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Pathology

A CROSS SECTIONAL STUDY OF BREAST CANCER HISTOMORPHOLOGY AND ITS CORRELATION WITH ER/PR, HER 2NEU AND KI-67 EXPRESSION IN A TERTIARY CARE HOSPITAL AT KANPUR

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ABSTRACT **BACKGROUND** Breast carcinoma is the most well-known malignancy in women. Different predictive and prognostic factors, for example, estrogen receptors (ER), progesterone receptors (PR) and human epidermal growth factor receptor (Her2neu), and Ki67 could influence breast carcinoma behaviour, yet to date no authoritative connection has been set up among them and breast carcinoma subtypes. In this way present study was done to determine the interrelationships of these predictive and prognostic factors for breast carcinoma.

METHODS In this cross sectional study, a total of 50 lumpectomy, modified radical mastectomy specimens of diagnosed carcinoma breast were included in this study. The histopathological grading of the breast carcinoma was performed by Nottingham modification of the Bloom Richardson grading system. All the cases went through immunohistochemistry for ER, PR, Her2neu and Ki67 expression. Association of ER, PR, Her2neu and Ki67 with different histomorphology was established.

RESULTS The ER positivity was significantly lower in tumors >5 cm size whereas Ki67was significantly increased with increased tumor size. The ER positivity was significantly lower in high grade tumors as compared to low grade tumors. The positive ER, PR, Her2neu and Ki67 were comparable in between premenopausal and post-menopausal age groups

CONCLUSION The present study concludes that ER, PR show inverse while ki67 showed a direct relationship with the tumor grade. Correlation of histomorphology of breast tumor and Her2neu status could not be established.

BACKGROUND

Breast cancer is one of the most widely recognized disease and causes the most noteworthy number of disease related deaths among ladies. The death rate has been fundamentally decreased lately due to its early determination and the latest treatment modalities for treatment; but it is as yet a major reason of death from malignant growth in ladies in European and Western nations, just behind lung cancer.[1,2] In 2018 around 20,88,849 new cases were accounted for and 6,26,679 ladies died of breast carcinoma, globally.[3] As indicated by the worldwide malignant growth observatory information, breast cancer has been positioned as the most well-known malignant growth among Indian females with 1,62,468 new cases and 87,090 deaths were observed.[3,4] Prior cervical malignant growth was most common in Indian women however now the incidence (14%) of breast malignancy has outperformed cervical cancer and is leading cause of malignant growth demise, albeit cervical disease actually stays generally in rural India.[5] The patient's outcome can be anticipated through prognostic factors. Various predictive and prognostic factors affect tumor progression.[4-6] Predictive factors are distinguished from prognostic factors in that the latter can be measured and are associated with the nature of the disease, whereas the former determine the response to treatments.[4] The patient's result can be predicted through prognostic elements. Tumor markers are utilized for the identification of danger, screening, diagnosis, staging and prognosis. It can likewise anticipate the therapy to treatment, screen treatment, recognize the presence of mysterious metastatic sickness and screen the course of the disease.[9]Immunohisto chemical articulation of tumor markers like estrogen and progesterone receptors, Ki-67, p53, cytokeratins, Her-2/neu, PCNA (Proliferating Cell Nuclear Antigen) etc assist to evaluate the tumor status.[10] Estrogen has impact on target tissues by binding to portions of cells called estrogen receptors and along these lines assume a significant role in the development and growth.[11] Estrogen causes tumorigenesis by binding to the estrogen receptor (ER) which causes multiplication of mammary cells prompting increment in cell division and DNA replication, bringing about transformations. The aftereffect of the two cycles is interruption of cell cycle, apoptosis and DNA repair and consequently, tumor formation.[12]ER positive breast malignancies for the most part have a superior anticipation and are regularly receptive to hormone therapy.[13]

Progesterone receptor (PR) is an intracellular steroid that explicitly ties progesterone communicated by a Chr 11 q 22 gene. Estrogen is important to initiate progesterone receptors. PR alongside ER is expressed in different histological kinds of breast cancer.[14] In Indian population, the positivity of PR is around 46% which is significant lower than those detailed in the Western literature revealed as around 79%.[15,16]Ki-67 is a non-histone protein that is available during the late G1, S, G2 and M periods of the cell cycle, mirroring the extent of cell proliferation.[17] Ki 67 articulation is likewise utilized for partitioning luminal-like breast cancer into luminal A and luminal B groups.[18] It has additionally been exhibited that high Ki-67 expression is related with a higher danger of recurrence and poor outcome in early breast cancer patients.[19]

In this study we aim to estimate the estrogen receptor (ER), progesterone receptor (PR), Human Epidermal growth factor Receptor 2 (HER-2neu) and Ki67 markers in breast cancer patients and their correlation with different histomorphologies.

AIMS & OBJECTIVES
1. To analyze histomorphology and lymph node status in breast carcinoma
2. To analyze the status of estrogen receptor(ER), progesterone receptor (PR), Ki-67 and human epidermal growth factor receptor-2 (HER2neu) in breast carcinoma with respect to histomorphology and lymph node status.

METHODS

This cross sectional was conducted in the Department of Pathology, GSVM Medical College Kanpur. Total of 50 lumpectomy, and modified radical mastectomy specimens of diagnosed carcinoma breast were included in this study based on convenient sampling. This study was conducted from Jan 2019 to September 2020. Patients with metastatic malignancy of breast, already treated for contralateral breast cancer and not willing to give written consent were excluded from the study.

In this study the histomorphology and estrogen receptor (ER), progesterone receptor (PR), Human Epidermal growth factor Receptor 2 (HER-2neu) and Ki67 markers were estimated. Specimen received in 10% formalin and furthermore put the specimen in 10% formalin after loafing for 6-8 hr. Take the samples from the nipple - areola complex, every one of the four edge (mark it referenced), base, growth and lymph nodes (if received) and send the cassettes for tissue processing and block formation. After the development of blocks, the

paraffin sections of 5μm thickness were stained by haematoxylin and eosin (H & E) for histopathological study.

Patient's clinical information was collected from histopathological requests and clinic records. This was a prospective study, all the histopathological slides were recovered and slides were assessed for composing and evaluating the tumor. The demonstrating blocks were chosen and sent to a standard research center for ER, PR, Her2neu and Ki67 in batches. Positive and negative controls were incorporated for each group. The association of ER, PR, Her2neu and Ki67 with different histomorphology was established

Histopathological type, stage of tumour, grade, progesterone receptor (PR), estrogen receptor(ER), and Human Epidermal growth factor Receptor 2 (HER-2 neu, Ki67) markers were used as assessment tools. The Scarff-Bloom-Richardson standard grading system was utilized for histopathological grading of the breast carcinoma. The tumors were graded as I, II, or III depending on the arrangement or non-formation of tubules, their nuclear pleomorphism, and the quantity of mitosis. Grade 1 is characterized as a well-differentiated tumor with the best prognosis, grade 2 is moderately differentiated and grade 3 is an exceedingly poorly differentiated tumor.

Interpretation of the IHC Scoring was Carried as Given Below
Allred scoring framework was utilized for ER and PR (a negative outcome was characterized as a score of 0 or 2 and positive between 3 - 8) (Table 1).[20-22]

Table 1. Allred Scoring System

	Percentage of Positive Cells		The Intensity of the Stain
0	No positive cells	0	No detectable stain
1	Positive cells <1%	1	Weak nuclear stain
2	Positive cells 2-10%	2	Moderate nuclear stain
3	Positive cells 11- 33%	3	Strong nuclear stain
4	Positive cells 34-66%		
5	Positive cells >66%		

Her2neu scoring of IHC slides was done as per the ASCO/CAP 2018 guidelines (American Society of Clinical Oncology and the College of American Pathologists) and US Food and Drug Administration-approved Herceptest score and cases were classified into scores of 0, 1+, 2+ and 3+ [23]

The level of Ki-67 positive cells < 15% was assigned as low articulation, while ≥ 15% was medium/high articulation.[24]

STATISTICAL ANALYSIS
The data was expressed as mean and standard deviation (SD) and percentage as appropriate. All the categorical data was compared by using chi square test/ Fischer's exact test. The p-value <0.05 will be considered as significant. The statistical analysis was done using SPSS 21.0 version (Chicago, Inc., USA) windows software.

RESULTS
Table 1 shows the baseline characteristics of the infiltrating breast carcinoma patients. Out of 50, total 7 (14.0%) patients had ≤40 years of age and 43 (86.0%) patients were >40 years old. Total 23 (46%) patients were premenopausal and 27 (54%) patients were post-menopausal status. On the basis of tumor size, a total 4 (8.00%), 38 (76.00%) and 8 (16.00%) patients had <2 cm, 2-5 cm and >5 cm of tumor, respectively. On the basis of histological types, total 46 (92.00%), 2 (4.00%), 1 (2.00%), and 1 (2.00%) patients were invasive ductal carcinoma, invasive lobular carcinoma, medullary carcinoma and mucinous carcinoma, respectively. The histological grade I, grade II and grade III were found in 4 (8.00%), 31 (62.00%) and 15 (30.00%) patients, respectively. The lymph node not identified, negative, positive with 1-3 nodes and 4 or more were 7 (14.00%), 16 (32.00%), 17 (34.00%), and 10 (20.00%), respectively

Table 2. Baseline Characteristics of the Infiltrating Breast Carcinoma Patients

Parameter	Category	n	%
Age	≤40 years	7	14.00
	>40 years	43	86.00
Menopausal status	Premenopausal	23	46.00
	Post-menopausal	27	54.00
Tumor size (in cm)	< 2 cm	4	8.00
	2– 5 cm	38	76.00
	>5 cm	8	16.00
Histological types	Invasive ductal carcinoma	46	92.00
	Invasive lobular carcinoma	2	4.00
	Medullary carcinoma	1	2.00
	Mucinous carcinoma	1	2.00
Histological Grades	Grade I	4	8.00
	Grade II	31	62.00
	Grade III	15	30.00
Lymph node status	Not identified	7	14.00
	Negative	16	32.00
	Positive (1-3 nodes)	17	34.00
	Positive (4 or more)	10	20.00

Out of 50, total 34 (68%) patients were ER positivity, 19 (38%) patients were PR positive, 7 (14%) patients were Her2neu positivity. Comparisons of ER, PR Her2neu and Ki67 test outcomes in between premenopausal and post menopausal age groups are shown in Table 2. The positive ER, PR, Her2neu and Ki6 were 55%, 26.09%, 9.09 and 17.39% in perimenopausal age group and 76.67%, 48.15%, 17.86% and 40.74% in post-menopausal age group, respectively. On the basis of distribution of frequencies of positive ER, PR, Her2neu and Ki67 were comparable in between premenopausal and post-menopausal age groups.

Table 3. Comparisons of ER, PR Her2neu and Ki67 test outcome in between premenopausal and post-menopausal age groups

Parameter	Category	Perimenopausal age Group		Post-menopausal age Group		p value
		n	%	n	%	
ER	Positive	11	55.00	23	76.67	0.194
	Negative	9	45.00	7	23.33	
PR	Positive	6	26.09	13	48.15	0.190
	Negative	17	73.91	14	51.85	
Her2neu	Positive	2	9.09	5	17.86	0.634
	Negative	20	90.91	23	82.14	
Ki67	Low (<15%)	4	17.39	11	40.74	0.137
	High (≥15%)	19	82.61	16	59.26	

Note: Fisher's exact test was applied where the expected frequencies were less than 5 in one of the cells

Comparisons of ER, PR Her2neu and Ki67 test outcome in between different tumor sizes are shown in Table 3. The positive ER, PR, and Her2neu were 75%, 75%, and 25% patients had <2 cm tumor size, 76.32%, 36.84%, and 10.53% patients had 2-5 cm tumor size and 25%, 25% and 25% patients had >5cm in post-menopausal age group, respectively. Moreover, low Ki67 levels were found in 75%, 31.58% and 0.0% patients in <2 cm, 2-5 cm and >5 cm tumor size respectively. The frequencies of ER positive were significantly lower with increase in >5 cm tumor size whereas the frequency of high Ki67 was significantly increased with increased tumor size. The frequencies of positive PR, and Her2neu were not significantly different with increased tumor size.

Table 4. Comparisons of ER, PR Her2neu and Ki67 Test outcome in between different Tumor Size

		< 2 cm (n=4)		2-5 cm (n=38)		>5 cm (n=8)		P-Value
		n	%	n	%	n	%	
ER	Positive	3	75.00	29	76.32	2	25.00	0.018[*]
	Negative	1	25.00	9	23.68	6	75.00	
PR	Positive	3	75.00	14	36.84	2	25.00	0.232
	Negative	1	25.00	24	63.16	6	75.00	
Her2neu	Positive	1	25.00	4	10.53	2	25.00	0.452
	Negative	3	75.00	34	89.47	6	75.00	
Ki67	Low (<15%)	3	75.00	12	31.58	0	0.00	0.026[*]
	High (≥15%)	1	25.00	26	68.42	8	100.00	

Note: Fisher's exact test was applied where the expected frequencies were less than 5 in one of the cells
[*]=Significant (p<0.05)

Comparisons of ER, PR, Her2neu and Ki67 test outcomes in between low (Grade I+II) and high grade (Grade III) tumors are shown in Table 4. The frequencies of positive ER were significantly lower in high grade as compared to low grade. Whereas the frequencies of PR, Her2neu and Ki67 were comparable in between low (Grade I+II) and high grade (Grade III) tumors.

Table 5. Comparisons of ER, PR, Her2neu and Ki67 test outcome in between Low (Grade I+II) and High Grade (Grade III) Tumor

		Grade I+II		Grade III		p
		n	%	n	%	
ER	Positive	30	88.24	5	31.25	0.001*
	Negative	4	11.76	11	68.75	
PR	Positive	15	78.95	20	64.52	0.446
	Negative	4	21.05	11	35.48	
Her2neu	Positive	6	85.71	29	67.44	0.594
	Negative	1	14.29	14	32.56	
Ki67	Low (<15%)	12	80.00	23	65.71	0.501
	High (≥15%)	3	20.00	12	34.29	

*=Significant (p<0.05)

Comparisons of ER, PR, Her2neu and Ki67 test outcome in between lymph nodes involved and not involved are shown in Table 5. The frequencies of positive ER, PR, Her2neu and Ki67 (low and high) were comparable in between lymph node involved and not involved.

Table 6. Comparisons of ER, PR Her2neu and Ki67 test outcome in between Lymph Node Involved and not Involved

		Lymph Node Involved		Lymph Node not Involved		Significant
		n	%	n	%	
ER	Positive	12	70.59	13	48.15	0.160
	Negative	4	23.53	14	51.85	
PR	Positive	2	11.76	10	37.04	0.167
	Negative	14	82.35	17	62.96	
Her2neu	Positive	2	11.76	4	14.81	0.832
	Negative	14	82.35	23	85.19	
Ki67	Low (<15%)	3	17.65	5	18.52	0.985
	High (≥15%)	13	76.47	22	81.48	

Note: Fisher's exact test was applied where the expected frequencies were less than 5 in one of the cells

DISCUSSION

This study comprised 50 cases of invasive breast carcinoma with maximum incidence of breast cancer (56%) was observed in the age-group of 41-50 years. Breast cancer is most common among women worldwide; its incidence is expected to increase by 26% by 2020 in developing countries.[25] In India, the average age of developing breast cancer has shifted over the last few decades and younger women (40-50 yr) are being affected. The lifestyle factors such as late age at marriage, reduced breast feeding, and westernization of diet may be associated with occurrence of breast cancer in the younger population in India. In our study 86% of patients belong to the more than 40 years age group. Similarly, Naggada et al. (2008) and Anders et al. (2009), reported that the increased age of women was significantly associated with increased risk of breast cancer. Previously a study reported that the maximum patients (36.1%) are in the 40-49 years age group.[26,27] Reddy et al. (2020) demonstrated that the maximum number of cases belong to 41-60 years (58% of patients), mean age of the patients being 49.52 years.[28]

In our study, out of 50 cases, total 23 (46% were premenopausal and 27 (54%) were post-menopausal. Similarly, Reddy et al. (2020) reported that the incidence of breast carcinoma was high in post-menopausal patients (54.0%). Another study also reported that the 48% patients were premenopausal and 52% had reached menopause.[29] Sebastiani et al. (2016) also reported that the body mass index was significantly associated with risk of postmenopausal breast cancer. Furthermore among post-menopausal obese women were at significantly higher risk.[30] In the present study most common histological type is invasive ductal carcinoma (NOS) comprises 96% of total cases. Similarly, Reddy et al. (2014) reported that the most common pathological type of carcinoma is IDC NOS (92%). Various other studies also

demonstrated that the NOS were most common histological type (more than 92%) Juneja et al (92.72%), Bhagat et al (94.82%).[31,32] In this study, we found that the total 62% cases belonged to grade II of Bloom Richardson grading, while (30%) and (8%) cases belonged to grade III and grade I respectively. It was similar to study conducted by Suvarchala et al. (2011), Sofi et al. (2012), Bhagat et al. (2012), who also recorded grade II as the predominant grade in 42.1%, 52.1%, 43.1% and 59% cases [33-35]. Grade of the tumor correlated inversely with the hormone receptor positivity. Grade I tumors were predominantly ER positive PR positive, grade III tumors were predominantly ER negative PR negative. In our study, a total of 38 (76%) cases had tumor size 2-5 cm, 8 (16%) cases had tumor size > 5 cm, 4 (8%) cases had tumor size less than 2 cm. Nabi et al. (2016) study showed that mean size of the lesion was 3.3 cm.[36] Majority of cases (57.5%) had tumor size of 2 to 5 cm. Pinto et al. (2001) also showed that one hundred and twenty tumours (39.2%) were pT1 (< 2 cm), 163 (53.3%) pT2 (2-5 cm), and 23 (7.5%) pT3 (>5 cm) that correlates with our study in which maximum number of cases were between 2 to 5 cms in size.[37] Cheang et al. (2009) also reported that maximum cases belong to tumor size between 2 to 5 centimeters which correlates with our study.[38] In our study out of 50 cases, total 27 cases were metastatic lymph node positive. Out of 27 cases, 10 cases were having 4 or more metastatic lymph nodes. In developed countries, in the majority of patients lymph node involvement was not present, but studies carried out in India documented a greater percentage of breast carcinoma with lymph nodal metastasis. In study done by Tiwari et al. (2015) cases with lymph node involvement was 74.3%.[39] Similarly, Munjal et al. (2009) and Ambroise et al. (2011) also reported that the lymph node involvement were more common.[40,41] In our study we found that the total 68% cases were ER positive and 32% cases were ER negative. Whereas total 38% cases were PR positive and 62% cases were PR negative. Total 14% cases were Her 2 neu positive cases and 86% cases were her 2 neu negative. These data are similar to study done by Nadji et al. (2005) most of the grade I and grade II tumor are ER positive and most of the cases of the grade III are ER negative.[42] Most of the grade I and grade II tumor were PR positive and most of the cases of the grade III were PR negative. It was observed that in grade III tumors 75% cases had unfavorable hormone receptor status, in contrast to grade I and grade II tumors which showed association with favorable hormone receptor status.

Receptor statuses and Ki67 expression showed no significant correlation with histological types in the present study because IDC NOS was the most predominant histological type of tumor contributing to 92% of all the types. It is similar to the study done by Thiygarajan et al. (2015).[25] Her2neu expression had no correlation with tumor grade in the current study which was similar to the studies done by Thiygarajan et al. (2005), Almari et al. (2007) and Barrios et al. (2008).[25,43,44] High Ki67 proliferation index was significantly correlated with a higher grade of the tumor in the present study similar to the studies done by Haroon et al. (2013), Inwald et al. (2012), Peng et al. (2012, Han et al. (2011) and Rhee et al. (2008).[45-49] ER/PR receptor status had not much of a correlation with nodal status in the current study similar to the observation in studies like Thiygarajan et al. (2015), Azizun- Nisa et al. (2008) and Fatima et al.[25,50,51] In our study, there was not much of a correlation between the nodal status and Her2neu status, in contrast, to studies done by Thiygarajan et al. (2015), Azizun- Nisa et al. (2008) and Naqvi et al. (2002) where a significant correlation was found between Her2neu over expression and lymph node status.[25,50,52] The high Ki67 expression was seen in lymph node metastasis positive cases similar to the studies done by Peng, (2012), Han et al. (2011) and Rhee et al. (2008).[47-49] Ki67 is a proliferative marker and its expression is seen in higher grade tumors and carries poor prognosis whereas ER/PR expression is associated with lower grade tumor and has a better prognosis. So all breast cancer patients should be subjected to all 4 markers (ER, PR, Her2neu and Ki67) where ER, PR, Her2neu expression defines the line of treatment and Ki67 expression defines the prognosis. Especially high Ki67 expression in ER, PR negative cases carries a poor prognosis. Breast cancer with high Ki67 expression responds better to chemotherapy.

CONCLUSION

The ER positivity was significantly lower with increase in tumor size whereas the frequency of high Ki6 was significantly increased with increased tumor size. The positivity of PR and Her2neu were not significantly different with increased tumor size. Moreover, the positive ER was significantly lower in high grade as compared to low grade. Whereas the PR, Her2neu and Ki6 were comparable in between low (Grade I+II) and high grade (Grade III) tumors. Ki 67 can be considered as another prognostic marker. Expanded Ki67 proliferative

file is related with expanded mitotic action and less expression of ER, PR; subsequently convey a poor prognosis and requires treatment likewise. Most normal histological type is invasive ductal carcinoma (NOS). Lymph Node metastasis was more common in high grade tumors. Correlation of histomorphology of breast tumor and Her2neu status couldn't be set up decisively.

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