Original Research Article

Molecular classification of breast carcinoma
Based on the prognostic marker- 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 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 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). Majority of 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.

Introduction

Breast cancer (BC) is the commonest malignancy among women globally. In 2020, globally 2.3 million women were diagnosed with breast cancer and 6, 85,000 deaths occurred due to breast cancer. It has been a leading cause of cancer amongst Indian females with an age-adjusted rate of 25.8 per 100,000 women and mortality of 12.7 per 100,000 women. [(1)] Family history, early menarche, late age of marriage, first child at a late age, poor practice of breast feeding, menopausal hormonal therapy, radiation exposure to the chest for other malignancies and environmental toxins with estrogenic effect increases the risk of sporadic breast cancer.Whereas patients with a mutation in specific genes, including BRCA1, BRCA2, TP53, and PTEN have a high risk of developing hereditary breast cancer.

Breast cancers are heterogeneous, showing distinct molecular expression, pathologic features, and biologic behavior. [(2)] Morphologically, there are 21 distinct subtypes of invasive breast carcinoma (IBC) as defined by the World Health Organization classification. [(3)] 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.[(4,5,6)] In clinical practice, the immunohistochemical status of estrogen receptor (ER), progesterone receptor (PR), and HER2/neu is used

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as a surrogate marker to classify these tumors into molecular subgroups.[7,8] 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. [9]
The usual surgical procedure for carcinoma breast is 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. Conversely, cancers that fail to express ER or PR have a less than 10% likelihood of responding to hormonal therapy but are more likely to respond to chemotherapy. [10] [2] HER2/neu overexpression is associated with poorer survival, but they respond to agents that target the trans-membrane protein (eg. Trastuzumab or Herceptin). [10] [11] Proliferative index, in addition to mitotic count as part of histological grading, proliferation 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 current therapeutic approach for breast carcinoma consists of combination of surgery, postoperative radiation, hormonal treatment, chemotherapy, and Trastuzumab.

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. Total of 150 cases were evaluated. Institutional ethical committee clearance was taken.

All the specimens were analyzed grossly and relevant data were noted . H&E slides were studied for the tumor type, modified Scarff-Bloom- Richardson (MBR grade), lymph node metastasis etc. Representative sections were processed for IHC. The immunostained slides were examined for nuclear staining in case of ER, PR and Ki-67, and membranous staining in case of HER2/neu. In each case, the proportion of positive staining tumour cells (expressed in percentage) and the average intensity of staining (expressed as 0, 1+, 2+ or 3+) were evaluated. The relationship between various parameters such as menopausal status, duration of disease, tumour size, tumour extent, histologic type, histologic grade, lymph node status, expression of ER, PR, HER2/neu and Ki-67 index were studied.

Statistical analysis

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

Results

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A total of 150 cases were included in present study, 40 underwent modified radical mastectomy and 110 were biopsy specimens. Out of 150 patients, 5 were males and 145 were females with age ranging from 22 to 83. Majority of the patients belonged to peri-menopausal age group (55.9%). Left breast (51.3%) involvement was more common than right breast (48%). 96.7 % of cases were invasive duct carcinoma (NOS) with grade II (47.3%) being the commonest grade. 60% patients presented with lymph-node metastasis. Maximum cases were triple negative, followed by luminal A subtype. There was significant correlation of ER and PR status with histological grades of tumor with a p value of <0.001. Majority of 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. 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% where as tumors which were both ER and PR negative had a high proliferative index (ki-67 > 30%).
On correlating molecular type with ki-67 score, majority of tumors with low proliferative index (ki-67<15%) were luminal type and tumors with a high proliferative index (Ki-67 >30%) were triple negative. This difference was statistically significant with a p-value of<0.01.

Discussion

The recent advances in breast cancer diagnosis and management modalities have shifted the focus to molecular studies for 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. .

In the present study the peak age was from 41-50 years followed by 31-40 years. Maximum numbers of females in our study were peri-menopausal (55.9%). Similar results were found by Karangadan S et al[12], MG Nabi et al[13], Pandit P et al.,[14] Naeem et al.,[15]Su et al,[16] and Dang and Mysorekar.[17]
In the present study, all triple negative and HER2 rich subtype patients had a tumour size of > 2 cm compared to 78% patients with luminal subtypes A and B.. Similar results were found by Nikhilesh et al,[18] Nabi M.G. et al,[13] Krishnamurthyet al, [19].However Adedayo A. et al,[20] and Zhu et al [21] reported maximum number of cases with tumors size less than (<2cm). The increased tumor size observed in our study and other studies might be due to late presentation because of lack of awareness, scarcity of breast cancer screening programs and social stigma associated with cancer leading to late consultation. In resource poor country like India, inadequate availability of mammography, lack of infrastructure, well-trained staffs and financial constraints lead to delay in diagnosis and further management.
IDC (NOS) was most common subtype followed by ILC, medullary carcinoma and papillary carcinoma of breast. Nahed A.et al[22] Ghanghoria S et al,[23] Pandit P et al, [14] Stierer M et al.,[24] Nadji M et al.[25]and Hoffetal R. E[26]also had similar results. In the present study, 12% cases were not given any grade as they belonged to histological types other than IDC (NOS) where grading is

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not applicable.
In the present study, maximum cases were ER, PR, and HER2/neu negative. ER and PR were negative in the studies done by many Indian authors,[12],[27] whereas in most international studies, hormonal receptors were usually positive.[28],[29],[30],[31],[32] This might be due to change of trend in Indian population where good number of patients present with high grade tumors with triple negative molecular profile. This discordance can partially be attributed to the lack of standardization in IHC laboratories of various centers of our country.
ER and PR positivity was more common among old patients (>60years) compared to younger women (<30 years) which is similar to the findings of studies by Sofi etal.[33] Nishimura et al.[31] and Inwald et al.[29] We also noticed higher Ki-67 index in women of younger age group.
Luminal A was the most common molecular subtype in most of the Indian and international studies.[16],[34],[28],[31],[35],[36],[37] . However in the present study the maximum patients were in peri-menopausal age group which attributed to the increased incidence of triple negative cases. Similar observation was found by study done by Mysorekar D [17] and Karangadan S [12]. We could conclude from our study that subtype with poor prognosis such as triple negative and HER2/neu positive were more common among younger women, whereas tumor subtypes with better prognosis such as the luminal tumors were more common among older women.

Number of HER2/neu enrich cases were more in the present study (21.33%) as compared to many Indian and International studies except in few studies as in Munjal etal.[38] done in the Indian population.

In our study, out of two cases of lobular carcinoma, one was HER2 rich and other was basal type. Two case of medullary carcinoma were triple negative which correlated with studies by Suetal,[16] ,Engstrøm et al.[36] and Karangadanetal S.[12]

Male breast cancer is a rare disease, accounting for approximately 1% of all breast cancer cases and < 1% of all malignancies in men.[39] 5 cases of carcinoma of 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.,[39] on 42 male breast cancer cases, 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 etal.,[40] studied 65 male breast cancer cases and found 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 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 Mysorekar D [17], Shabnamkarangadan[12]and Onitilo et al[36].

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Conclusion

In our study of 150 breast carcinoma cases, most of the cases were of triple negative subtype with high proliferative index (Ki-67). In our study we observed young women with higher tumor grade and advanced stage. A significant statistical correlation between molecular classification, ER/PR status, and Ki67 were observed. Correlation of histological grade with molecular classification and hormonal status were also statistically significant. We conclude from our observation that evaluation of hormone receptor status has major implication 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 better selection of patients who will benefit from treatment.

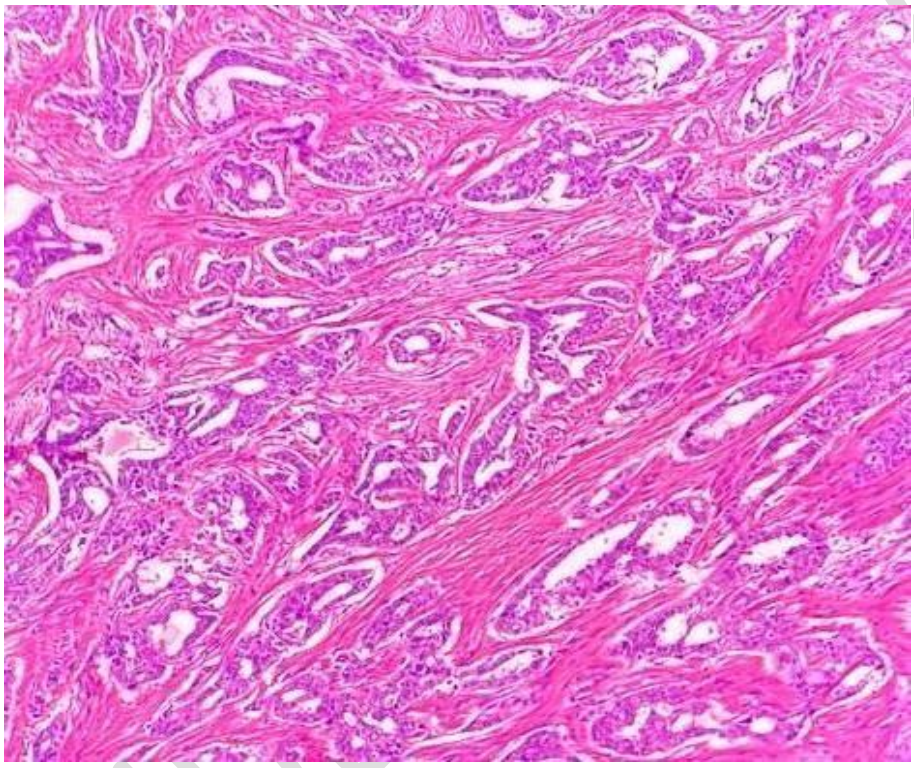

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Fig 1: INVASIVE DUCTAL CARCINOMA(H&E:10X)

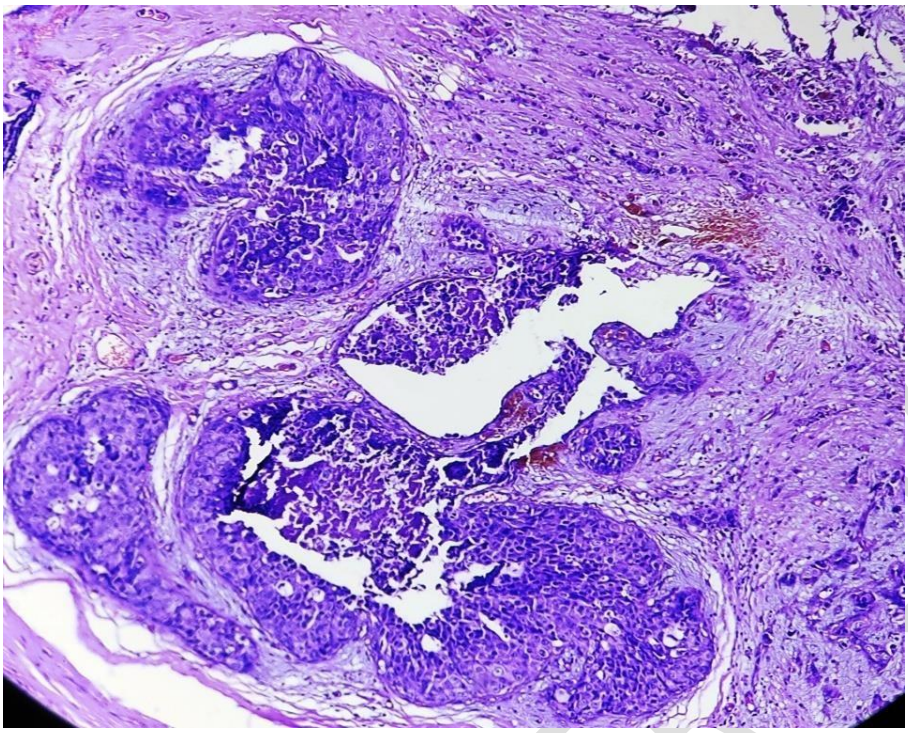

Fig 2: DCIS WITH COMEDO NECROSIS (H&E:10X)

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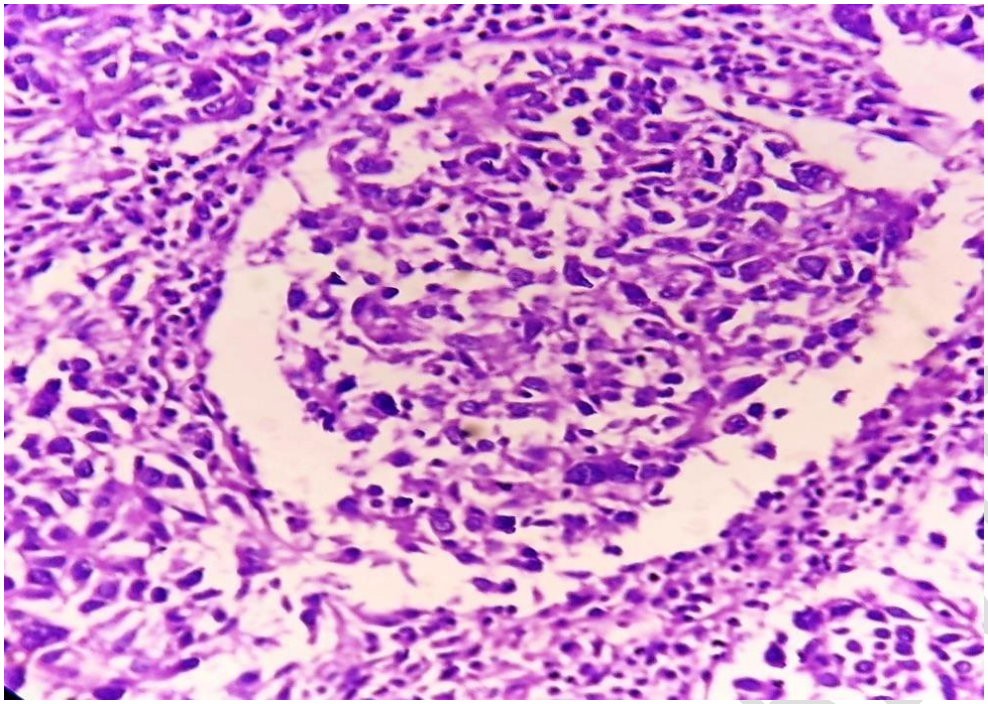

Fig 3: IDC WITH MEDULLARY PATTERN (H&E:10X)

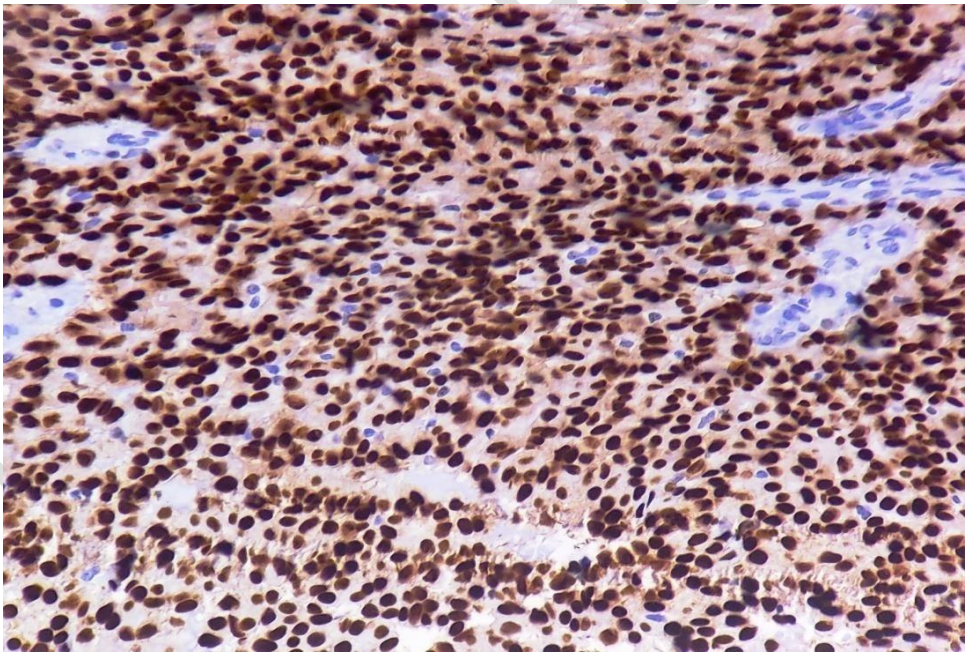

Fig 4: ER positivity3+ in 100% of the tumor cell nuclei (Anti-ER-polyhorseradishperoxidase-DAB chromogen, 40X)

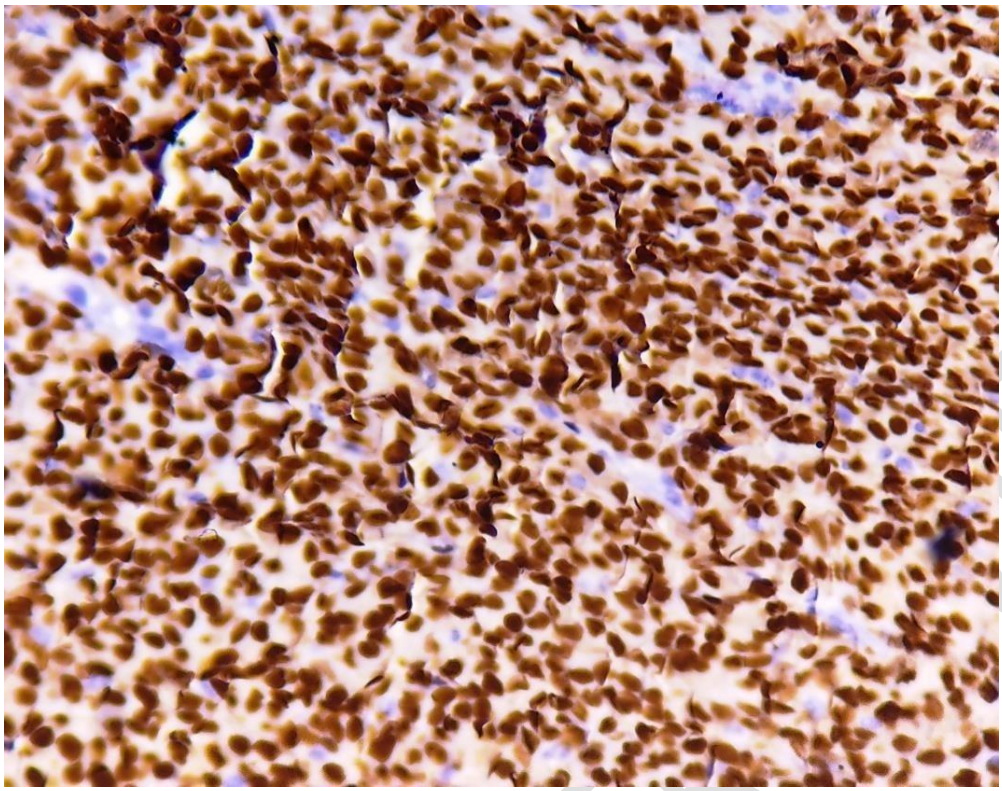

Fig 5: PR positivity 3+ in 100% of tumour cell nuclei (Anti-PR- poly horseradishperoxidase-DAB chromogen, 100X)

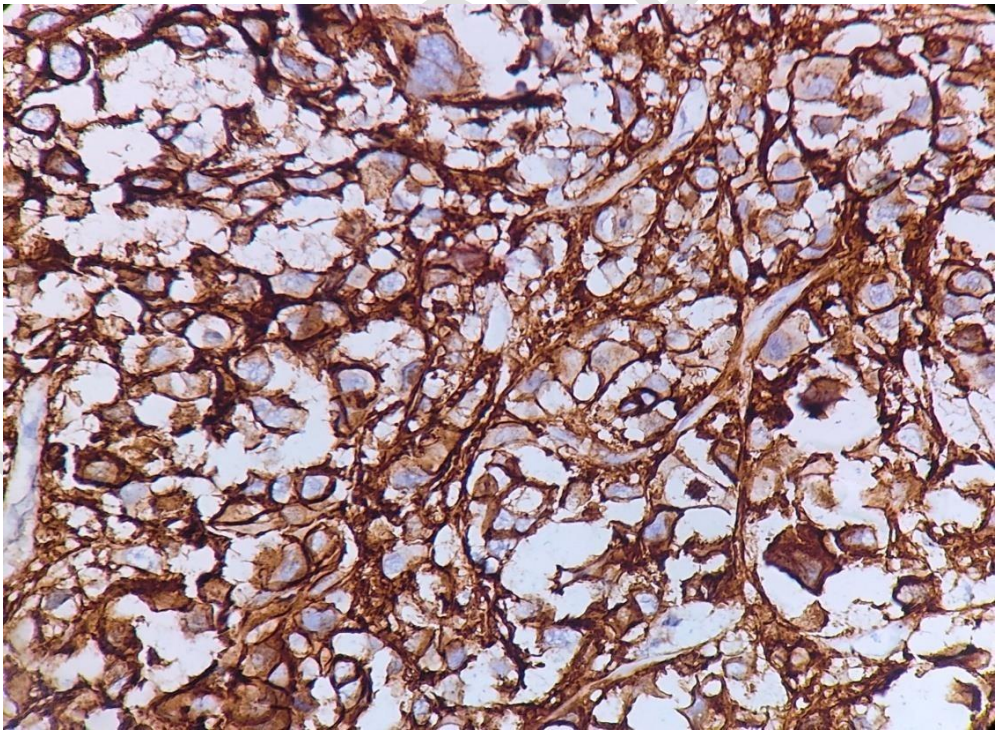

Fig 6: HER2/neu 3+ in100% of tumor cells with complete membrane Staining(Anti-

HER2/neu-polyhorseradishperoxidase-DABchromogen,40X)

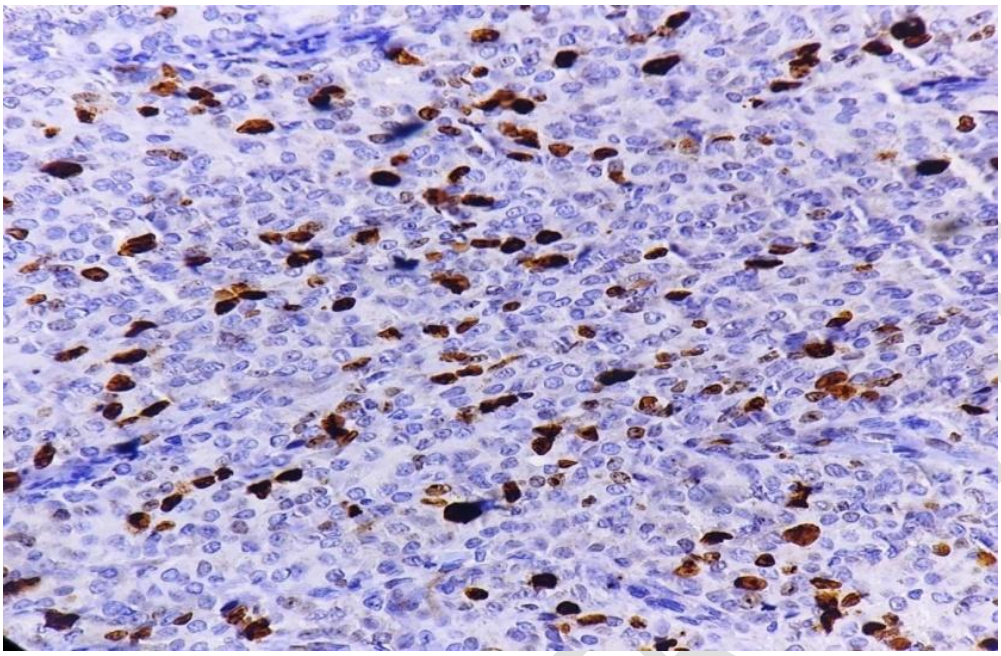

Fig 7: Ki-67 positivity in 40% of tumor cells nuclei (MIB1Antibody-polyhorseradish peroxidase-DAB chromogen,x100)

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TABLE 1: ER, PR AND HER2/NEU STATUS IN RELATION TO AGE

AGE	ER+PR+ Her2+	ER-PR- &Her2+	ER+PR- &Her2-	ER+PR+ &Her2-	ER-PR- &Her2-	Total
<= 30 years	1	2	0	2	5	10
	10.0%	20.0%	.0%	20.0%	50.0%	100.0%
31-40 year	5	4	4	10	13	36
	13.9%	11.1%	11.1%	27.8%	36.1%	100.0%
41-50 year	3	14	3	14	11	45
	6.7%	31.1%	6.7%	31.1%	24.4%	100.0%
51-60 year	0	4	7	7	10	28
	.0%	14.3%	25.0%	25.0%	35.7%	100.0%
61-70 year	2	6	4	7	6	25
	8.0%	24.0%	16.0%	28.0%	24.0%	100.0%
>70 year	0	3	0	1	2	6
	.0%	50.0%	.0%	16.7%	33.3%	100.0%
Total	11	33	18	41	47	150
	7.3%	22.0%	12.0%	27.3%	31.3%	100.0%

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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	χ^2 value	Pvalue
GRADEI	1	0	5	10	0	16	50.092	0.00 (significant)
	6.2%	.0%	31.2%	62.5%	.0%	100.0%		
GRADEII	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%		

TABLE 3 :ER, PR AND HER2/neu STATUS IN RELATION TO THE LYMPHNODE STATUS

Lymph nodemetastsis	ER+PR+Her2+	ER-PR-&Her2+	ER+PR-&Her2-	ER+P+&Her2-	ER-PR-&Her2-	TOTAL	χ^2value	Pvalue
	3	2	5	4	10	24		

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Present	12.5%	8.3%	20.8%	16.7%	41.7%	100.0%		
Absent	2	1	1	4	8	16		
	12.5%	6.2%	6.2%	25.0%	50.0%	100.0%	1.898	0.754
TOTAL	5	3	6	8	18	40		
	12.5%	7.5%	15.0%	20.0%	45.0%	100.0%		

TABLE 4 : FREQUENCY OF ER AND PR POSITIVITY IN PRE AND POSTMENOPAUSALWOMEN

	ER+&PR+	**ER+&PR-**	**ER-&PR-**	**Total**	**χ^2 Value**	**p-value**
premenopausal	26	10	45	81		
	32.1%	12.3%	55.6%	100.0%	0.444	0.801 (not significant)
Postmenopausal	23	9	32	64		
	35.9%	14.1%	50.0%	100.0%		
Total	49	19	77	145		
	33.8%	13.1%	53.1%	100.0%		

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