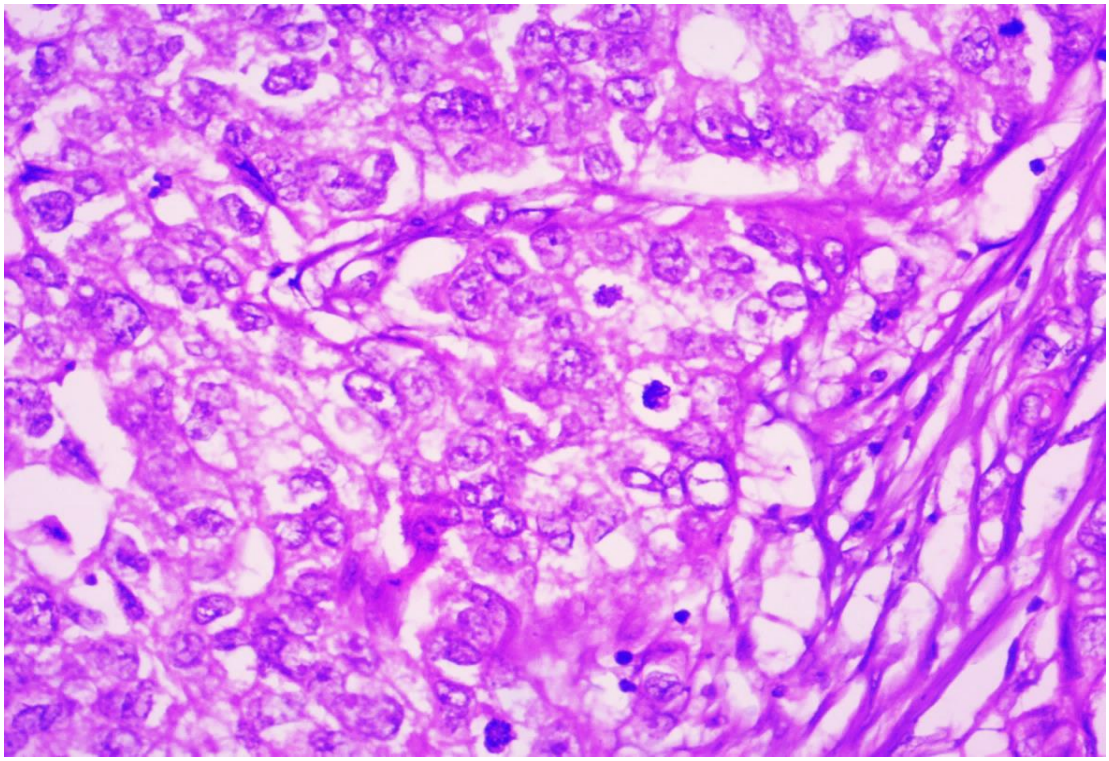

Figure 12- Invasive Ductal carcinoma Grade 3 , H & E – 400x

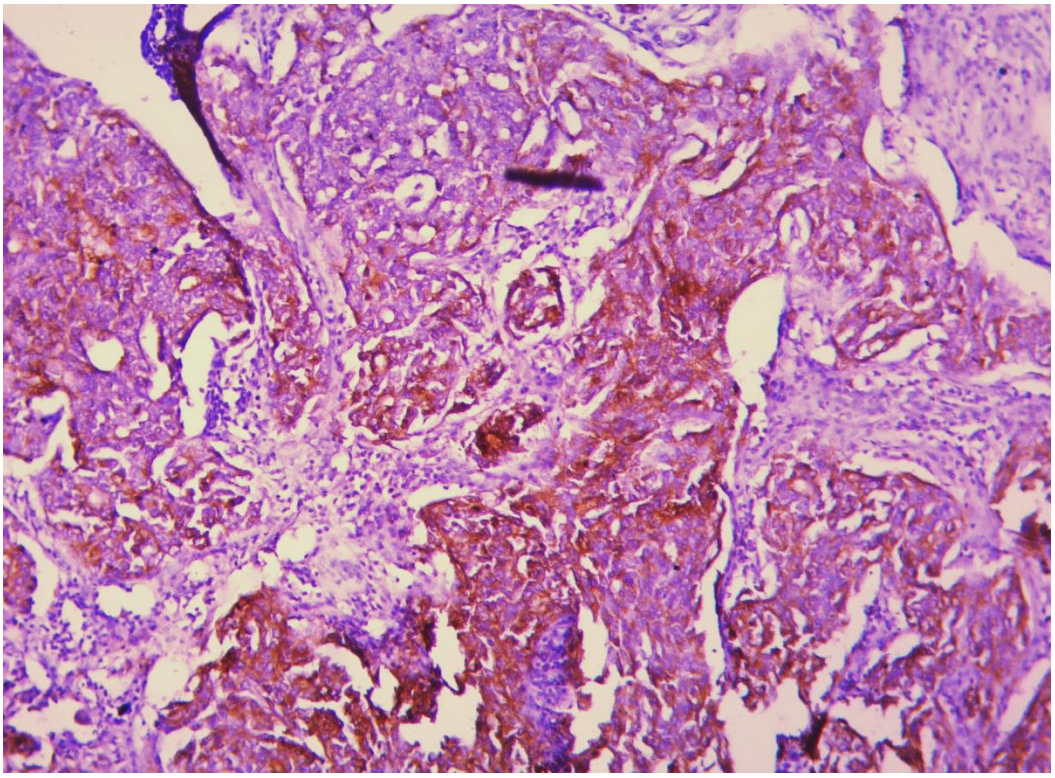

Figure 13-IDC- CD 133 IHC – 100x (Extent of positivity >75%- Score 4)

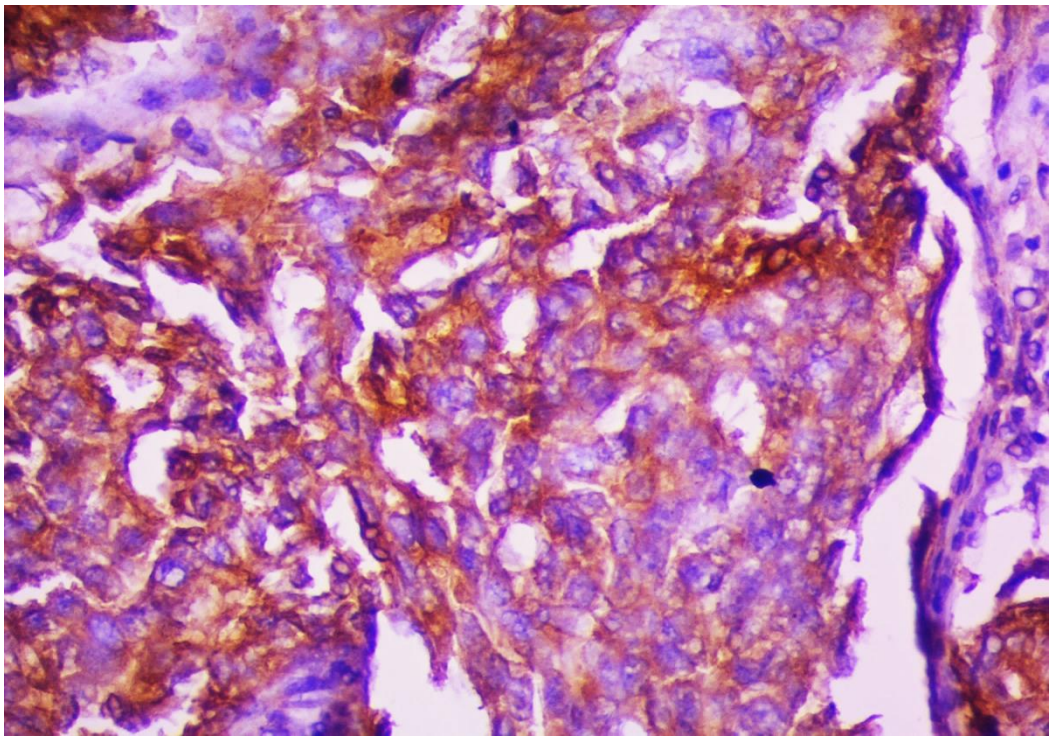

Figure 14- IDC- CD 133 IHC – 400x (Extent of positivity >75%- Score 4)

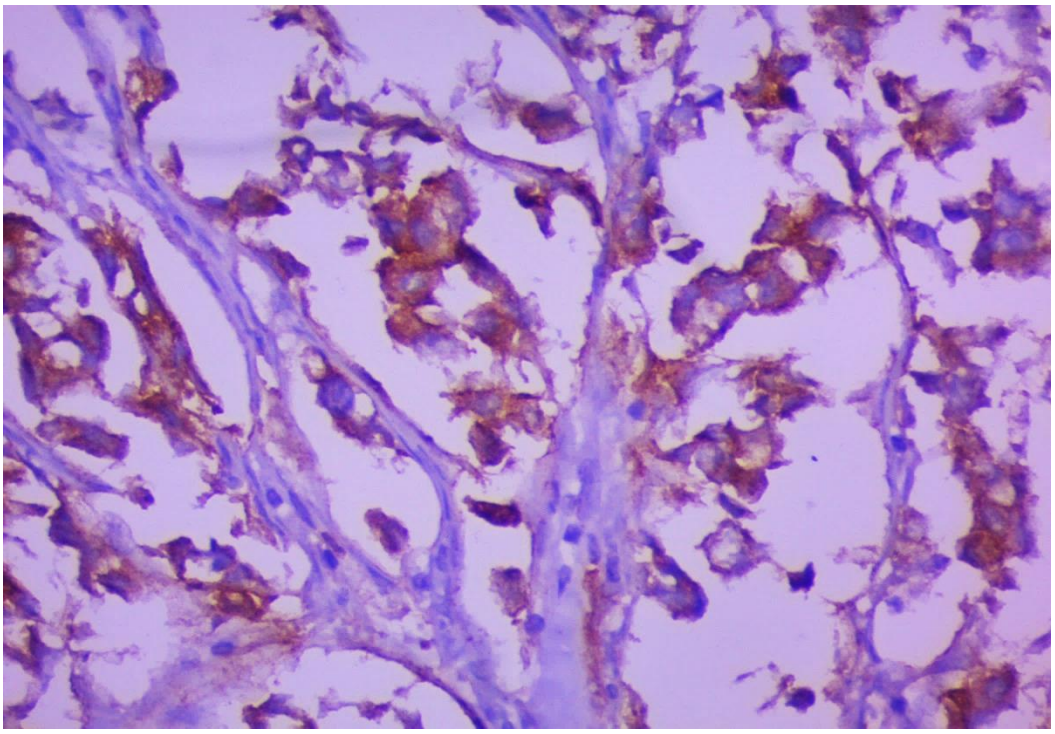

Figure 15- IDC-CD 133 IHC – 400x (Intensity of positivity - Score 3)

RESULTS

RESULTS

The study was done in the time period of November 2017 to October 2019. Total 57 cases were collected and stained with CD133 and scoring was done.

AGE DISTRIBUTION

Out of total 57 cases, the youngest age was 28 years and oldest age was 92 years. The average age of presentation is 55 years. Majority of the patient belonged to 41- 60 years which constituted 31 cases (54.38%) , followed by 21-40 years constituting 15 cases (26.31%), 09 cases (15.78%) belonged to 61 -80 years age group , 02 cases (3.53%) belonged to 81 -100 years age group .

Table 12 – Age distribution of subjects

Age (in years)	No. of subjects	Percentage (%)
21-40	15	26.31
41- 60	31	54.38
61-80	09	15.78
81-100	02	3.53

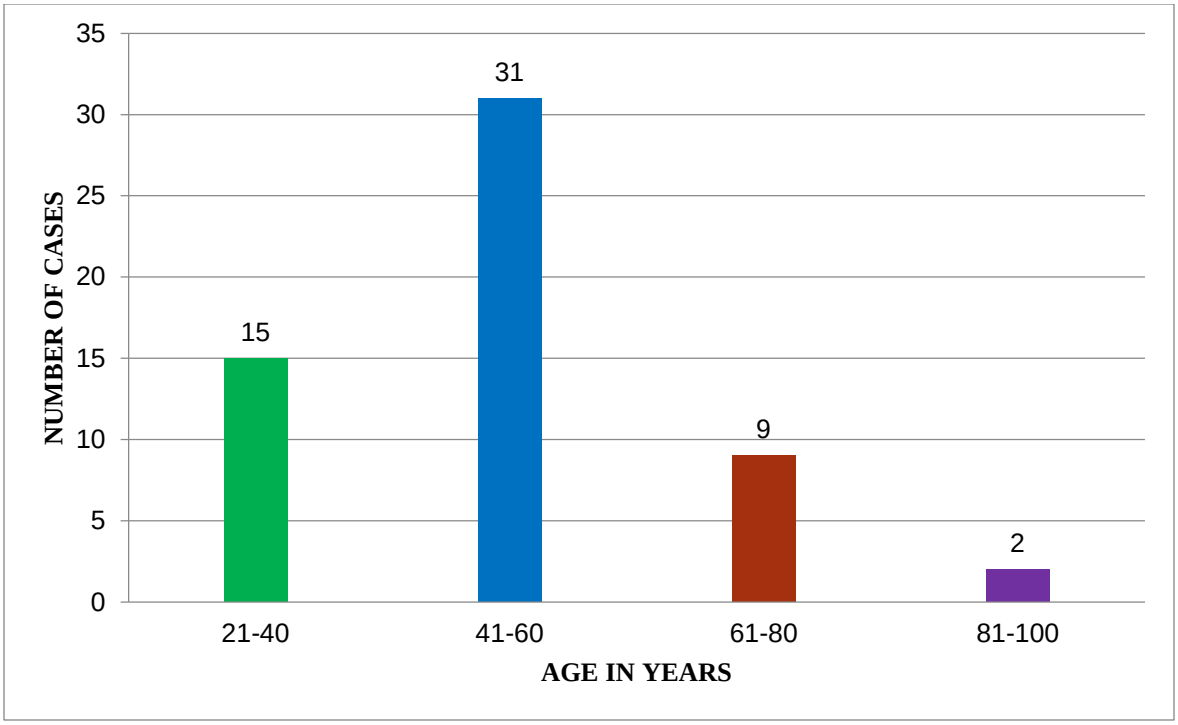

Chart 1 - Age distribution of subjects

DISTRIBUTION OF THE CASES INTO GRADE OF TUMOR

Table 13 – Distribution of subjects into Tumor grade

GRADE	CASES	PERCENTAGE (%)
I	33	57.9
II	17	29.82
III	7	12.28

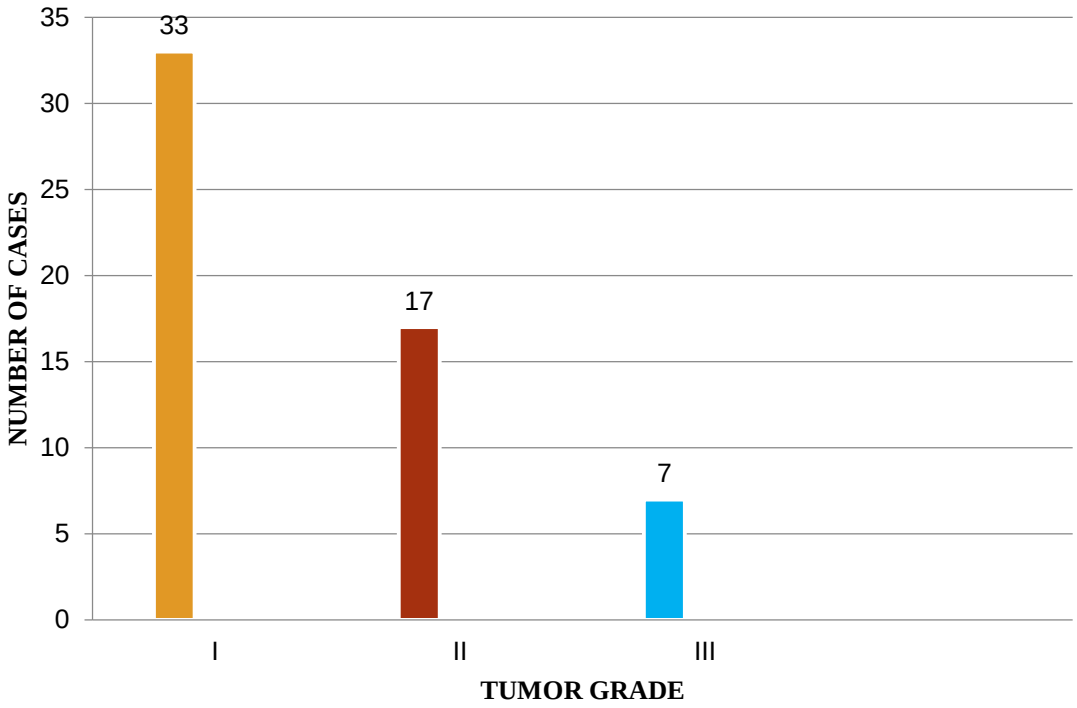

Chart 2 - Distribution of subjects into Tumor grade

TUMOR SIZE

Table 14 – Distribution of subjects based on Tumor size

Tumor size	cases	Percentage
T1 (<2CMS)	8	14.04
T2 (2- 5 CMS)	23	40.35
T 3 (>5CMS)	19	33.33
T4 (CHEST WALL)	7	12.28

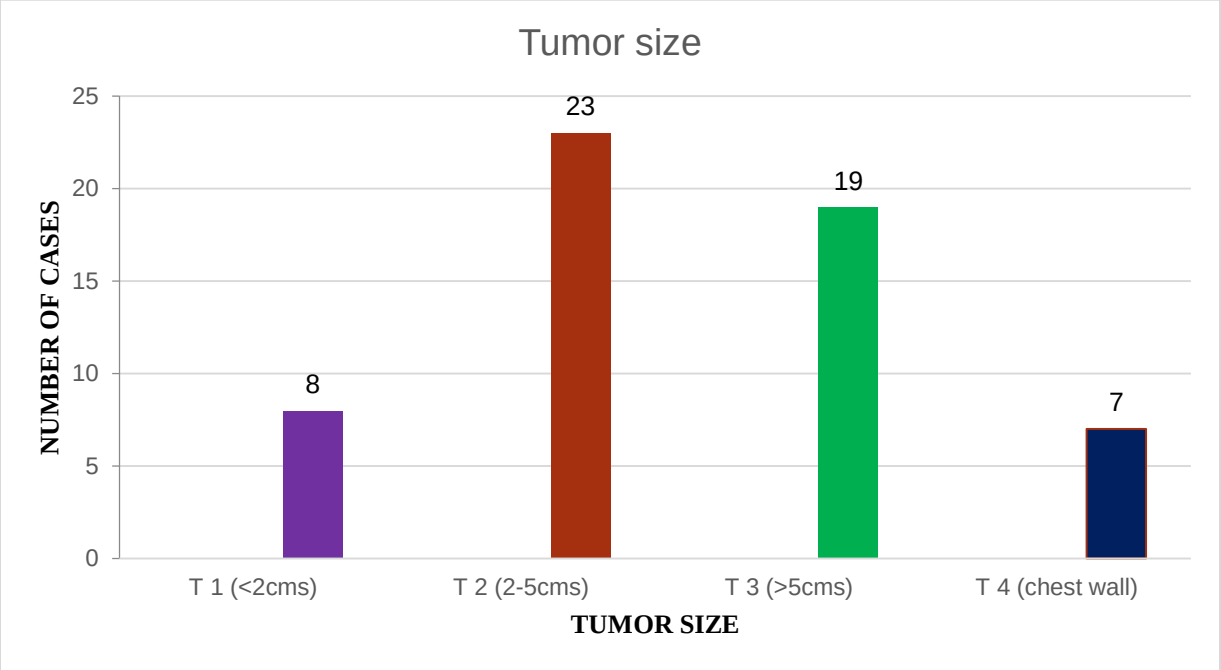

Chart 3 -Distribution of subjects based on Tumor size

LYMPH NODE CATEGORY

Table 15 – Distribution of subjects based on Lymph node stage

LYMPH NODE STAGE	CASES	PERCENTAGE
Stage N0 (0)	13	22.80%
Stage N1 (1-3)	18	31.57%
Stage N2 (4-9)	7	12.28%
Stage N3 (>9)	19	33.33%

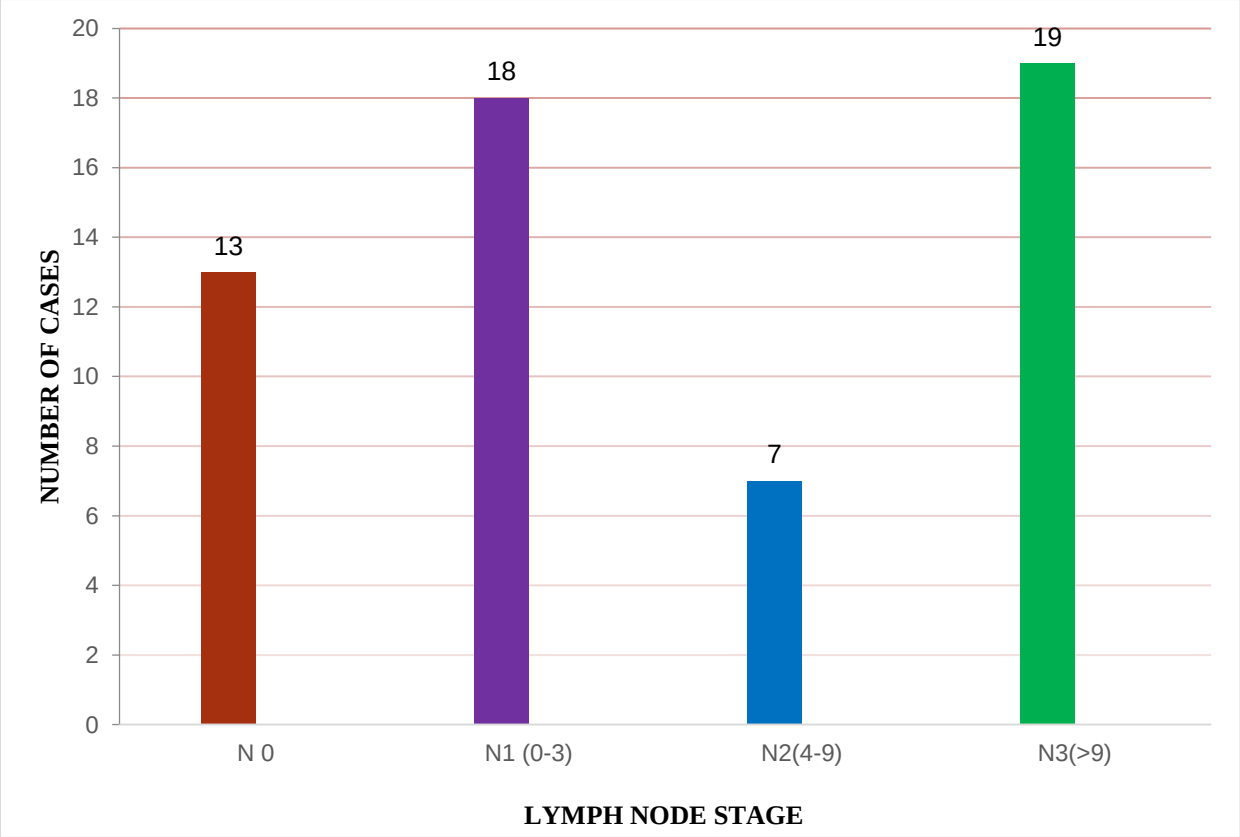

Chart 4- Distribution of subjects based on Lymph node stage

NOTTINGHAM PROGNOSTIC INDEX

Table 16 – Distribution of subjects based on Nottingham prognostic index

NPI CATEGORY	CASES	PERCENTAGE
I	1	1.75%
II	16	28.07%
III	28	49.12%
IV	12	21.05%

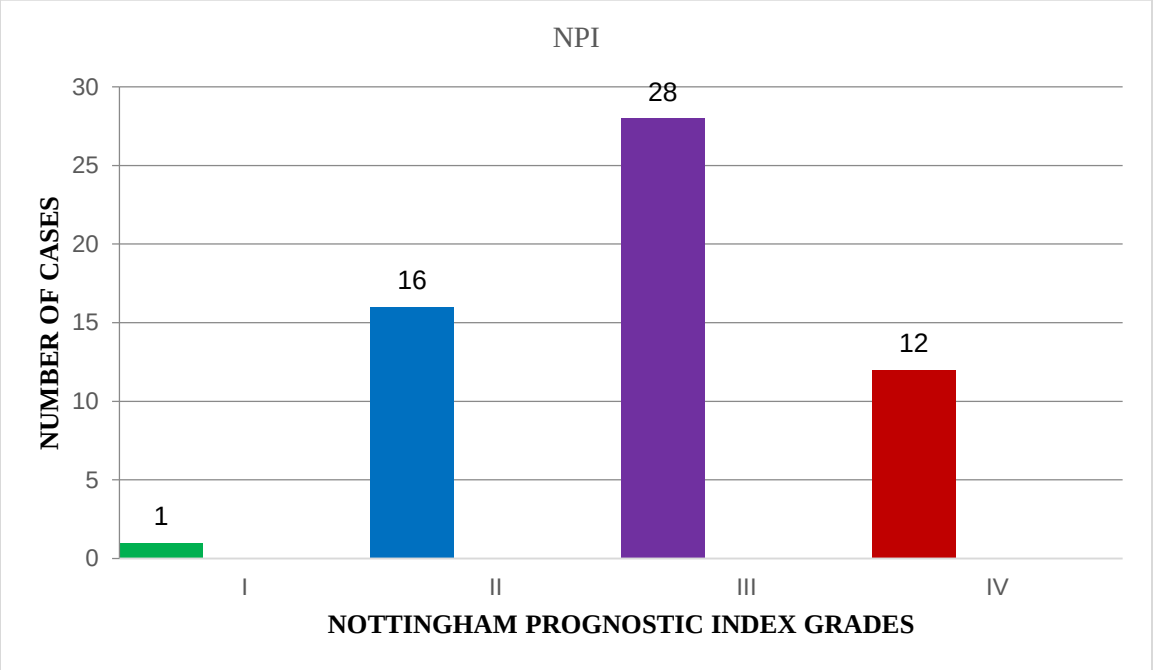

Chart 5 - Distribution of subjects based on Nottingham prognostic index

CORRELATION CD 133 EXPRESSION WITH AGE

Table 17 – Correlation of Age and CD 133 expression of cases

Age group	Number (percent)	CD 133 status		Total
		Negative	Positive	
21-40	Count	6	9	15
	% within ages	40.0%	60.0%	100.0%
41-60	Count	9	22	31
	% within ages	29.03%	70.97%	100.0%
61-80	Count	3	6	9
	% within ages	33.34%	66.66%	100.0%
81-100	Count	1	1	2
	% within ages	50%	50%	100.0%

Table 18 – Correlation of Age and CD 133 correlation

Chi-Square Tests					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.073[a]	1	.787		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.073	1	.787		
Fisher's Exact Test				1.000	.505
Linear-by-Linear Association	.072	1	.789		
N of Valid Cases	57				

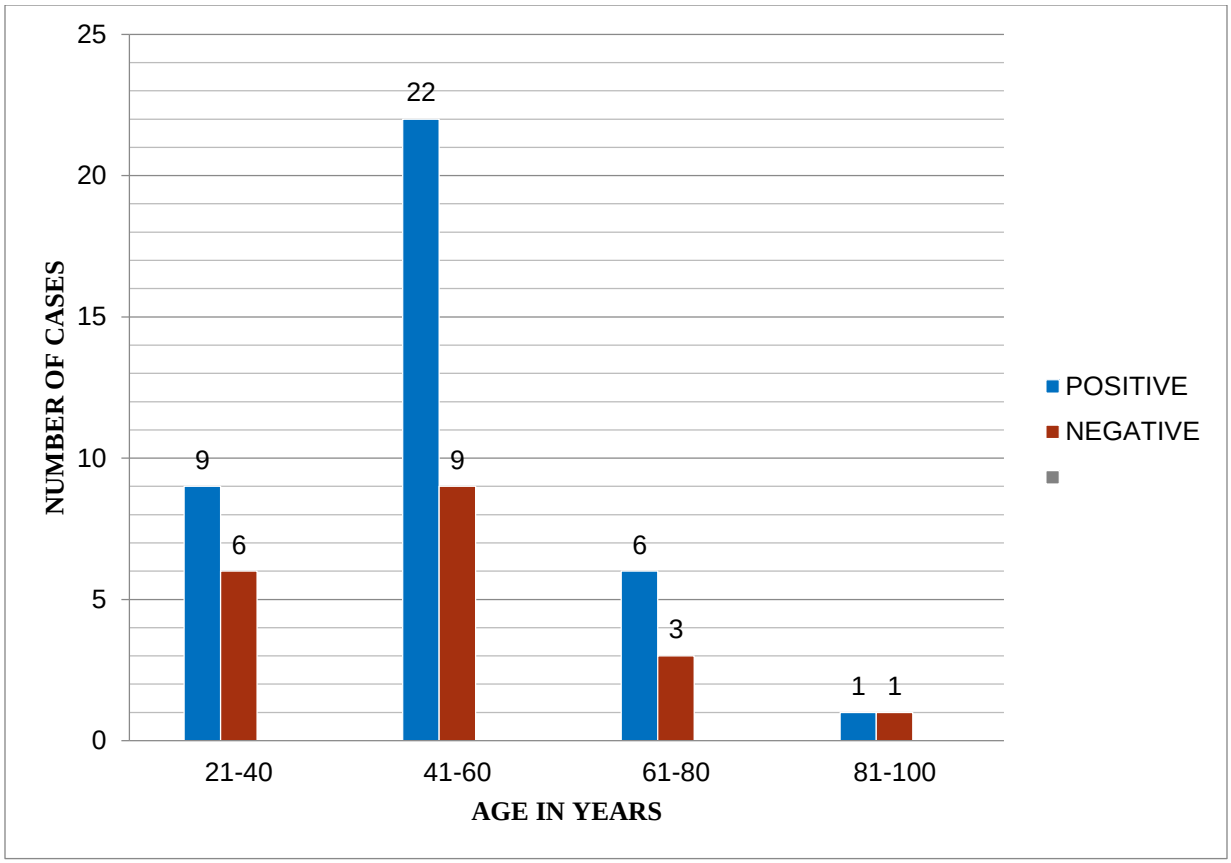

Chart 6 - Correlation of Age and CD 133 expression of cases

FREQUENCY OF CD133 EXPRESSION IN INFILTRATING DUCTAL CARCINOMA BREAST (NOS)

Table 19 - Frequency of expression of CD 133

	Number of cases	Percentage
CD 133 positive cases	**44**	**77.18%**
CD 133 negative cases	**13**	**32.82%**
Total	**57**	**100%**

Out of 57 cases collected 44 cases showed CD 133 total score of $\geq$ 3 which was considered positive. The frequency of CD133 expression in Infiltrating Ductal of breast is 77.18%.

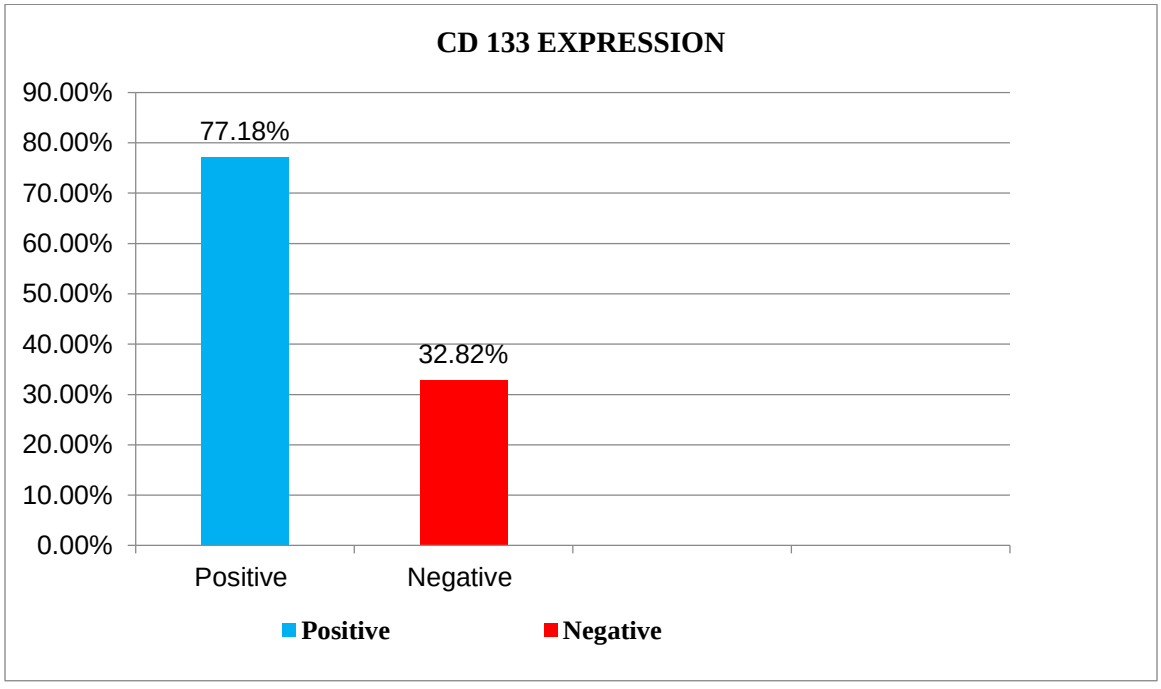

Chart 7 - Frequency of expression of CD 133

CORRELATION BETWEEN CD 133 SCORE AND TUMOR GRADE

Table 20 - Chi-Square Tests of CD 133 score and Tumor grade

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.608[a]	2	0.447
Likelihood Ratio	1.745	2	0.418
Linear-by-Linear Association	1.564	1	0.211
N of Valid Cases	57		

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is 2.21.

Table 21 - Correlation between CD 133 score and tumor grade

Tumor grade	Positive	Negative	Total	P Value
1	20	12	32	0.447
2	13	5	18	
3	6	1	7	
Total	39	18	57	

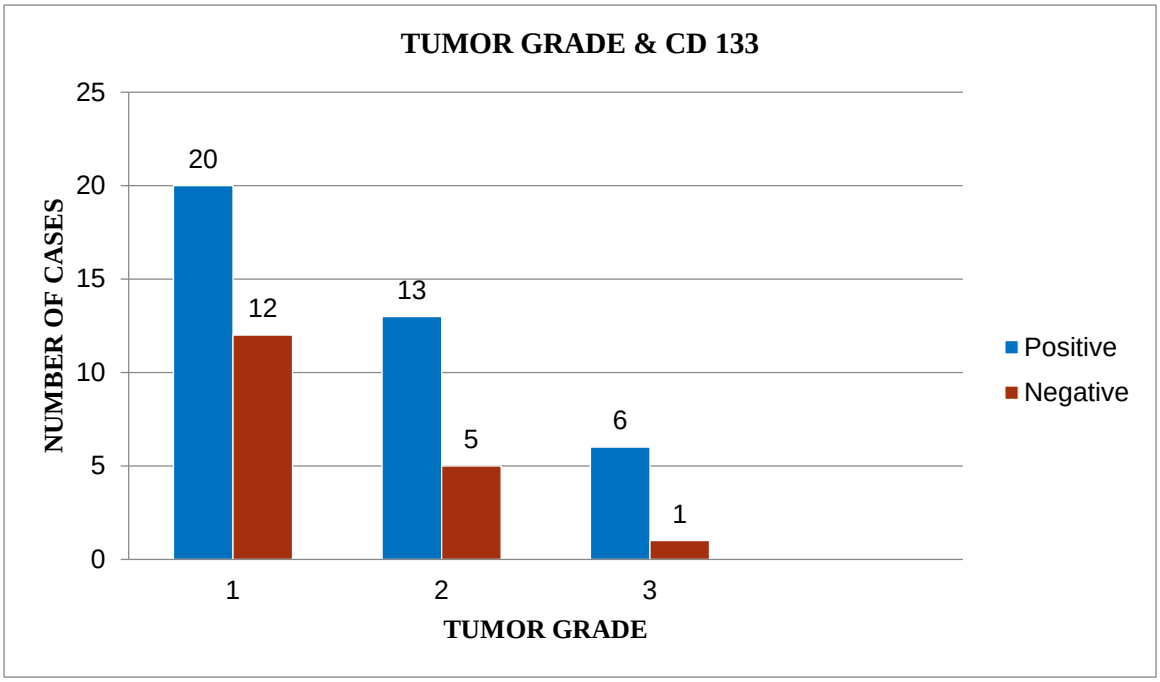

Chart 8 - Correlation between CD 133 score and tumor grade

CORRELATION BETWEEN CD 133 AND TUMOR SIZE

Table 22- Chi-Square Tests Of CD 133 expression and Tumor size			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	8.332[a]	6	0.215
Likelihood Ratio	9.865	6	0.130
N of Valid Cases	57		

a. 10 cells (71.4%) have expected count less than 5. The minimum expected count is 0.63.

Table 23 - correlation between CD 133 and Tumor size

		IHC		Total	p Value
		CD 133 Positive	CD133 negative		
T	1(<2cms)	5(62.5%)	3(37.5%)	8	0.218
	2(2-5 cms)	12(52.17%)	11(47.83%)	23	
	3(>5cms)	16(82.21%)	3(15.79%)	19	
	4(Chest wall)	6(85.71%)	1(14.29%)	7	
Total		39	18	57	

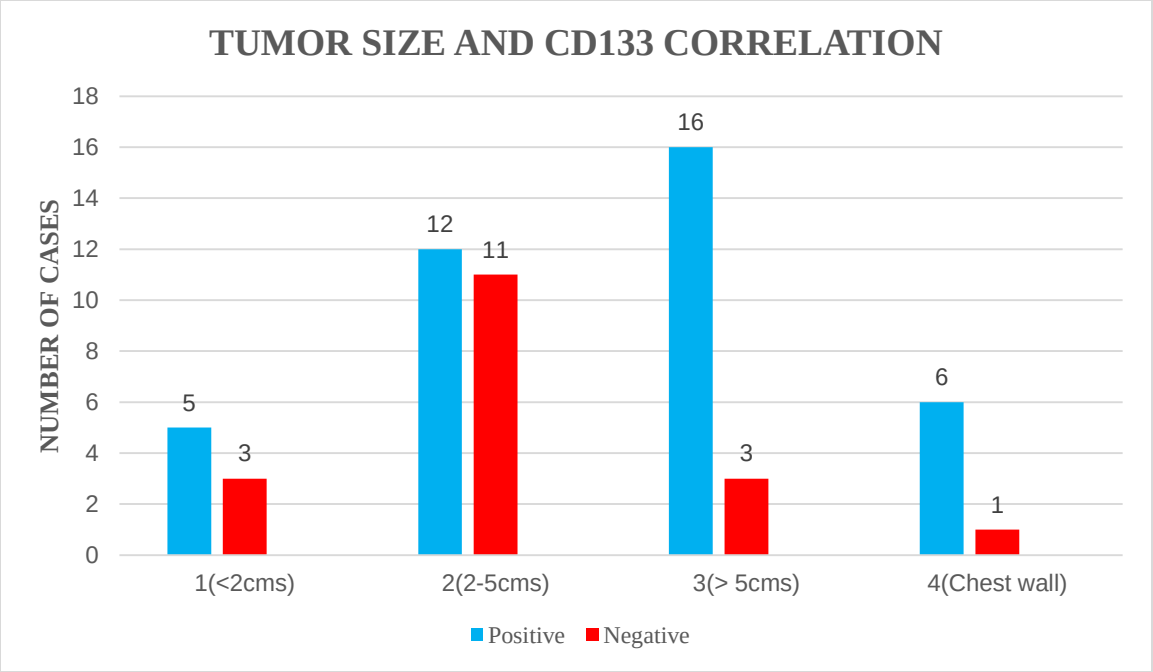

Chart 9 - Correlation between CD 133 and Tumor size

CORRELATION BETWEEN CD 133 AND LYMPH NODES (N CATEGORY)

Table 24 - Chi-Square Tests of CD 133 expression and Lymph node stage

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	16.250[a]	7	0.023
Likelihood Ratio	20.428	7	0.005
N of Valid Cases	57		

a. 13 cells (81.2%) have expected count less than 5. The minimum expected count is 0.63.

Table 25 - CD 133 expression and Lymph node stage

Lymphnodes		**CD 133**		**Total**	**p Value**
		Positive	**Negative**		
N	0 (0)	11	13	24	0.005
	1(1-3)	17	3	20	
	2(4-9)	7	1	8	
	3(>9)	4	1	5	
Total		39	18	57	

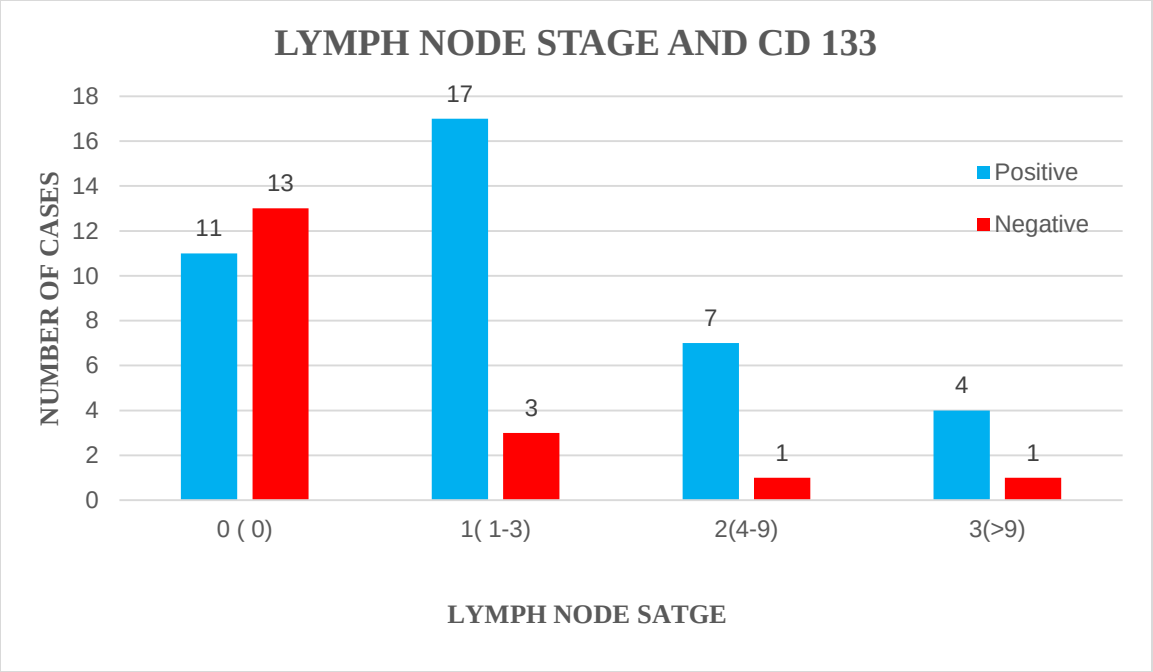

Chart 10 - Correlation between CD 133 and lymph nodes (N category)

CORRELATION BETWEEN CD 133 AND METASTASIS

CD 133 and metastasis did not correlate as there were no distant metastasis cases.

CORRELATION BETWEEN CD133 AND TUMOR STAGE

Table 26 - Chi-Square Tests of Tumor stage and CD133 expression

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	10.375[a]	2	0.006
Likelihood Ratio	11.048	2	0.004
Linear-by-Linear Association	7.114	1	0.008
N of Valid Cases	57		

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is 0.95.

Table 27 – Correlation of Tumor stage and CD133 expression

		CD 133		Total	pValue
		Positive	Negative		
Stage	I	2	1	3	0.006
	II	13	14	27	
	III	24	3	27	
Total		39	18	57	

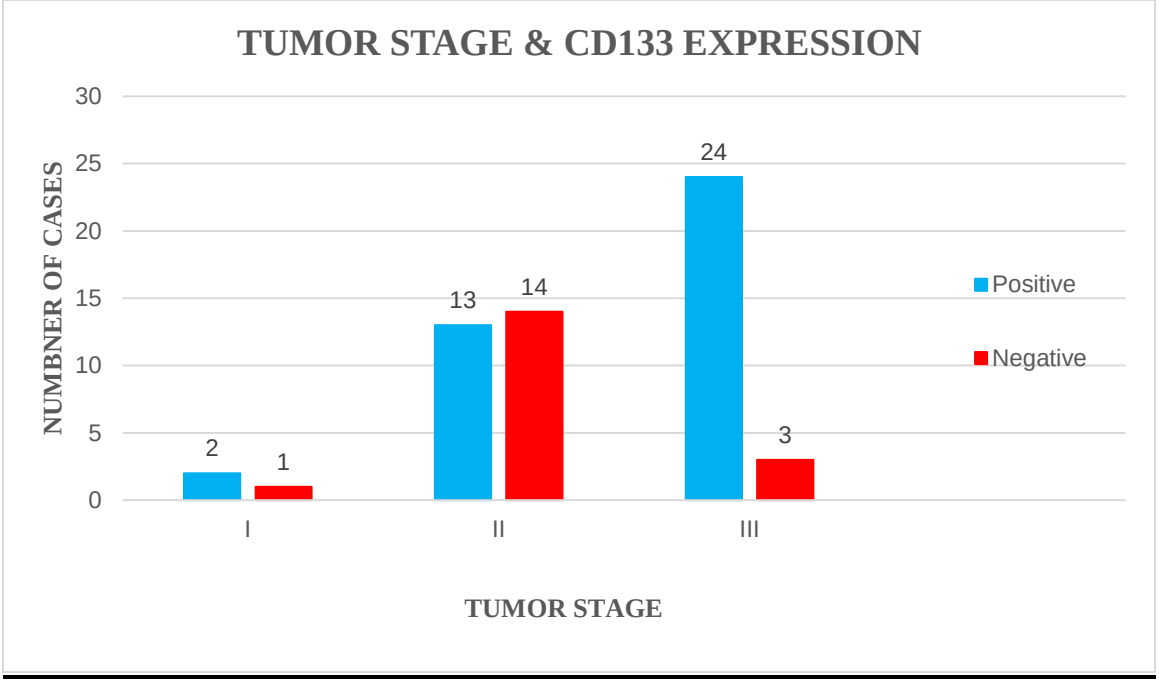

Chart 11 - Correlation of Tumor stage and CD133 expression

CORRELATION BETWEEN CD 133 AND NOTTINGHAM PROGNOSTIC INDEX

Table 28 - Chi-Square Tests CD 133 and Nottingham prognostic Index

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	17.839[a]	3	0.001
Likelihood Ratio	18.061	3	0.001
Linear-by-Linear Association	14.802	1	0.001
N of Valid Cases	57		

a. 3 cells (37.5%) have expected count less than 5. The minimum expected count is 0.32.

Table 29 – Correlation between CD 133 and Nottingham prognostic Index

		CD 133		Total	pValue
		Positive	Negative		
NPI	I	0	1	1	0.001
	II	5	11	16	
	III	23	5	28	
	IV	11	1	12	
Total		39	18	57	

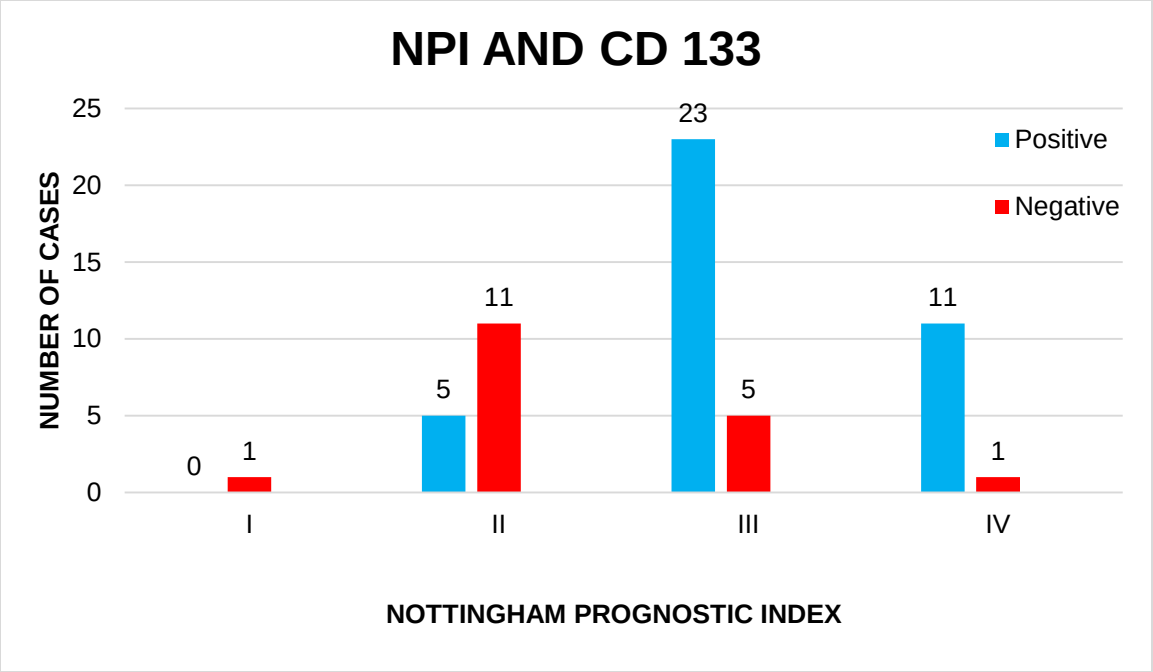

Chart 12 - Correlation between CD 133 and Nottingham prognostic Index

DISEASE FREE SURVIVAL RATE

Kaplan-Meier graph

Correlation of CD 133 positive expression and disease free survival

Table 30 - Means and Medians for Survival Time

Mean[a]				Median		
Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval
		Lower Bound	Upper Bound			Lower Bound
22.530	2.381	17.863	27.197	16.000	3.760	8.630

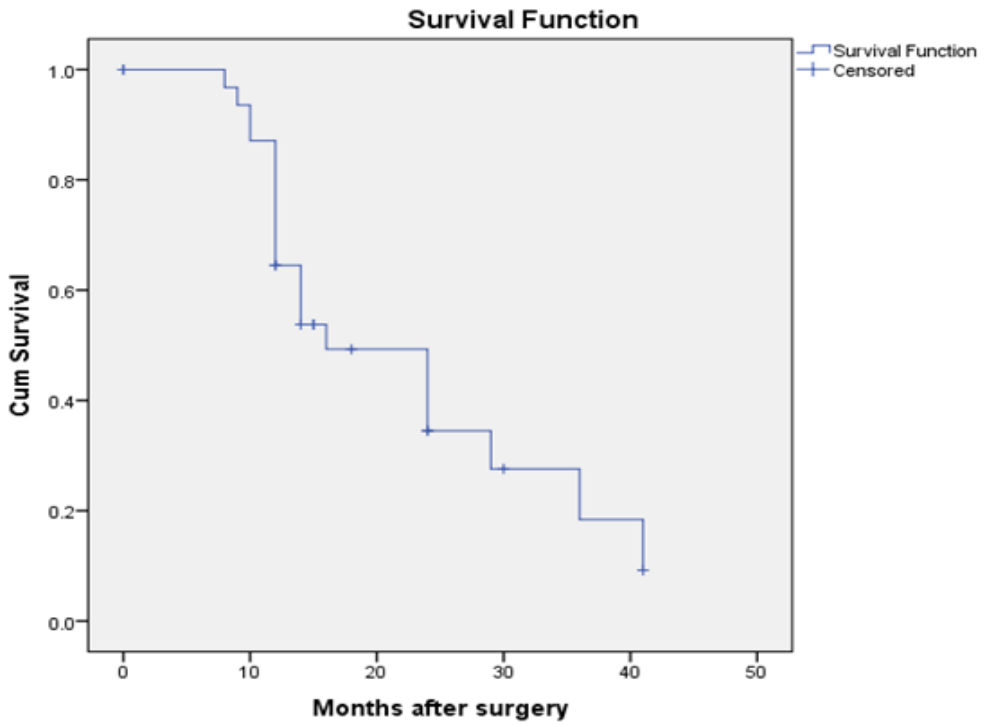

Chart 13 - correlation of CD 133 positive expression and Disease free survival

Table 31 - Correlation of CD 133 Negative expression and disease free survival

Means and Medians for Survival Time

Mean[a]				Median		
Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval
		Lower Bound	Upper Bound			Lower Bound
27.084	3.109	20.991	33.177	30.000	6.675	16.917

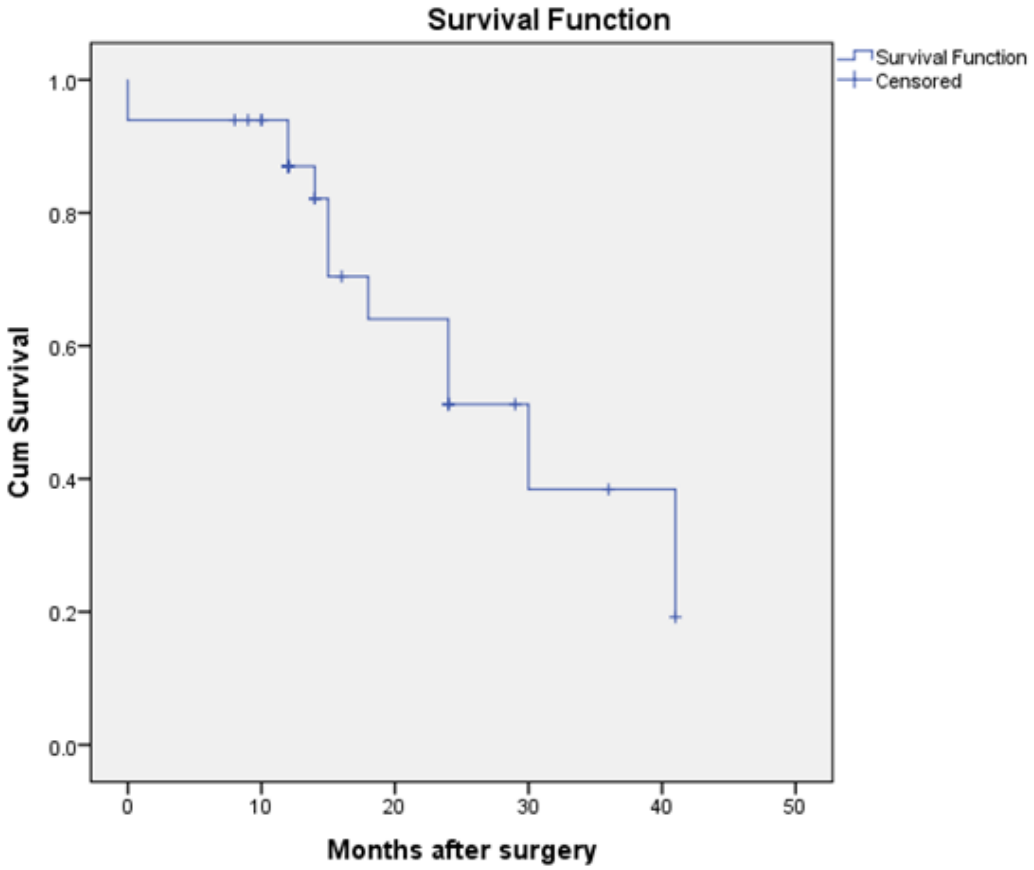

Chart 14 - Correlation of CD 133 negative cases and Disease free survival

Table 32 – Cases with ER ,PR and HER 2 neu staining

BIOPSY NUMBER	AGE	HOSPITAL NUMBER	CD 133	ER	PR	HER 2 neu
B-2373-18	38	600105	4	Positive	Positive	Equivocal
B-2471-18	55	630252	6	Negative	Negative	Positive
B-31-18	48	713964	9	Negative	Negative	Negative
B-369-18	82	681338	0	Positive	Positive	Negative
B-371-18	58	681638	12	Negative	Negative	Negative
B-386-18	80	682643	1	Positive	Positive	Negative

33 – Overall results and statistical significance

HISTOPATHOLOGICAL PARAMETRES	p VALUE
TUMOR GRADE	0.447
TUMOR SIZE	0.215
NODAL METASTASIS	**0.005**
TUMOR STAGE	**0.006**
NOTTINGHAM PROGNOSTIC INDEX	**0.001**

DISCUSSION

DISCUSSION

"In the field of malignant tumor biology, the Cancer stem cells (CSCs) have become the topic of debate since its fundamental theory was put forward. It has been considered that the tumor is composed of tumor cells and CSCs, which are a rare subpopulation of cells in solid tumors with the capability of self-renewal, differentiation potential and initiating tumors.[80] Cancer Stem Cells are at the root of tumor formation that can lead to various degrees of differentiation and are the source that enables the tumor to keep growing and spreading.[81] The CSC hypothesis has basic implications for cancer biology, in addition to clinical implications for cancer risk assessment, early detection, prognostication and prevention."

CSCs can be distinguished from the tumor cells through identification of specific molecular surface markers such as CD24, CD44, ALDH1, ESA (epithelial specific antigen) and CD 133. Initially CD 133 was found in hematopoietic stem cells and was considered as specific molecular biomarker for hematopoietic stem cells.[82] Several studies have been done on CD 133 expression and its correlation in breast carcinomas, colorectal carcinoma, ovarian tumors , liver malignancies and brain tumors like Glioblastoma etc. These studies and meta-analysis show that the CD 133 has been associated with high grade, high tumor stage and poor prognosis and disease free survival rate. In view of the inconsistent conclusions on CD 133 expression and poor prognosis this study was undertaken.

AGE DISTRIBUTION

In the present study, the age group ranged from 28 years to 80 years with mean age of 54 years, which is similar to the study done by Margaret et al[6] with 29.22% cases less than 50 years and 70.78% cases above 50years. Whereas the age distribution did not correlate with the study done by Anwar et al[83].

Table 34 – Comparison of distribution of the cases into tumor grade with other studies

GRADE	Anwar et al[83] (2019)	Margaret JC et al[6] (2012)	Kim SJ et al[5] (2015)	PRESENT STUDY
I	1(2.5%)	5(5.63%)	149(51.2%)	33(57.9%)
II	11(27.5%)	32(35.95%)		17(29.82%)
III	28(70.0%)	52(58.42%)	142(48.8%)	7(12.28%)
Total	40	89	291	57

In the present study the highest cases belonged to grade 1 i.e 33 cases (57.9%) and least were of grade 3 i.e 7 cases (12.28%) which did not correlate with the studies done by Mansour et al[86], Margaret et al[6] and Kim SJ et al[5]. This may be because of the pathogenesis or the tumor biology may be different in our area compared to other countries.

Table 35 – Comparison of frequency of CD 133 expression with other studies

FREQUENCY	Anwar et al[83] (2019)	Margaret JC et al[6] (2012)	Kim SJ et al[5] (2015)	Joshi et al[84] (2018)	PRESENT STUDY
POSITIVE	31(77.5%)	22(24.72%)	72(24.74%)	67(87%)	44(77.18%)
NEGATIVE	09(22.5%)	67(75.28%)	219(75.26%)	10(13%)	13(22.82%)
Total	31/40	22/89	72/291	67/77	44/57

Table 36 - Comparison of CD 133 expression and tumor grade with other studies

Tumor grade (Histological grade)	CD 133 expression (Positive)			
	Present study	Mansour et al[86] 2015	Han et al[85] 2015	Joshi et al[84] 2018
1	20/32	24	27/76	06 (11)
2 & 3	19/25	40	131/259	58(67)
Total	39/57	64/120	258/335	79/100

In present study the frequency of CD 133 expression was 77.18% (44cases) which correlated with the study done by Anwar et al[83] and Joshi et al[84], in contrary to the study done by Margaret et al[6] and Kim SJ et al[5] where the frequency of CD 133 expression was low. Other studies have reported in the range from 40%-50% [87& 88] . This may be explained by the theory that these two studies had more of grade 3 cases and less of grade 1 cases in contrary to our study where grade 1 cases are more. Further there was no correlation between the CD 133 expression and grade of the tumor but there was consistent increase in the expression of CD 133 as the grade increases similar to study done by Han et al[85]. In present study the highest cases belonged to grade 1 so the CD 133 expression was more seen in grade 1 cases i.e 20/ 32 cases, but after looking at the grade 2 and grade 3 cases out of 25 cases 19 cases were positive for CD 133 staining which was similar in study done by Mansour et al[86] where and Joshi et al[84] where out of 76 grade 1 cases 26 showed CD 133 positivity and out of total 259 grade 2& 3 cases 131 showed CD 133 positivity . These studies also prove that the higher the grade the more is the CD 133 expression. A trend of higher incidence of CD133 expression was noted with advanced histology grade which was consistent with result obtained in a study by Han et al[85].Different studies show varying percentage of CD 133 expression. Indian studies by Anwar et al[83] and Joshi et al[84] have shown results similar to our study. However studies by Kim SJ et al[5] and Margaret et al[6] did not show similar findings inspite of having more of grade 3 cases. Han et al showed statistical significance between CD 133 expression and grade of the tumor.No statistical correlation was seen in our study due to less sample size as compared to Han et al(N=259).

TUMOR SIZE

The present study showed highest number of cases belonging to T2 i.e 23 cases (40.35%) which correlated with the study done by Anwar et al[83].

Table 37 - Comparison of CD 133 expression and tumor size with other studies

		CD 133 expression (Positive)			
		Present study	Mansour et al[86] 2018	Kim SJ et al[5] (2015)	Joshi et al[84] 2018
T	1(<2 cms)	5/8	50	20	6/6
	2(2- 5 cms)	12/23		52	58/70
	3(>5cms)	16/19	14		18/19
	4(Chest wall)	6/7			05/05
Total		39/57	64/120	72	87/100

In present study there was no correlation between the tumor size and the CD 133 expression. Similar to the studies conducted by Mansour et al[86] and Kim SJ et al[5] in contrary to the study done by Joshi et al[84] where there is correlation between CD 133 expression and tumor size. In this study nearly almost all the cases of T 3 and T 4 had CD 133 expression which correlated with the studies conducted by Mansour et al[86], Kim SJ et al[5] and Joshi et al[84] . This shows that CD 133 correlates with the higher tumor size of infiltrating ductal carcinoma of Breast.Our study showed CD 133 expression last tumors (tumors >5cms) which was similar to studies done by Mansour et al[86], Kim SJ et al[5] and Joshi et al[84] . However our study did not meet statistical significance.

Table 38 - Comparison of CD 133 expression and lymph node metastasis with other studies

Lymphnodes		CD 133 expression (Positive)				
		Present study	Mansour et al[86] 2018	Kim SJ et al[5] 2015	Joshi et al[84] 2018	Collina et al[87] 2015
N	Positive	39(68.42%)	49(76.56%)	34(47.22%)	42(48.28%)	11(36.67%)
	Negative	18(31.58%)	15(23.44%)	38(52.78%)	45(51.72%)	19(63.33%)
Total		57	64	72	87	30

In present study the expression of CD 133 and lymph node metastasis was statistically significant which correlated with the studies done by Mansour et al[86], Kim SJ et al[5],Joshi et al[84] and Liu et al [66]. However the study done by Collina et al[89] did not show any correlation between CD 133 expression and lymph node metastasis. This shows that CD 133 expression is important for tumor to spread along the lymphatic channels by the process of epithelial mesenchymal transition.

Table 39 - Comparison of CD 133 expression and Tumor stage with other studies

		CD 133 Expression(Positive)			
		Present study	Mansour et al[86] 2018	Kim SJ et al[5] 2015	Joshi et al[84] 2018 -
Stage	I	2/3	10	16	
	II	13/27	54	35	58
	III	24/27		21	29
Total		39/57	64/120	72/291	87/100

In present study there was statistical correlation between CD 133 expression and tumor stage. The higher the stage the more was the CD 133 expression. The similar findings were seen in the study done by Kim SJ et al[5] , Joshi et al[84] and Mansour et al[86].However our study may not reflect the true staging of the disease since no data was available on hematogenous spread (M) of the tumor.

Table 40- Comparison of CD 133 expression and NPI with other studies

		CD 133 Expression (Positive)	
		Present study	Mansour et al[86] 2018
NPI	I	0/1	-
	II	5/16	24
	III	23/28	40
	IV	11/12	
Total		39/57	64/120

Nottingham prognostic index is an indirect indicator of prognosis and helps in knowing the disease free survival of the breast carcinoma cases. In present study there was statistical correlation between Nottingham prognostic index and CD 133 expression which was seen in other study done by Mansour et al[86].Hence CD 133 may also reflect the prognosis.

So in this study histopathological prognostic parameters such as nodal metastasis, tumor stage and Nottingham prognostic index suggesting breast cancer cells with high expression of CD133 harbors invasive properties. Similar findings were seen in a meta-analysis study done by Zhan et al[4] and Liu et al [66].

In our study we tried to correlate CD 133 expression with Esrtogen receptors (ER), Progesterone receptors(PR) and HER 2 neu receptors Immuno Histo Chemistry. Out of 57 we could get the data of only 6 patients. Among these 6 patients 2 patients are triple negative breast cancer patients where ER, PR and HER2 neu is found negative and these patients had high CD 133 expression with score ranging from 9 to 12. The other 4 patients 2 patients belonged to Luminal A (ER/PR positive and HER2 neu Negative) , 1 patient in Luminal B (ER/PR positive and HER2 neu - Equivocal) and 1 patient Luminal C (ER/PR Negative and HER2 neu positive) . These cases we could not correlate statistically as the cases obtained are very less. The studies done by Han et al showed CD 133 expression correlation with triple negative breast carcinomas, which explains the association of CD133 expression with the poor prognosis and triple negative breast cancers. So our number of cases are too less for the comparison.

We also tried to correlate CD 133 expression with Disease free survival. Out of 57 cases we could follow up 33 patients out of which 3 patients expired.These3 patients that expired are not because of tumor metastasis as these patients did not have any metastasis during the surgery.

Among these one is of age 92.Out of 33 patients 21 cases showed CD 133 positivity and the mean survival was 16months.And the other 12 patients who were negative for CD 133 expression had mean survival of 30months. By the Kaplan miere graph it is evident that the more the CD 133 expression the lesser was the disease free survival of the patients similar to the study done by Han et al[85] , Zhan et al[4], Wu et al[90],Martin et al[91] and Zhao et al[92], contrary to the study done by Joshi et al[84] where the CD 133 negative expression showed poor disease free survival.

Few studies like Mansour et al[86] and Joshi et al[84] studied CD 133 expression with histological types of breast carcinomas but without any significant correlation. In our study we included only infiltrative ductal carcinoma cases and did not correlate with histological types.

However more studies including more number of cases subgrouped into CD133 positive and CD 133 negative with longer follow up needs to be done

CONCLUSION

CONCLUSION

This study was taken up to know the expression of CD 133 in infiltrating ductal carcinoma and its correlation with all histopathological parameters.

In our study CD 133 expression was seen in 77.08% cases and was associated with histopathological parameters higher tumor grade , higher tumor size , lymphnode metastasis, higher tumor stage and poor Nottingham prognostic index and worse DFS.

CD133 markers may potentially serve as prognostic markers and novel potential therapeutic targets in breast cancer. However similar studies with more number of samples are required to study CD 133 expression in molecular subtypes of breast carcinoma.

SUMMARY

SUMMARY

- The present study was undertaken in Department of pathology, Sri Devaraj Urs Medical College, Tamaka, Kolar from June 2017 – November 2019.
- Retrospective cases were also collected from January 2015 to May 2017.
- Total of 57 cases were collected out of which majority belonged to the age group of 41 – 60 years (54.38%)
- Majority cases were of Grade I tumor (57.9%)
- Majority of the cases (40.356%) had Tumor size between 2 – 5 cms
- Lymph node stage N1 had majority of the cases 18 (31.57%)
- Majority of cases were Nottingham Prognosis Index category III (49.12%)
- In this study there was no statistically significant correlation between CD 133 expression and Age , Tumor Size and Tumor grade. Even though these did not show statistically significant correlation, there was increase in trend of CD 133 positivity with increase in the age, tumor size and Tumor grade. Out of 7 cases of age >60 years 4 cases were showed CD 133 positivity. Out of 7 Grade 3 cases 6 cases were positive for CD 133 expression and only 1 was negative. Out of 26 cases of Tumor stage 3 and 4, 22 cases were positive for CD 133 and only 4 cases did not show CD 133 expression.
- Out of 57 cases the CD 133 expression was seen in 44 cases (77.18%)
- Out of 57 cases 24 cases were negative for lymph node metastasis, out of which 11 cases were positive for CD 133 expression. And 33 cases showed lymph node metastasis, out of which 28 cases showed CD 133 positivity. The CD 133 positivity and lymph node metastasis was statistically significant (p value - 0.005).

- There was statistically significant correlation between CD 133 expression (p value - 0.006).Out of 57 cases, 3 cases were of Stage I among which 2 cases showed CD 133 positivity. 13 cases out of 27 Stage II cases were positive for CD 133.And 24 cases out of 27 Stage III cases were showing CD 133 expression.
- Out of 57 cases, 1 cases was in Nottingham Prognostic Index category I which was negative for CD133 positivity. 16 cases were of NPI category II out of which only 5 cases were CD 133 positive. 28 cases were of NPI category III , out of which 23 cases were CD 133 positive and 5 were negative. 11 cases out of 12 NPI category IV cases showed CD 133 positivity .The CD 133 expression and NPI category was statistically significant (p value - 0.001)
- Out of 57 cases we could follow up 33 cases from 8 months to 41 months after the surgery. These was no distant metastasis during the surgery. Out of these 33 cases 3 cases expired during the follow up and 1 case was of age 92 and the death of these cases were not related to breast carcinoma. Out of these 33 cases 21 cases showed CD 133 positivity with a mean survival of only 16 months. And 12 cases showed CD133 negativity with the mean survival of 30 months. Thus indicating that the CD 133 expression has got poor disease free survival rate compared to CD 133 negative cases.
- Further studies are required with large sample size to prove the role of CD 133 expression in Infiltrating Ductal carcinoma of breast and its relation to distant metastasis, poor Disease free survival and Overall survival.

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ANNEXURES

ANNEXURES

PROFORMA

Name

Age

Hospital number

Chief complaint

History of presenting illness

Past history

Personal history

Family history

Menstrual history

Local examination

Clinical diagnosis with TNM staging

Radiological findings

Biopsy number

Histopathological diagnosis

Gross Features

Nature of Specimen:
Tumour site:
Tumour size:
Tumor shape:
Specimen size:

Complete gross description:

Microscopy

Invasive tumour type:
Histological grade:
Disease extent:
Microscopic extension of tumour:

- Skin changes
- Nipple
- Skeletal muscle

Lymph node:
Axillary nodes present: No □ Yes □
Total present: .....
Total positive: .....
Extracapsular spread: Present □ Not identified □

NPI scoring.

CD133 expression

- Intensity of CD positivity
- Extent of CD 133 positivity
- Total score

INFORMED CONSENT FORM

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 that will be collected and disclosed during the study.

I have had the 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

PATIENT INFORMATIOM SHEET

STUDY TITLE: "EXPRESSION OF CD133 IN INVASIVE DUCTAL CARCINOMA OF BREAST"

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

The main aim of the study is to check for the presence of the cancer stem cell marker CD133 and its correlation with the histopathological parameters such as tumor size, lymph node metastasis, aggressiveness of the IDC.

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

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

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

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

For any clarification you are free to contact the investigator**.**

PRINCIPAL INVESTIGATOR: Dr. Preeti ashok Utnal.

Contact no: 8147177015

Email ID: utnalpreeti@gmail.com

KEYS TO MASTER CHART

Age Distribution in years	1- 21-40 2- 41- 60 3- 61-80 4- 81-100
Grade of the tumor	1- Grade I 2- Grade II 3- Grade III
Tumor size	1- T1 (<2CMS) 2- T2 (2- 5 CMS) 3- T 3 (>5CMS) 4- T4 (CHEST WALL)
Lymph nodes positive	1- Stage N0 (0) 2- Stage N1 (1-3) 3- Stage N2 (4-9) 4- Stage N3 (>9)
Tumor stage	1- Stage I 2- Stage II 3- Stage III
Nottingham Prognostic Index	1- NPI I 2- NPI II 3- NPI III 4- NPI IV
CD 133 Expression	1- Positive 2- Negative

MASTER CHART

S.NO	YEAR	Biopsy no	Name	Age	Hosp N	PHONE NUMBER	TS	TG	LN	T	N	M	Stage	NPI	CD 133 - Int	CD133 - %	Total	ER	PR	HER 2 neu	Months after surgery
1	2018	146	Zareen	50	508481		6x3	I	0/7	4	0	x	IIIB	3.2	0	0	0				
2	2018	191	Bhagyamma	50	529217	8722757086	5x4x3	II	0/7	3	0	x	IIB	4.2	2	1	2				18
3	2018	275	Gohar Taj	50	537450	7338084976	2x1.3x2	I	3/16	1c	0	x	I	3.4	3	1	3				14
4	2018	510	Rubystella	35	421835	9980859140	4.2x4x3.2	III	0/2	2	0	x	IIA	4.84	3	2	6				0
5	2018	602	Lakshmidevamma	49	559352	9611916557	4x2x1.5	II	0/0	2	x	x	IIA	3.8	0	0	0				14
6	2018	732	Amaravathi	40	562743	9629283493	6.5x6x3.5	III	0/0	3	x	x	IIB	4.3	0	0	0				15
7	2018	952	Parvathamma	50	570525	99800732787	4x2.8x1.8	I	1/10	2	1	x	IIB	4.8	2	2	4				14
8	2018	1236	Thiruvasugi	65	581449	9740482540	4.5x4x3	I	1/5	2	1a	x	IIA	3.6	3	4	12				12
9	2018	1250	sakamma	55	560942	9740636643	4x2x1.9	I	0/13	2	0	x	IIA	2.8	2	2	4				14
10	2018	1279	Jubeda	53	562840	9986729866	1.2x0.9x1.2	I	3/15	1	1	x	IIA	3.24	0	0	0				12
11	2018	1293	Thabasum Kousar	53	582456	9900657371	1.3x1.1x1.3	II	4/13	1c	2	x	IIIA	5.26	3	2	6				12
12	2018	1309	Channabasava	50	582129	9980060474	6.5x4.1x3	II	7/8	3	2	x	IIIA	5.2	2	3	6				12
13	2018	1371	Lakshmamma	46	584126	8618452467	8x6x1.5	I	2/9	3	1a	x	IIIA	4.6	2	2	4				10
14	2018	1475	Jareena Begum	56	585075	9591854898	6x5.5x3	I	2/9	3	1a	x	IIIA	4.2	2	2	4				9
15	2018	1917	Lalitha	59	601433	8748915150	5x2x2	I	1/12	1	1a	x	IIA	4	3	2	6				8
16	2018	1918	Venkatalakshmamma	65	608018	9845548175	3.8x2.7x3.2	I	0/10	2	0	x	IIA	2.76	1	2	2				15
17	2018	1987	Chinnamma	50	611791	9380545797	12x5x2	I	3/10	3	1a	x	IIIA	4.5	3	2	6				16
18	2018	2028	shradamma	55	609669	9481862729	2.5x3x1	I	0/20	2	0	x	IIA	2.6	2	1	2				12
19	2015	2086	Pushpa	32	618289		4x3.8x3.4	I	1/23	2	1	x	IIB	2.8	1	2	2				
20	2015	2093	Padma	45	622032		3.5x3.5x2	I	0/0	2	x	x	IIA	2.7	2	1	2				
21	2015	2101	Anasuyamma	60	616799		3x2x2	I	0/4	2	0	x	IIA	2.6	2	1	2				
22	2015	2334	Gangamma	60	603691		4.5x4x3	II	2/8	4	1	x	IIIB	4.9	2	2	4				
23	2018	2373	Lakshmamma	38	600105		4x4x4	I	1/4	4a	1	x	IIIB	3.8	2	2	4	P	P	Equivocal	
24	2018	2390	Reddamma	55	635438		4x3x2.5	I	0/4	2	0	x	IIA	1.8	0	0	0				
25	2018	2431	Sarojamma	55	630252	8971110201	6x5x3.3	II	10/13	3	3	x	IIIC	6.2	3	2	6	0	0	Positive	10
26	2018	2741	Kanthamma	45	608809	9448249601	5.5x4x2.6	I	5/5	3	2a	x	IIIA	5.1	2	2	4				
27	2017	24	Fareeda	40	355502	opd register	13x9.5x8	II	0/7	3	0	x	IIB	4.6	3	4	12				24
28	2017	43	Ramakka	92	380287		10x5.5x4	I	0/2	3	0	x	IIB	4	3	3	9				0
29	2017	444	Gowramma	65	402801		1.2x1x1.3	II	0/12	1c	0	x	I	3.26	2	3	6				24
30	2017	559	Gayathri	38	408977	9741334237	4x2x1	I	0/3	2	0	x	IIA	2.8	3	1	3				24
31	2017	585	Vanaja	40	407106	opd register	2x1.5x0.5	I	0/7	1	0	x	I	2.4	1	1	1				30
32	2017	1717	Susheela nagendra	68	461171	9448411795	4x3x2	I	1/9	2	1	x	IIB	4.8	0	0	0				24
33	2017	1831	Fierdose Kousar	35	465862	9242312654	5.3x2.5x2.5	II	0/1	3	0	x	IIB	3.06	2	1	2				24
34	2017	1878	Haseena Banu	39	461944		4.2x4.2x3.5	III	6/12	2	2	x	IIIA	5.84	3	4	12				
35	2017	2180	Yalagamma	60	445817		5.5x4.5x4	III	2/7	3	1	x	IIIA	6.1	3	2	6				
36	2017	2505	Zareen Begum	75	501731		12x9x3.5	II	1/8	4b	1	x	IIIB	7.4	3	4	12				
37	2017	2762	Zubeda Begum	40	517278		5.3x5x2.5	I	1/12	3	1	x	IIIA	3.06	3	1	3				
38	2016	30	Chowdamma	60	236685		11.5x0x7.5cms	I	8/8	4b	0	x	IIIB	6.3	3	2	6				
39	2016	64	Saraswathamma	56	236685		7x6.5x2.1	II	20/25	3	3a	x	IIIC	6.4	3	1	3				
40	2017	806	Reshma Taj	37	236685	opd register	2.5x2.4x2	II	19 / 26	2	3a	x	IIIC	5.5	3	4	12				29
41	2016	1719	Hanumakka	48	302256		7.5x6.8x3.6	I	0/10	3	0	x	IIIA	3.5	2	2	4				
42	2016	1721	Sarasamma	55	302185		5.5x4x1.7	III	11/11	3	2	x	IIIA	7.1	3	4	12				
43	2016	1731	Malathi	47	293825	opd register	12.5x11x5	III	22/22	3	2	x	IIIA	8.5	3	4	12				36

44	2016	2573	Parveen Taj	45	310945	8867886270	2.5x2x2.5	II	4/6	2	2	x	IIIA	4.5	3	3	9				41
45	2016	2574	Gangamma	45	295125	9741495235	3x1.5x1.5	III	2/5	2	1	x	IIB	5.6	3	4	12				41
46	2018	2299	sridevi	35	622435.00		7x5x2	I	2/10	3	1	x	III A	4.4	3	3	9				
47	2018	2695	Gowramma	70	642200	9740830354	3x3x2.7	I	0/11	2	0	x	IIA	2.6	3	3	9				
48	2018	2732	laxmi	28	647618	9663793708	3.5x3x2	II	7/10	2	2a	x	IIIA	5.7	0	0	0				12
49	2018	2964	Sakamma	78	651335	9945376859	9x8x7.8	I	0	4b	0	x	IIIB	3.8	3	2	6				12
50	2018	1200	farida Begum	62	575396	9743998496	4x4x5	II	\3/12	2	1	x	IIB	4.8	3	3	9				12
51	2018	2804	Gowramma	36	650777		3.5x3.5x3.5	I	1/12	2	1	x	IIB	3.7	2	3	6				12
52	2018	2373	lakshmamma	41	600105		4x4x4	I	1/4	4a	1	x	IIIB	3.8	3	1	3				
53	2018	31	savithramma	48			8x4x2.8	II	11/15	3	3	x	IIIC	6.6	3	3	9	0	0	Negative	
54	2018	369	Chowdamma	82	681338		2.9x2.5x2	I	00/09	2	0	x	IIA	2.5	0	0	0	p	p	Negative	
55	2018	371	Peddammaiah	58	681638		4x2x2	II	01/14	1	1	x	IIA	4.8	3	4	12	0	0	Negative	
56	2018	386	munimaramma	80	682643		2x1.5x1.3	II	25/25	1c	3a	x	IIIC	5.4	1	1	1	p	p	Negative	
57	2018	1454	Munirathnamma	52.21428571	713964		3.5x3x3	I	0	2	0	x	IIA	2.7	1	1	1				