Research Article

Assessment of ER, PR & HER2-neu in carcinoma breast.

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INTRODUCTION

Breast carcinoma is the commonest malignancy among women around the world.[1] Annually many cases of breast carcinoma are reported worldwide.[2] In India, it has become the most common carcinoma and replaced the cervical carcinoma in females, mainly in the urban areas.[3] Every year the incidence of breast carcinoma is increasing in India.[4] In India, most patients with breast carcinoma belong to middle to old age group.[5] The incidence of breast carcinoma increases with age and reaches its peak at menopausal age. Various histopathological parameters have prognostic significance in breast carcinoma. This includes histologic subtype, grade, lymph node status, Estrogen receptor/Progesterone receptor status, growth factor and its receptors.[6] It has been found that the assessments of immunohistochemical markers have assumed a significant role in influencing the clinical decisions about the treatment and it is helpful in accessing the prognosis of the patients in breast carcinoma.

Currently, neo-adjuvant chemotherapy has become the standard approach for locally advanced breast tumors as it helps to shrink the tumor in the early stage of carcinoma and make it convenient for breast conservative surgery.

Chemotherapy is also the mainstay treatment for almost all patients of metastatic breast carcinoma.[7] Several markers such as Estrogen receptor, Progesterone receptor and Human epidermal growth factor receptor 2-neu (HER2-neu) and their expressions have been used to study the breast carcinoma. Assessment of the status of these tissue markers have significant role in accessing the diagnosis, treatment and prognosis of breast carcinoma in patients.

Hormonal markers such as Estrogen receptor and Progesterone receptor are important and useful predictive factor in breast carcinoma. In invasive breast carcinoma whose tumor cells lack Estrogen Receptor/Progesterone Receptor, they do not respond to hormonal therapy. Their status also has a prognostic value. Patients with ER/PR positivity has low risk of mortality in comparison to the patients with ER+/PR- or ER-/PR+ or both negative.[8,9]

HER2-neu is also known as Epidermal growth factor receptor 2 (ERBB2). It has gained an importance as a significant prognostic marker.[10] Its amplification and over expression is associated with the poor prognosis in breast carcinoma patients with axillary lymph node metastases but there is no association with negative lymph nodes.[11] HER2-neu can also be a predictive marker.[12]

This study was planned with the aim of assessing the expressions of ER, PR, HER2-neu and its correlation with tumor grade & stage in breast carcinoma.

MATERIAL & METHODS

This study was conducted in the Department of pathology, Era's Lucknow Medical College & Hospital, Lucknow. 32 cases of breast carcinoma were taken which underwent Modified radical mastectomy. Tissue processing was done by fixing the tissues in 10% buffered formalin overnight. The tissues were grossed and representative sections were taken and submitted for processing. The processed tissues were embedded into paraffin wax blocks and sections were taken onto the slides and routine H&E staining was done for histological diagnosis. Grading was done according to modified Bloom Richardson grading system of WHO.[35,36] Most suitable tissue block was selected for ER, PR and HER2-neu markers on formalin fixed paraffin embedded tissue. The ER/PR expression shows the amount of estrogen receptors (ER) and progesterone receptors (PR) present in tumor cells. HER2-neu assay measures the amount of HER2-neu staining present on the membrane of tumor cells. Allred's scoring was used for IHC.[37] Statistical Analysis was done by using statistical software Med Calc and the data were expressed as percentages.

RESULTS

Table 1: Demographic details of the population under the study

Age (mean value)	46.4±12.7
Gender	FEMALE – 32 MALE – 0
Hormonal Therapy	5 Cases with chemotherapy before MRM
Menopausal	25 Cases [78.1%] out of 32 Cases
Parity	10 Nulliparous [31.2%] 22 Multiparous [68.7%]

Table 2: Histopathological variants of Breast Carcinoma cases under study

DIAGNOSIS	TOTAL NUMBER – 32 cases
Infiltrating Ductal Carcinoma	27 cases
Lobular Carcinoma	3 cases
Mucinous Carcinoma	1 case
Medullary Carcinoma	1 case

Table 3: Clinical features of cases under study

Laterality	Left Breast - 12 cases [37.5%] Right Breast - 20 cases [62.5%]
Lymph node Status	Nx - 2 cases N0 - 11 cases N1 - 6 cases N2 - 7 cases N3 - 6 cases
Lymphovascular invasion	10 cases [31.2%]

Table 4: IHC Status of cases under study

IHC MARKER STATUS	NUMBER OF CASES
ER positive	7 cases [21.8%] out of 32
PR positive	7 cases [21.8%] out of 32
HER2neu positive	6 cases [18.7%] out of 32
Triple negative	18 cases [56.2%] out of 32
Triple positive	1 case [3.1%] out of 32

Table 5: Luminal classification of cases under study

Types	Number of Cases	Diagnosis
Luminal A [ER+/PR+;HER2neu -]	7	IDC- 4 Lobular - 2 Mucinous – 1
Luminal B [ER+/PR+;HER2neu +]	1	Lobular – 1
HER2-neu positive [ER-/PR-;HER2neu+]	6	IDC – 6
Triple Negative [ER-/PR-;HER2neu -]	18	IDC – 17 Medullary – 1

Table 6: Association of Tumor Grade with ER/PR and HER2-neu Positivity

Grade	Number of Cases (32)	ER positive	PR positive	HER2neu positive
Grade 1	4 [12.5%]	3 cases out of 4 [75%]	3 cases out of 4 [75%]	1 case out of 4 [25%]
Grade 2	22 [68.7%]	5 cases out of 22 [22.7%]	5 cases out of 22 [22.7%]	5 cases out of 22 [22.7%]
Grade 3	6 [18.7%]	-	-	1 case out of 6 [16.6%]

Table 7: Association of Tumor Stage with ER/PR and HER2-neu Positivity

Stage	Number of Cases (32)	ER+	PR+	HER2-neu+
Stage 2	17 (53.1%)	5 cases out of 17 (29.4%)	5 cases out of 17 (29.4%)	4 cases out of 17 (23.5%)
Stage 3	15 (46.8%)	3 cases out of 15 (20%)	3 cases out of 15 (20%)	3 cases out of 15 (20%)

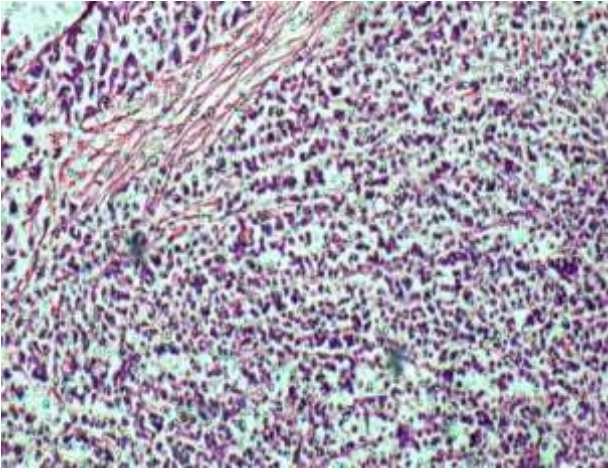**Note- No case of Stage 1 and Stage 4 was found in our study.**

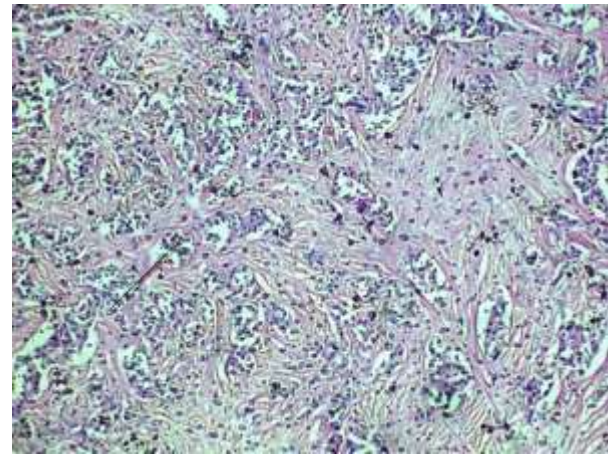**Figure 1: INFILTRATING DUCTAL CARCINOMA**
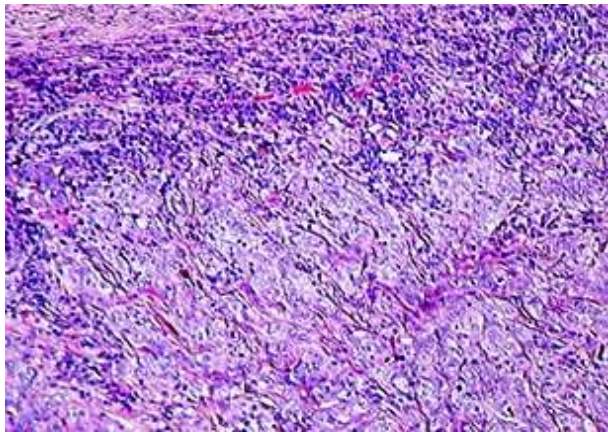

Figure 2: LOBULAR CARCINOMA

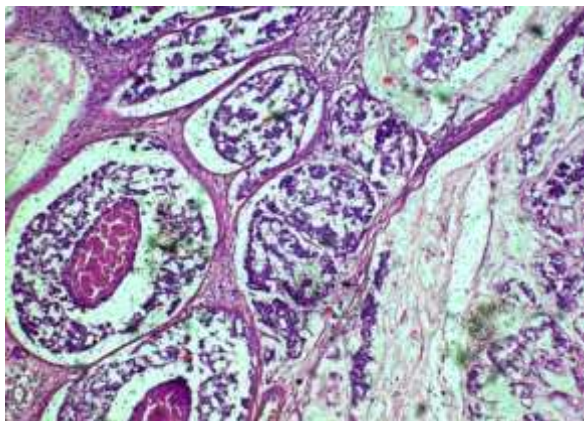**Figure 3: MEDULLARY CARCINOMA**
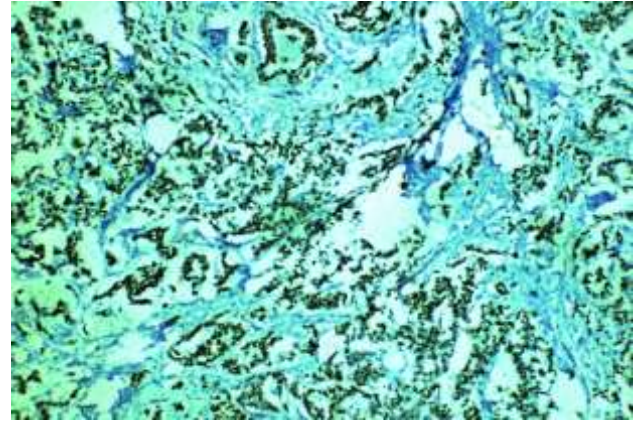

Figure 4: MUCINOUS CARCINOMA
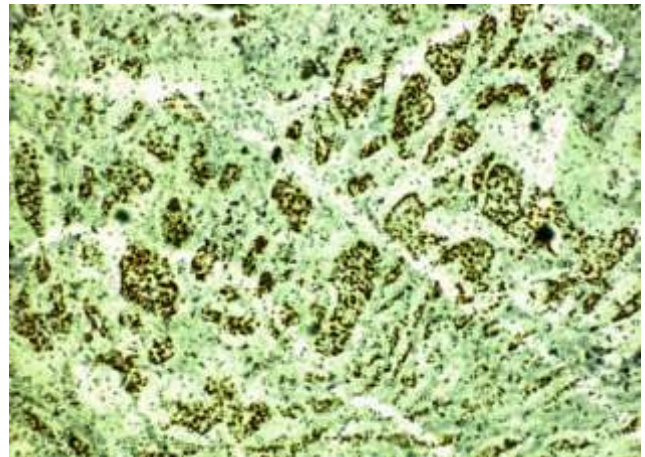

Figure 5: ER POSITIVITY
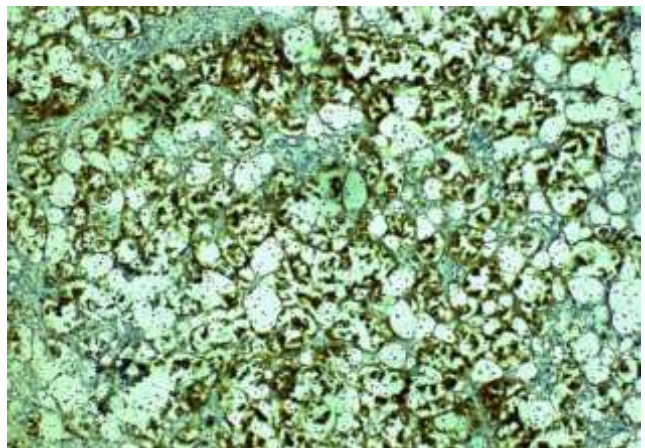

Figure 6: PR POSITIVITY

Figure 7:HER2-neu POSITIVITY

DISCUSSION

Breast carcinoma is the second most common cancer in the world and the most common cancer in females, with high incidence and increasing mortality rate every year.[13] The WHO classification of breast carcinoma not only covers epithelial tumors like invasive breast carcinomas but also their precursor lesions, benign epithelial proliferations, myoepithelial, fibroepithelial and mesenchymal tumors.[14] The expressions of the ER/PR and HER2-neu in breast carcinoma has become an integral part of histopathological evaluation as it has both predictive and prognostic importance in addition to histological subtype, grade and lymph node status. This study was done to view the correlation of the

expression of ER/PR and HER2-neu with histological subtypes, grade and lymph node status.

Estrogen receptors are a group of proteins that are found in breast tissue. These receptors are activated by the hormone estrogen and its exposure is a risk factor for breast carcinoma.[15] It is a predictive marker and has significant therapeutic effects.[16] It consists of two subtypes such as ER-alpha and ER-beta.[17] ER-alpha is expressed in majority of the breast carcinoma cases. However, its predictive value is not ideal, as around one-third of ER+ breast carcinoma patients show no response to the hormonal therapy.[18]

Progesterone receptors are also an estrogen-regulated protein found in the cells of breast tissue. Therefore its expression is believed to function as in ER pathway.[19] Assessment of both ER and PR is helpful in predicting response to hormonal therapy more accurately. There are few proposals which indicate that PR positive tumors are more likely to respond to tamoxifen.[20] The predictive value of PR positivity in the absence of ER is controversial.[21] Breast tumors which are ER+ and/or PR+ show low mortality risk in comparison with ER- and/or PR- tumors.

Human epidermal growth factor receptor 2 (HER-2)

The human epidermal growth factor receptor 2 is a member of epidermal growth factor receptor (EGFR) family of tyrosine kinases. It is located on chromosome 17q21.[22] It is a protein found in humans which is encoded by ERBB2 gene and called as HER2-neu. Amplification or over-expression of HER2-neu has been seen in 18–20% of breast cancers.[23] It plays an important role in the development and progression of certain aggressive types of breast carcinoma. HER2-neu positivity is associated with high grade tumors, lymph node involvement, mortality and poor prognosis.[24] HER2-neu expression is a predictive factor in response to trastuzumab [Herceptin] therapies.[25] HER-2 positivity may also predict for resistance to hormonal therapy.

Molecular breast carcinoma classification is into four groups:[38]

1. ER/PR+,HER2-neu+ = ER+/PR+,HER2-neu+;ER-/PR+,HER2-neu+; ER+/PR,HER2-neu+ ;
2. ER/PR+,HER2-neu−=ER+/PR+,HER2-neu−;ER-/PR+,HER2-neu−; ER+/PR,HER2neu−;
3. ER/PR-,HER2-neu+ = ER-/PR-, HER2-neu+
4. ER/PR-,HER2-neu− = ER-/PR-, HER2-neu−

The classification of Molecular subtypes of breast carcinoma in correlation with IHC expression:

- Luminal A- ER/PR+, HER2-neu− ;
- Luminal B- ER/PR+, HER2-neu+ ;
- HER2 enriched- ER/PR−, HER2-neu+;
- Triple negative- ER/PR−,HER2-neu−.

This study was conducted in Era's Lucknow Medical College & Hospital, Lucknow, India. In this, we studied the varied presentations of breast carcinoma and association of ER, PR and HER2-neu with grade and stage. The study comprised of 32 cases of breast carcinoma out of which; 27 cases were of Infiltrating Ductal Carcinoma [IDC] [84.3%], 3 cases of Lobular carcinoma [9.3%], 1 case of Mucinous carcinoma [3.1%] and 1 case of Medullary carcinoma [3.1%]. Our findings were similar to other studies done by, Bhagat et al [26], Adedayo et al [27] and Ghosh et al [28] and it was concluded that Invasive Ductal Carcinoma is most common histological subtype. Our findings were almost similar to an another study done by Nikhra et al. who saw that out of 43 cases of breast carcinoma, 41 cases were of Infiltrating ductal carcinoma (95.34%), 1 case of medullary carcinoma (2.32%) and 1 case of Invasive lobular carcinoma (2.32%). [30]

The mean age of female population in our study was 46.40 years, which was close to the age group of females in a study done in Iran, a similar study showed that the mean age of the patients was 40.2±2.3. [29] Hence, it shows that in recent years young women are more prone to the risk of developing breast carcinoma. In our study, we also saw that 12 tumors involved the left side breast [37.5%] while 20 tumors arose from the right side breast [62.5%] and most occurring in the outer upper quadrants. Whereas in a study conducted by Nikhra et al; left breast was found to be more commonly involved, comprising of 23 cases (53.4%) than right breast comprising of 20 cases (46.51%). [30]

Among the 32 cases of breast carcinoma; 4 cases were of Grade 1 [12.5%], 22 cases were of Grade 2 [68.7%] and 6 cases were of Grade 3 [18.7%]. In a study done by Bhagat et al, also showed the similar finding that the majority of the breast tumors were of Grade 2(43.10%) followed by Grade 3 (29.31%). [26]

Out of total 32 cases; 17 cases were of Stage 2 [53.1%] and 15 cases [46.8%] were of Stage 3. In Stage 2; 52.9% [9 cases] were triple negative; 23.5% [4 cases] were ER/PR positive; 17.6% [3 cases] were HER2-neu Positive and 5.8% [1case] was triple positive. In Stage 3- 60% [9 cases] were triple negative; 20% [3 cases] were HER2-neu positive and 20% [3 cases] were ER/PR positive. The Stage 3 tumors have a higher number of triple negative tumors as compared to Stage 2, which is associated with poor outcome. This result allowed us to find a correlation between ER/PR and HER2-neu status with lymph node positivity, which is also seen in the study done by Aysha S. Alzaman et al. Molecular subtypes of breast carcinoma based on their hormonal status. Breast carcinomas are more aggressive if they were HER2-neu positive or triple negative for ER, PR, and HER2-neu. [31]

HER2-neu expression is associated with poor prognosis while ER and PR expression are indicators of response to hormonal therapy and better prognosis. The triple negative subtype of breast carcinoma (ER/PR,HER2-neu) have the worst prognosis and survival rate. In our study, we found that there is an association of triple negative as well as her2neu positive tumors with high grade.

In our study, Grade 1 had 4 cases out of which 75% [3cases] showed ER/PR positivity. Grade 2 had 22 cases out of which 22.7% [5cases] showed ER/PR positivity. No case was found to be ER/PR positive in Grade 3. Our findings were similar to a study in which ER positivity was expressed 100% [2cases] in Grade I, 42.35% [11cases] in Grade II and 23.07% [3cases] in Grade III respectively and PR positivity was expressed 100% [2cases] in Grade I, 50% [13cases] in Grade II and 15.3% [2cases] in Grade III tumors respectively. [30] HER2-neu positivity in our study was expressed in 1 case [25%] of Grade 1; 5 cases [22.7%] of Grade 2 and 1 case [16.6%] of Grade 3. Our findings are contrary to the findings of another study in which HER2-neu positivity was expressed 50%, 15.3% and 69.2% in Grade I, II and III respectively. Similar results were observed by Azizun et al showing ER positivity in 70% cases in Grade I, 48.2% cases in Grade II and 3.5% cases in Grade III tumors respectively[31]. PR positivity was expressed 70 %cases in Grade I, 36.1% cases in Grade II and 1.75% cases in Grade III tumors respectively while HER2-neu

positivity was expressed in 0%, 22.9%, 31.6% in Grade I, II and III respectively. [32]

As per the size of the tumor, we saw that out of 32 cases; 21 cases [65.6%] were between 2 to 5cms and 11 cases [34.3%] were more than 5cms in size. No cases was found of tumor measuring less than 2cms. Similar results were observed by Bhagat et al (91.38%), Azizun et al (88%), Moses et al (91.6%) and Mona et al in their study[26,32-34]. While study done by Adedayo et al showed 71.4% cases with ≤ 2cm size, this could be due to early cancer detection programs[27]. In India due to lack of awareness and absence of breast cancer screening program, the majority of breast carcinomas are diagnosed at an advanced stage. [26,27]

Study by Nikhra et al have found a significant association between increasing tumor size and HER2-neu expression. [30] Tumors size more than 2cms had high rate of HER2-neu expression than those of tumors less than 2cms in size. In our study we saw that tumor size of more than 2cms were inversely related with increased HER2-neu expression with Stage 2 [29.4%] showing more expression as compared to stage 3[20%]. Similar result was also seen in Bhagat et al study.[26]

HER2-neu positivity in Nikhra et al study was 32.5% while in our study it showed 23.5% of Stage 2 and 20% of Stage 3 tumors. In study by Bhagat et al showed 27.58% positivity and Moses et al showed 27.10% positivity. This reaffirms that the Indian population has a high incidence of HER2-neu positivity. So it would be profitable to have IHC profiling of breast carcinomas in our country.[26,33]

In the present study, there was one case of Mucinous carcinoma showing ER/PR positive and HER2-neu negative and one case of Medullary carcinoma with triple negative status which matches with the findings of Nikhra et al[30] showing Medullary carcinoma with basal-like gene expression pattern with triple negativity for (ER/PR/HER2-neu), so Medullary carcinomas has poor prognosis and no response to hormonal therapy and herceptin.

In the present study, there were 3 cases of Invasive Lobular carcinoma showing 2 cases with ER/PR positive and HER2-neu negative status which matches with the study done by Nikhra et al, showing 1 case of Invasive Lobular carcinoma with luminal-A gene expression pattern with positivity for ER/PR and negativity for HER2-neu, so Lobular carcinomas has good prognosis and better response to hormonal therapy.[30]

The relation of HER2-neu with ER/PR showed inverse correlation with just 1 case showing triple positivity out of 32 cases.

Conclusion-

From our study, it was concluded that Infiltrating Ductal carcinoma (NOS) was the most common histopathological subtype of breast carcinoma. Young women are more prone to the risk of the development of breast carcinoma. Most cases of Grade 1 showed ER/PR positivity expression implying a better prognosis. As the tumor grade increases, ER/PR expression decreases and HER2-neu expression increases and Grade 3 had maximum number of triple negative cases indicating for poor prognosis. So as the Stage 3 had high number of triple negative tumors associated with poor outcome. Breast carcinoma with HER2-neu positivity or with triple negativity shows more aggressive nature. IHC improves the more prognostic accuracy into the histopathological assessment along with staging and grading of breast carcinoma.

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