

# Molecular classification of breast carcinoma

Based on the prognostic marker: A Clinico-pathological correlation

## Abstract

### Background

Breast cancer (BC) has now surpassed lung cancer as the leading cause of global cancer incidence in 2020, with an estimated 2.3 million new cases, representing 11.7% of all cancer cases. It is a highly heterogeneous disease with a variety of morphologic and clinical manifestations which results in a range of responses to treatment. Recently, targeted therapies based on the genetic, hormonal, or immunohistochemical (IHC) subtypes of breast cancer have been used.

**Objective:** The study was a prospective hospital-based observational study with a sample size of 150 cases. The study aimed to determine the distribution of various molecular subtypes of breast carcinoma and also correlate the expression status of ER, PR, Her2neu, and Ki 67 with the patient's age, tumor size, tumor type, histological grade, lymph node status, and TNM staging.

**Result:** 96.7 % of cases were invasive ductal carcinoma (NOS). Most of the grade 1 tumors (68.8%) were both ER and PR positive. Grade 2 tumors had almost equal distribution with 39.4% of patients being ER & PR positive and 43.7% being both ER & PR negative. Almost all grade 3 tumors (88.9%) were both ER and PR -negative.

**Conclusion:** IHC markers are cost-effective and easily available worldwide even in resource-poor countries like India. A greater understanding of the molecular classification of breast carcinoma based on triple markers will help in the development of targeted therapies that will lead to increased efficacy, decreased toxicity, increased disease-free survival, and better selection of patients who will benefit from treatment.

**Key words :** Breast cancer , ductal carcinoma ,mER & PR negative, BRCA1, BRCA2, TP53

### Introduction

“Breast cancer (BC) is the commonest malignancy among women worldwide. In 2020, globally 2.3 million women were diagnosed with breast cancer, and 6, 85,000 deaths occurred due to breast cancer”. (1) “It has been a leading cause of cancer amongst Indian females with an age-adjusted rate of 25.8 per 100,000 women and a mortality of 12.7 per 100,000 women”. (2.

“Breast cancers are heterogenous disorders showing distinct molecular expressions, pathological features, and biological behavior”. (3) “Morphologically, there are 21 distinct subtypes of invasive breast carcinoma (IBC) as defined by the World Health Organization classification”. (4) “However, from a therapeutic perspective, this classification has its limitations as most breast carcinomas fall under the category of ductal carcinomas not otherwise specified. A new therapeutically relevant molecular classification has been developed, based on gene expression profiling using complementary DNA microarrays. In this classification, breast carcinomas are divided into 5 intrinsic subtypes of IBC: Luminal A, Luminal B, normal breast-like, Human epidermal growth factor receptor 2 (HER2) enriched, and Basal type”. (5) “In clinical practice, the immunohistochemical status of estrogen receptor (ER), progesterone receptor (PR), and HER2/neu are used as surrogate markers to classify these tumors into molecular subgroups”. (6,7) “Currently, morphologic classification, histologic grade, the status of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2), along with tumor stage are used to guide clinical management. The routine immunohistochemical (IHC) analysis for ER, PR, and HER2 provides critical prognostic and predictive information for IBC”. (8)

“The usual surgical procedure for carcinoma breast is modified radical mastectomy. The outcome after surgery varies widely. Prognostic information is important in counseling patients about the likely outcome of their disease and planning further management. Some of these factors are estrogen and progesterone nuclear hormone receptors which correlate with a better outcome and are an important predictor of response to hormonal (anti-estrogen) therapy. About 80% of carcinomas that are both ER and PR positive respond to hormonal manipulation, whereas only about 40% of those with either ER or PR positivity respond to hormonal manipulation. ER-positive cancers are less likely to respond to chemotherapy”. (9) “HER2/neu overexpression is associated with poorer survival, but they respond to agents that target the trans-membrane protein (eg. Trastuzumab or Herceptin)”. (9) “Proliferative index can also be measured by immunohistochemical detection of cellular proteins produced during the cell cycle, eg Ki-67. Carcinomas with high proliferation rates have a poorer prognosis but may respond better to chemotherapy”. (10) Thus, the current therapeutic approach for breast carcinoma consists of a combination of surgery, postoperative radiation, hormonal treatment, chemotherapy, and Trastuzumab. The choice between hormonal therapy which has minimal side effects and chemotherapy with well-known morbidity is a major responsibility of the clinician. Accurate and reliable assessment of the ER, PR,HER2/neu and Ki67 status of breast cancers by the pathologist is therefore crucial as these markers not only prognostic but also predictive of response to therapy. Hence the present study was undertaken to establish a correlation between ER and PR status, HER2/neu overexpression, proliferative activity, clinical features, and tumor histopathology to effectively use these parameters to prognosticate and treat breast cancer patients.

## Material and Methods

A prospective hospital-based observational study was carried out at Indira Gandhi Institute of Medical Sciences, Patna in the department of Pathology from October 2018 to September 2020. Trucut biopsies and mastectomy specimens of all ages were included in the study. Malignancies other than epithelial in origin, cases with extensive tumor necrosis, post-chemotherapy, and recurrent breast cancers were excluded from the study. A total of 150 cases were evaluated. Institutional ethical committee clearance was taken.

All the specimens were analyzed grossly and multiple sections of the tumor were taken along with surgical margins, nipple and areola, non-neoplastic breast, and all lymph nodes were collected after a thorough description of the specimen. The tumor size were retrieved from ultrasonography in biopsy specimens and grossly in MRM specimens .It was further grouped into three category : < 2cm , 2-5 cm and > 5cm .H&E slides were studied for the tumor type, modified Scarff-Bloom- Richardson (MBR grade), lymph node metastasis, etc. Additional microsections were subjected to immunohistochemistry (IHC) to look for ER, PR, HER2/neu overexpression, and the Ki-67 proliferative index. With each set of slides, positive and negative controls were applied. The immune-stained slides were analyzed for membrane staining for HER2/neu and nuclear staining for ER, PR, and Ki-67. The percentage of tumor cells that stained positively in each case, as well as the average staining intensity (reported as 0, 1+, 2+, or 3+), were assessed. For ER and PR evaluation, Allred semi-quantitative scoring system was employed. the HER2 was scored from 0 to +3 in which score 0 or 1 is negative, 2+ is equivocal and 3+ is positive. For Ki-67 only nuclear staining is considered positive without any relevance of staining intensity. Scoring is done by counting of at least 500 malignant cells. The Ki-67 score or index should be expressed as the percentage of positively staining cells among the total number of malignant cells in the area scored.

## Statistical analysis

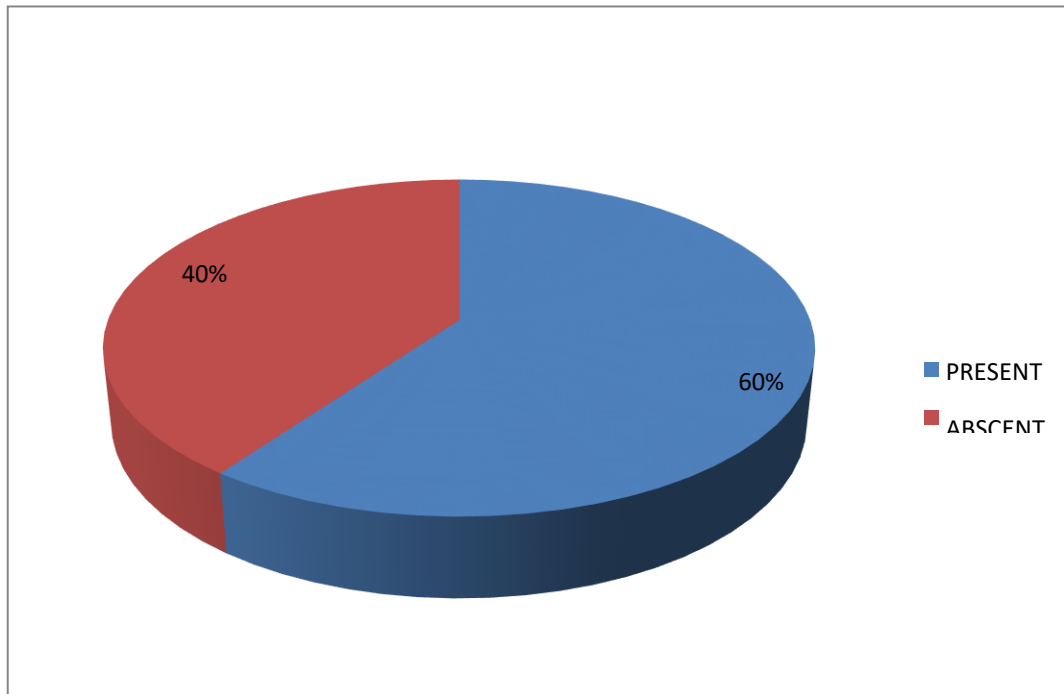
The percentage was calculated for categorical variables. Mean and standard deviation (SD) were calculated for numerical variables. Continuous variables were compared using a student's t-test for normally distributed variables. The chi-square test was used to compare proportions. All p values <0.05 were considered statistically significant. The software used for data analysis was SPSS version 20.

## Results

A total of 150 cases were included in the present study, 40 underwent modified radical mastectomy and 110 were biopsy specimens. Out of 150 patients, 5 were males and 145 were females with ages ranging from 22 to 83. The majority of the patients belonged to the peri-menopausal age group constituting 55.9% while 44.1% were post-menopausal females. A total of

149 patients presented with unilateral breast involvement and 01(0.7%) had bilateral breast carcinomas. Left breast (51.3%) involvement was more common than right breast (48%). Total 40 specimens were received as MRM, out of which 26 patients (65%) had tumor size between 2-5cm, 10% had tumor size less than 2cm and 25% had a tumor size of more than 5cm. Out of 40 MRM specimens, 24 patients (60%) presented with lymph-node metastasis while 16 patients were negative for lymph node metastasis.

**GRAPH 1: DISTRIBUTION OF LYMPH-NODE METASTASIS**



The most common histological type of breast carcinoma was invasive ductal carcinoma (NOS) type. 145 patients (96.7%) out of total 150 had IDC NOS type [table1].

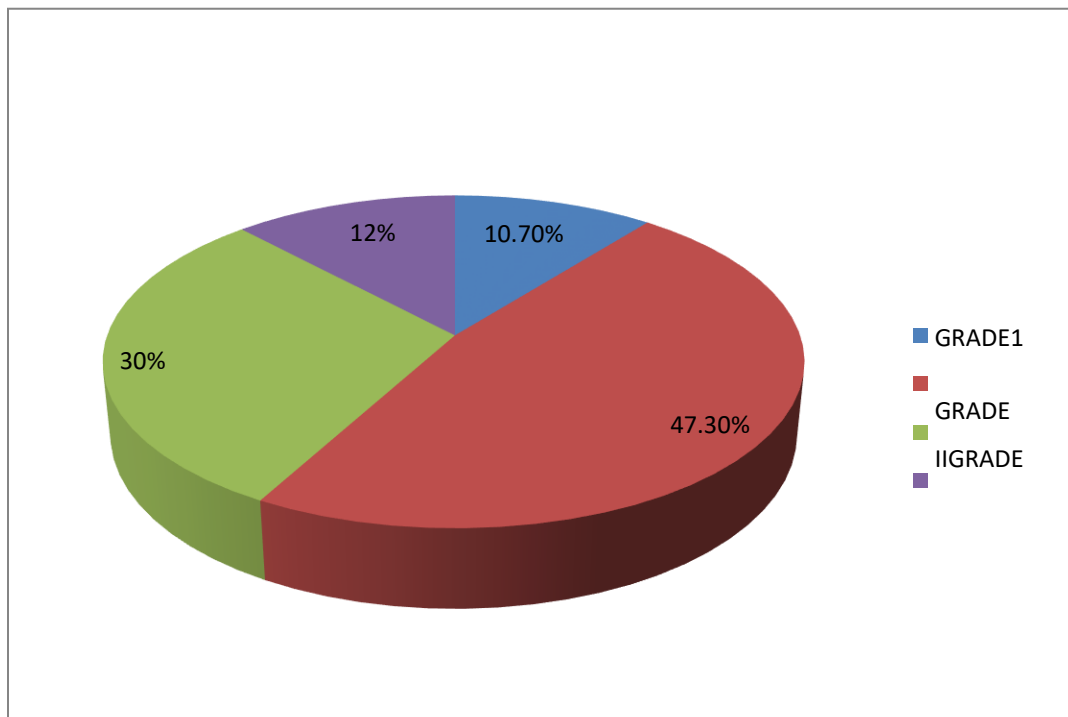
**TABLE1: HISTOLOGIC TYPES OF BREAST CARCINOMA**

HISTOLOGICALTYPE	FREQUENCY	PERCENTAGE
INVASIVEDUCTAL CARCINOMANOS	145	96.7

INVASIVELOBULAR CARCINOMA	2	1.3
MEDULLARY CARCINOMA	2	1.3
PAPILLARY CARCINOMA	1	0.7
TOTAL	150	100.0

Out of 150 patients 10.7% of tumors were grade I and grade III tumors made up 30%. Grade II tumors predominated, accounting for 47.3% of all cases. Because of low tumor cellularity, extensive necrosis, and crush artefact, 12% of tumors could not be graded.

**GRAPH2: DISTRIBUTION OF GRADE IN THE PATIENTS STUDIED(N=150)**



All the 150 cases were processed for IHC. Estrogen receptor positivity was observed in 46% of cases while PR was positive in 34.7% of cases. Majority of cases were Her2/neu negative constituting 71.3%.

Grade 1 tumors (68.8%) were both ER and PR positive and HER2/neu negative. Grade 2 tumors had almost equal distribution with 39.4% of patients being ER& PR positive and 43.7% being both ER & PR negative. 22.5% of grade 2 tumors were triple negative. In grade 3 tumors 58% were triple negative, followed by 31.1% HER2-rich subtypes. The difference was statistically significant with a p-value of <0.01. Most of the ER& PR positive tumors had a Ki-67 score of less than 30% whereas tumors that were both ER and PR negative had a high proliferative index (ki-67 > 30%). The majority of tumors with low proliferative index (ki-67 < 15%) were luminal type whereas tumors with a high proliferative index (Ki-67 > 30%) were triple negative. This difference was statistically significant with a p-value of <0.01

**TABLE 2: ER, PR AND HER2/neu STATUS IN THE VARIOUS HISTOLOGIC GRADES OF BREAST CARCINOMA**

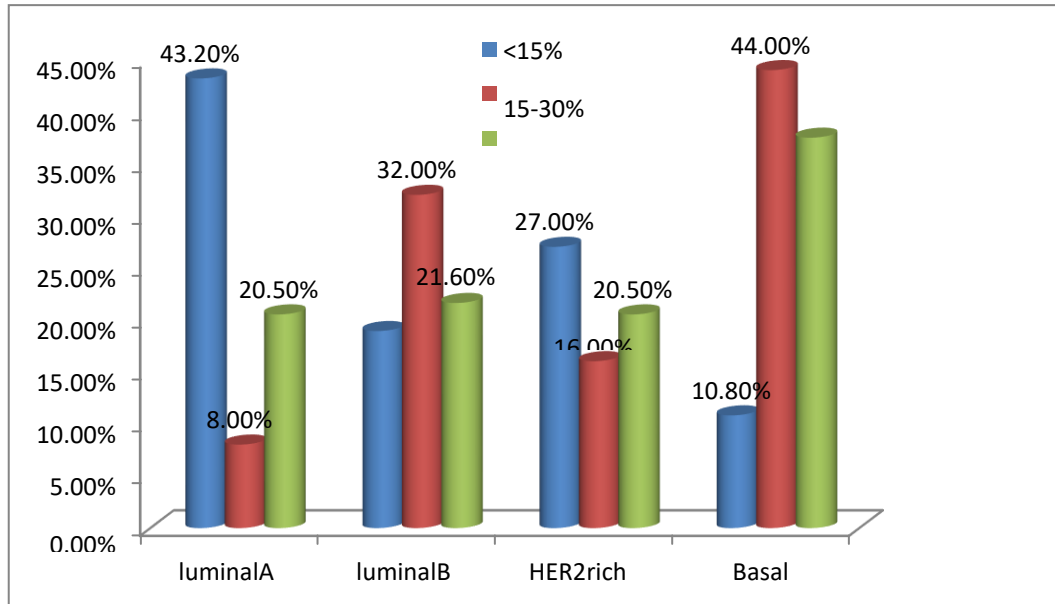
GRADE	ER+PR + Her2+	ER-PR- &Her2+	ER+PR - &Her2-	ER+PR+ &Her2-	ER-PR- &Her2-	Total	$\chi^2$ value	Pvalue
GRADE I	1	0	5	10	0	16	50.092	0.00 (significant)
	6.2%	.0%	31.2%	62.5%	.0%	100.0%		
GRADE II	7	16	11	21	16	71		
	9.9%	22.5%	15.5%	29.6%	22.5%	100.0%		
GRADE III	1	14	1	3	26	45		
	2.2%	31.1%	2.2%	6.7%	57.8%	100.0%		
NO GRADE	2	3	1	7	5	18		
	11.1%	16.7%	5.6%	38.9%	27.8%	100.0%		
TOTAL	11	33	18	41	47	150		
	7.3%	22.0%	12.0%	27.3%	31.3%	100.0%		

Majority of cases with high proliferative index were basal type and HER2/neurich type whereas majority of cases with low ki-67 score were luminal. (TABLE 3)

**TABLE3: CORRELATION OF MOLECULAR TYPE WITH Ki-67 SCORE**

Ki-67	luminalA	luminalB	HER 2 rich	Basal	TOTAL	$\chi^2$ value	Pvalue
<15%	16 43.2%	7 18.9%	10 27.0%	4 10.8%	37 100.0%	18.147	0.006
15-30%	2 8.0%	8 32.0%	4 16.0%	11 44.0%	25 100.0%		
>30%	18	19	18	33	88		

**GRAPH3: CORRELATION OF MOLECULAR TYPE WITH KI-67 SCORE**



## Discussion

“The recent advances in breast cancer diagnosis and management modalities have shifted the focus to molecular studies for a better understanding of the etiology and risk factors. Molecular classification based on surrogate triple markers which classify breast carcinoma into luminal A, luminal B, triple negative, and HER2 positive is considered a better predictive factor for prognosis and treatment than routine histopathology. It has also provided prognostic stratification which is comparable to the other costly and less available multigene markers such as Mammaprint, Oncotype DX, PAM50, and Endopredict”.(11)

In the present study, the peak age was from 41-50 years followed by 31-40 years. The maximum numbers of females in our study were peri-menopausal (55.9%). Similar results were found by Karangadan S et al(10), Nabi MG.et al(12).

In the present study, all triple negative and HER2-rich subtype patients had a tumor size of > 2 cm compared to 78% of patients with luminal subtypes A and B. Similar results were found by Nabi MG. et al,(12)Kumar N. et al,(13) . However, Adedayo A. et al, (14) and Zhu et al (15) reported a maximum number of cases with tumor sizes less than (<2cm). The increased tumor size observed in our study and other Indian studies might be due to late presentation because of lack of awareness, scarcity of breast cancer screening programs, inadequate availability of mammography, financial constraints and social stigma associated with cancer leading to late consultation.

IDC (NOS) was the most common subtype followed by invasive lobular carcinoma ( ILC), medullary carcinoma, and papillary carcinoma of the breast. Ghanghoria S et al,(16) Stierer M et al.,(17) Nadji M et al.(18) and also had similar results. In the present study, 12% of cases were not given any grade as they belonged to histological types other than IDC (NOS) where grading is not applicable.

“In the present study, the maximum cases were ER, PR, and HER2/neu negative. ER and PR were negative in the studies done by many Indian authors”, (10) “whereas in most international studies, hormonal receptors were usually positive”. (19-21) .This might be due to a change of trend in the Indian population where a good number of patients present with high-grade tumors with the triple-negative molecular profile. This discordance can partially be attributed to the lack of standardization in IHC laboratories of various centers in our country.

ER and PR positivity were more common among old patients (>60years) compared to younger women (<30 years) which is similar to the findings of studies by Inwald et al.(20), Nishimura et al.(21) Sofi et.al (22). **We also noticed a higher Ki-67 index in women of the younger age group.**

“Luminal A was the most common molecular subtype in most Indian and international studies”. (19),(21),(23),(24) . However, in the present study, the maximum number of patients were in the peri-menopausal age group which attributed to the increased incidence of triple-negative cases. A similar observation was found in a study done by Karangadan S (10). We could conclude from our study that subtypes with poor prognoses such as triple negative and HER2/neu positive were more common among younger women, whereas tumor subtypes with better prognosis such as luminal tumors were more common among older women.

The number of HER2/neu enrich cases were more in the present study (21.33%) as compared to many Indian and International studies except in a few studies as in Munjal et al.(25) done in the Indian population.

In our study, out of two cases of lobular carcinoma, one was HER2-rich, and the other was a basal type. Two cases of medullary carcinoma were triple negative which correlated with studies by Karangadan S. et al(10) and Engstrøm et al.(26) .

“Male breast cancer is a rare disease, accounting for approximately 1% of all breast cancer cases and < 1% of all malignancies in men”. (27) 5 cases of carcinoma of the male breast were found in the present study which included 2 cases of luminal A, one case of each luminal B, HER2 rich and basal subtype. In the study by Ge et al.,(27) “on 42 male breast cancer cases, the most common subtype was luminal A (83%) followed by luminal B (17%). No triple-negative or HER2-positive cases were identified in their study”. Wang-Rodriguez et al.,(28) studied “65 male breast cancer cases and found the mean Ki-67 score to be 10.6%, which indicates low proliferative activity which is in concordance with the present study”.

In the present study, we observed more cases of the younger age group presenting with large tumor size (2-5 cm), higher grade, and more tendency to metastasize to lymph node, molecular profile on IHC as triple negative and HER2/neu positive. Similar observations were made by Onitilo et al(24).

The limitation of this study was the absence of FISH to determine the status of HER2/Neu for equivocal (score 2+) cases. The absence of CK 5/6 from the study's scope prevented a comprehensive molecular categorization into basal and non-basal subtypes.

## Conclusion

In our study of 150 breast carcinoma cases, most of the cases were of a triple-negative subtype with a high proliferation index (Ki-67). We observed younger women with higher tumor grades and advanced stage. We concluded from our observation that the evaluation of receptor status has major implications in predicting the outcome, management, and prognostication of invasive breast carcinoma. It will help in the development of targeted therapies that will lead to increased efficacy, decreased toxicity, increased disease-free survival, and a better selection of patients who will benefit from the tailored treatment.

## **Ethical Approval:**

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

## **Consent**

As per international standard or university standard, patient(s) written consent has been collected and preserved by the author(s).

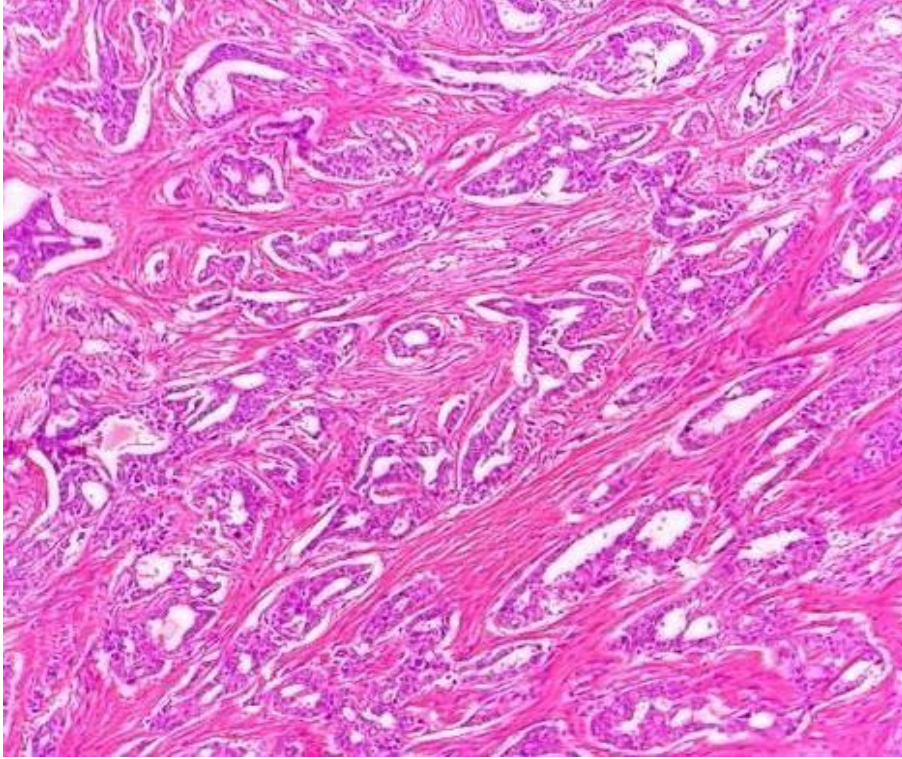


Fig 1: INVASIVE DUCTAL CARCINOMA(H&E:10X) shows infiltrative nests of tumor cells with moderately enlarged nuclei

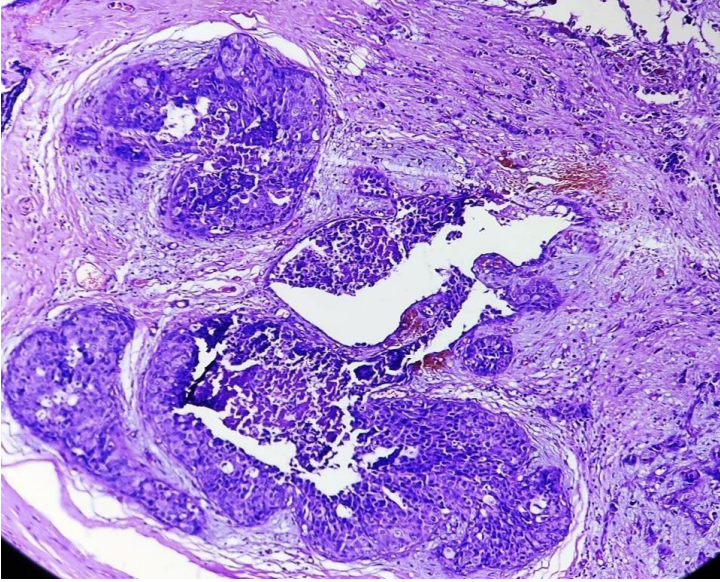


Fig 2: DCIS WITH COMEDO NECROSIS ( H&E:10X) shows intraductal epithelial proliferation with high grade nuclear atypia, solid growth pattern with comedo necrosis

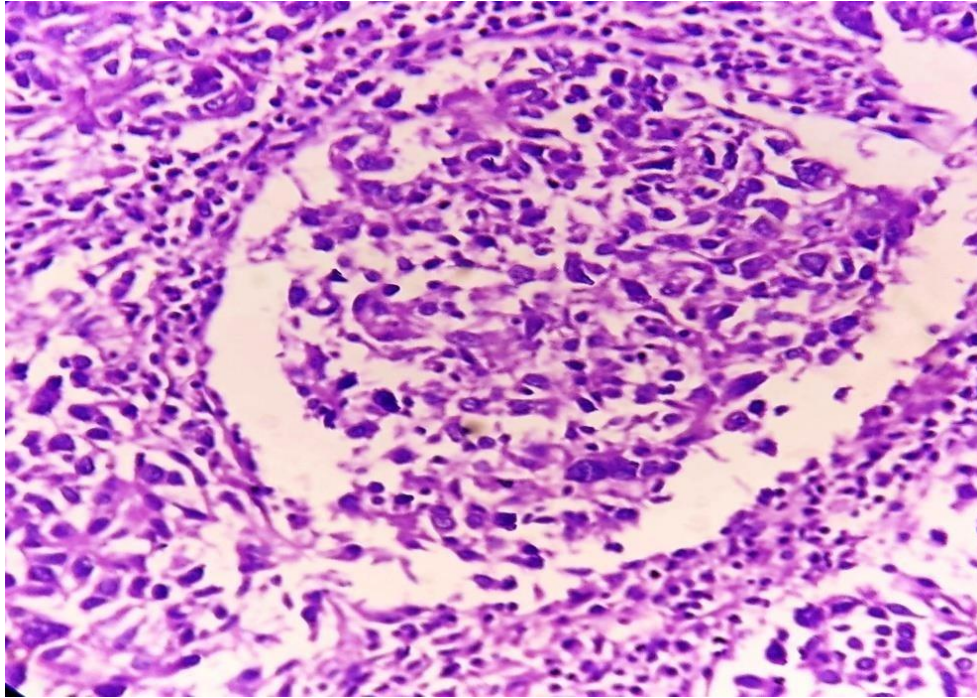


Fig 3: IDC WITH MEDULLARY PATTERN (H&E:10X) shows tumor cells with high nuclear grade and prominent nucleoli

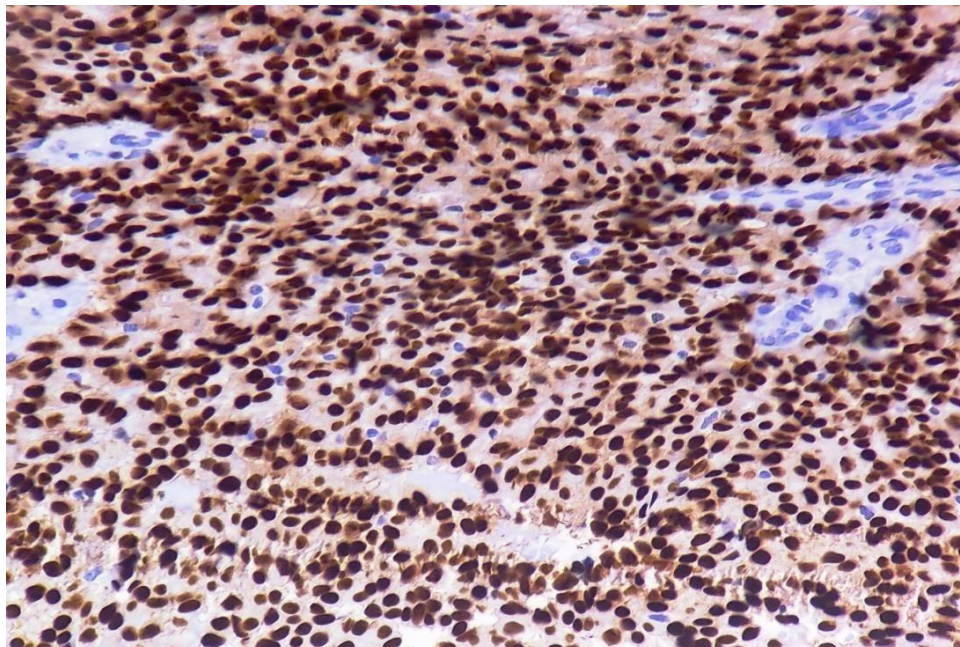


Fig 4: ER positivity<sup>3+</sup> in 100% of the tumor cell nuclei (Anti-ER-  
polyhorseradishperoxidase-DAB chromogen, 40X)

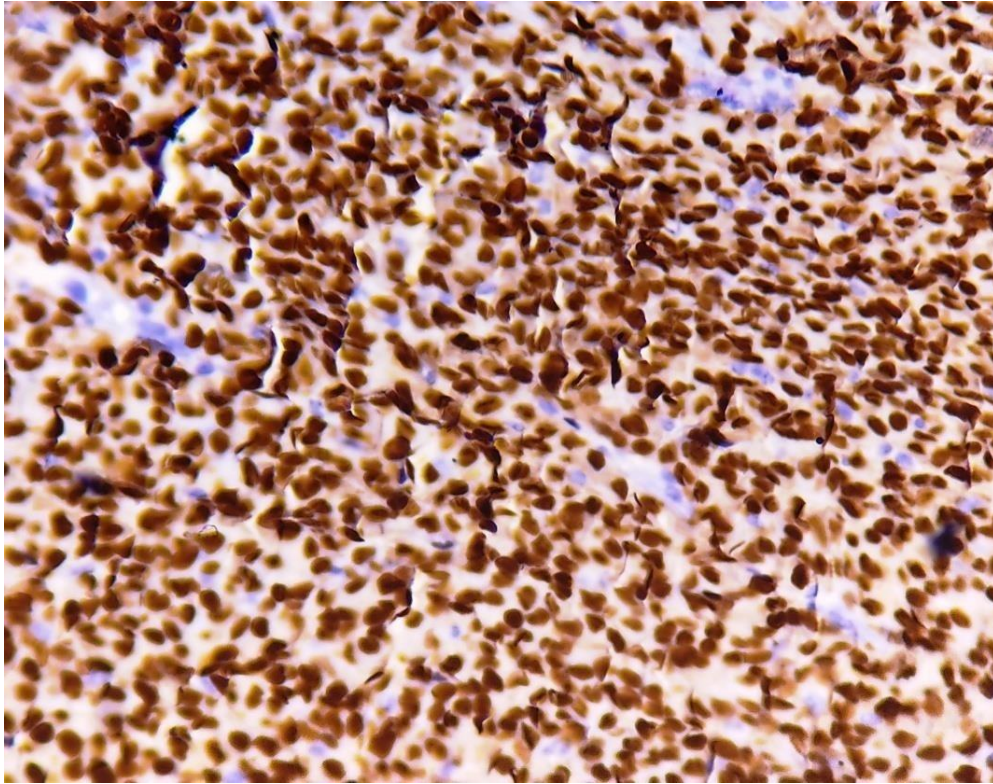


Fig 5: PR positivity <sup>3+</sup> in 100% of tumour cell nuclei (Anti-PR- poly  
horseradishperoxidase-DAB chromogen, 100X)

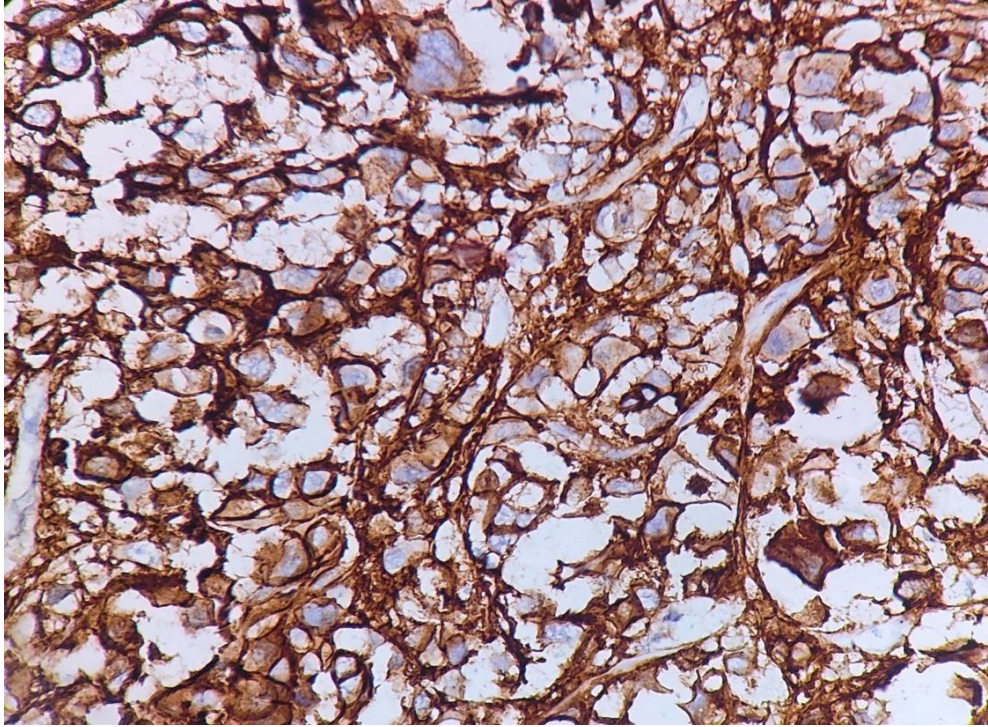


Fig 6: HER2/neu 3+ in 100% of tumor cells with complete membrane staining (Anti-HER2/neu-polyhorseradishperoxidase-DAB chromogen, 40X)

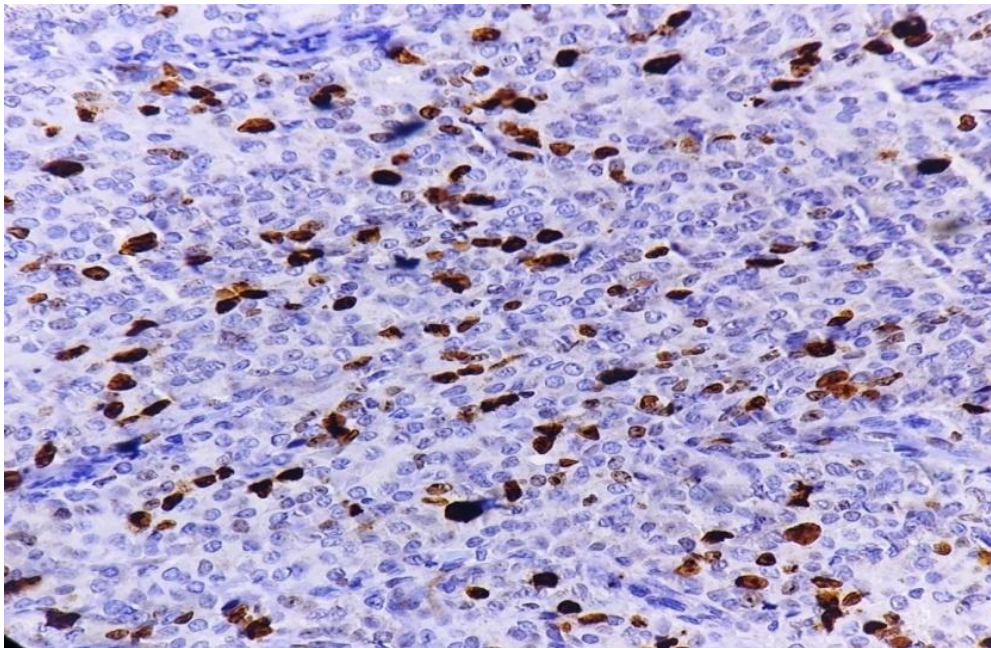


Fig 7: Ki-67 positivity in 15% of tumor cells nuclei (MIB1 Antibody-polyhorseradish

peroxidase-DAB chromogen,x100)

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