

Research Article

## Study of ER (Estrogen Receptor), PR (Progesterone Receptor) & HER-2/NEU (Human Epidermal Growth Factor Receptor) expression by immunohistochemistry in breast carcinoma

Pawan Nikhra<sup>1\*</sup>, Smita Patel<sup>2</sup>, Dilip Taviad<sup>3</sup> and Shalu Chaudhary<sup>4</sup>

<sup>1</sup>Resident, Department of Pathology, Medical college & S.S.G Hospital, Vadodara, Gujarat

<sup>2</sup>Professor & Head, Department of Pathology, Medical college & S.S.G Hospital, Vadodara, Gujarat

<sup>3</sup>Tutor, Department of Pathology, Medical College & S.S.G Hospital, Vadodara, Gujarat

<sup>4</sup>Resident, Department of Pathology, Medical college & S.S.G Hospital, Vadodara, Gujarat

### \*Correspondence Info:

Dr. Pawan Nikhra,  
Resident, Department of Pathology,  
Medical College & S.S.G Hospital,  
Vadodara, Gujarat, India.  
E-mail: [pawan.nikhra11@gmail.com](mailto:pawan.nikhra11@gmail.com)

### Abstract

**Aim:** The present study was undertaken with the view of correlating the histopathology of the tumor by way of tumor grade, various traditional prognostic markers and its Immunohistochemistry profile with respect to Estrogen/Progesterone hormone receptors and Human epidermal growth factor receptor/neu status.

**Introduction:** Over the last few decades there have been outstanding advances in breast cancer management leading to early detection and treatment of disease. Recent attention has been directed to Immunohistochemistry- based classification of both Estrogen Receptor/Progesterone Receptor and Human epidermal growth factor receptor/neu status which provides prognostic and therapeutic information and is inexpensive and readily available.

**Material and Methods:** 43 patients with breast neoplasm who were diagnosed to have carcinoma breast, after undergoing preliminary FNAC/Trucut biopsy/open biopsies and subsequently underwent Modified radical mastectomy were included in the study. The surgical specimen were then evaluated histopathologically and immunohistochemically for ER, PR, HER-2/neu markers.

**Results:** Out of 43 cases studied, Majority of the cases {41cases (95.34%)} were of Invasive ductal carcinoma. Grade I tumors were seen in patients more than 60 years of age implying a better prognosis. Percentage of ER positivity was 39.5%, PR positivity was 41.8%, Her2/Neu positivity was 32.5%. Only case was triple positive for all the three markers (ER/PR+ Her2+) which was in the 71-80 yrs age group implying a better predictive response to hormonal therapy.

**Conclusion:** From the present study it was concluded that with incorporation of Immunohistochemistry-based classification of both ER/PR and HER-2/neu status into the histopathology report using the traditional TNM staging and histological grading of breast carcinoma help in better therapeutic management and increases prognostic accuracy and is inexpensive and readily available.

**Keywords:** Breast cancer, Immunohistochemistry, Estrogen receptor, Human epidermal growth factor receptor

### 1. Introduction

Worldwide breast cancer is the most common invasive malignancy in women. It comprises 22.9% of invasive cancers in women and 16% of all female cancers<sup>1</sup>. The number of cases worldwide has significantly increased since the 1970s, a phenomenon partly attributed to the modern lifestyles. In the year 2010, one million new cases were diagnosed and more than five hundred thousand lives were claimed by breast cancer globally. In India, the average age of breast cancer patients range from 44.2 years to 49.6 years.<sup>2</sup>

Over the last few decades there have been outstanding advances in breast cancer management leading to early detection of disease and the development of more effective treatments resulting in significant decline in breast cancer deaths and improved outcome for women living with the disease<sup>3</sup>. Recent attention has been directed singularly at molecular classification of breast cancer. While molecular and genetic testing is very elegant, prognostic and predictive, it is expensive and not yet widely available<sup>4</sup>.

The Immunohistochemical (IHC) classification provides both therapeutic and prognostic information, is inexpensive and readily available. IHC-based classification of both ER/PR and HER-2/neu status provides prognostic and therapeutic information not achievable from either alone. Prior classifications separating breast cancer into one of two categories based on ER expression alone is less discriminatory in terms of prognosis, and the additional sub-classification based on Her2/neu expression provides enhanced and important therapeutic guidance<sup>5</sup>.

Thus this study was carried out with the aim of helping to correlate IHC and histopathological grade of breast carcinomas using the modified Bloom-Richardson system and TNM (Tumour(T), regional lymph nodes(N) and distant metastasis(M)) staging of breast carcinoma and hence, help in therapeutic management and prognosis of breast carcinomas.<sup>6</sup>

### 2. Material and Methods

The present study was conducted at the Department of Pathology, Medical College Baroda and SSG hospital between October 2012 and October 2013 after obtaining approval from the Ethical committee. The test population comprised of 43 patients with breast neoplasm. The patients who were diagnosed to have carcinoma breast, after undergoing preliminary Fine Needle Aspiration Cytology /Trucut biopsy/ open biopsies, and subsequently underwent Modified radical mastectomy were included in the study.

## 2.1 Tissue collection

The tissues of the test population submitted as MRM (Modified Radical Mastectomy) specimen were evaluated by histopathological processing and examination (HPE). The most suitable tissue block was selected for Immunohistochemical evaluation for ER, PR and HER-2/neu markers.

## 2.2 Tissue processing

Tissues were fixed in 10% Buffered formaline overnight, for an average period of 16 hrs. The tissue was grossed and blocks were processed in the histokinette with a cycle of 24 hours, after which the processed tissue was embedded into paraffin wax blocks. The wax blocks were trimmed using the rotary microtome. Sections were taken onto slides and stained by the routine H&E stain. During the HPE reporting, the best section representing the tumor was selected for Immunohistochemistry.

## 2.3 Immunohistochemistry procedure

The Peroxidase antiperoxidase (PAP) method of Immunohistochemistry was followed. Biogenex reagents were used for the antigen retrieval and IHC staining process. Biogenex Tris- EDTA based antigen retrieval solution with a pH of 9 was used for ER, PR staining and of pH 6 for HER-2/neu staining. The heating cycles followed in the Biogenex temperature controlled microwave were two cycles of 10 minutes and 5 minutes each at 95°C, with intermittent refilling of the antigen retrieval solution. Thereafter the slides were taken through the steps of wash with TRIS buffer, peroxide block, power block and monoclonal antibodies. After this, slides were again washed in TRIS buffer, the secondary antibody exhibited; thereafter Diamino benzidine chromogen was added. The slides were washed with water, and counterstained with hematoxylin. Then slides were serially dehydrated in alcohol, cleared in xylene and thereafter mounted using Distrene dibutyl phthalate xylene. After drying, the test slides were examined along with the control sections stained simultaneously.

## 2.4 IHC Markers

The ER/PR assay measures the amount of estrogen receptors (ER) and progesterone receptors (PR) in cancer cells. Human Epidermal growth Factor-2/neu is a receptor found on the surface of breast cancer cells. It accepts stimulatory growth signals from a substance called epidermal growth factor. The HER-2/neu assay measures the amount of HER-2/neu on the surface of cancer cells. Allred method <sup>7</sup> of ER/PR scoring system and criteria was used in this study.

## 2.5 Statistical Analysis:

Analysis was done by using statistical software Med Calc and Data were expressed as percentages.

## 3. Results

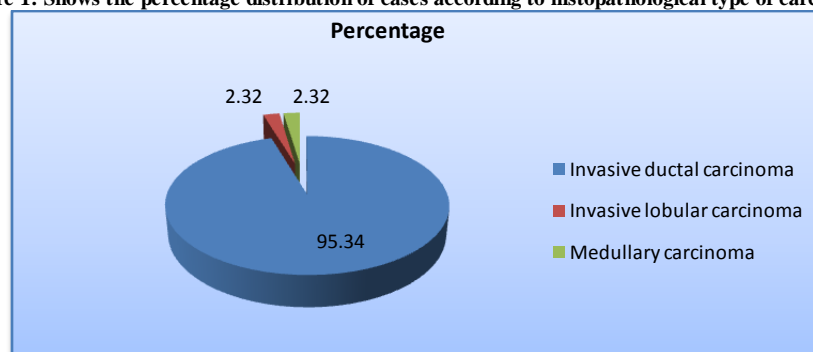
Out of 43 cases which were studied, the age of patients ranged from 31 to 75 years, with a mean age group of 49.2 years. Maximum numbers of cases were seen in the age group of 31- 40 years (30.23%) and 41-50 years (30.23%). Only 1 (2.32%) case occurred in a male and remaining 42 (97.67%) cases were females. The male to female ratio was 1:42. According to side of affected breast left breast was found to be more commonly involved, comprising of 23 cases (53.4%) including the only male case, right breast was involved in 20 cases (46.51%) while bilateral involvement was not seen in any case. Grade I tumors were seen in patients more than 60 years of age implying a better prognosis, while Grade II and Grade III tumors were more common in 40-60 years of age.

**Table 1 shows distribution of cases according to age and histological grade of Breast carcinoma**

Age	Grade I	Grade II	Grade III
21-30	-	-	-
31-40	-	9(34.61%)	3(23.07%)
41-50	-	7(26.92%)	5(38.46%)
51-60	-	5(19.23%)	4(38.46%)
61-70	1(50%)	5(19.23%)	1(30.76%)
71-80	1(50%)	-	-
<b>Total</b>	2(4.6%)	26(60.4%)	13(30.2%)

Out of the 43 cases in this study, majority were of Invasive ductal carcinoma {41cases (95.34%)}, followed by Invasive lobular carcinoma NOS type {1 case (2.32%)} and Medullary carcinoma {1 case (2.32%)}. (figure 1)

**Figure 1: Shows the percentage distribution of cases according to histopathological type of carcinoma.**



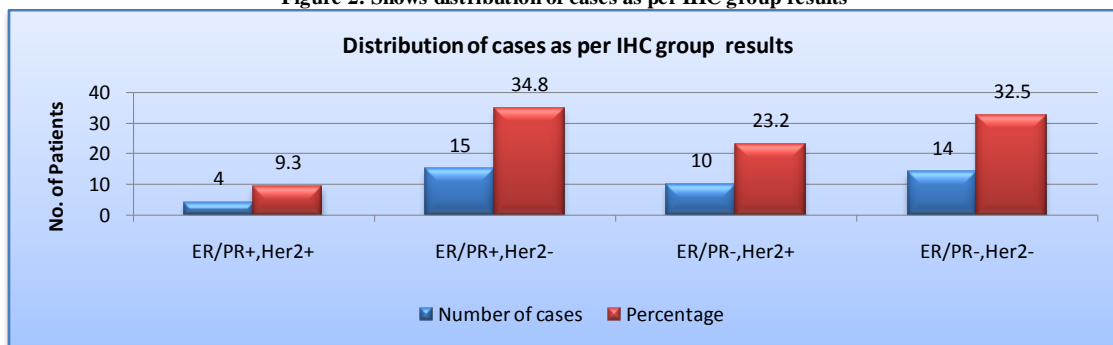
Grade I tumors showed 50% positivity in IHC groups {ER/PR+,Her2+} and {ER/PR+, Her2-}, while 44.4% of Grade II tumors showed {ER/PR+,Her2-} and 58.3% Grade III tumors shows {ER/PR-,Her2+}.

**Table 2. Distribution of cases according to Histological grade and IHC results**

	Grade I	Grade II	Grade III	Total
<b>ER/PR+,Her2+</b>	1(50%)	01(3.7%)	02(16.6%)	4(9.7%)
<b>ER/PR+,Her2-</b>	1(50%)	12(44.4%)	01(8.3%)	14(34.1%)
<b>ER/PR-,Her2+</b>	-	03(11.1%)	07(58.3%)	10(24.3%)
<b>ER/PR-,Her2-</b>	-	10(37.0%)	03(25%)	13(31.7%)
<b>Total</b>	2(100%)	27(100%)	12(100%)	41(100%)

Out of 43 cases studied, ER positivity was shown by 17 cases (39.5%), PR positivity was shown by 18 cases (41.86%) and HER-2/neu positivity was shown by 14 cases (32.55%). Only 04 cases (9.3%) were positive for all three markers (ER/PR+, HER-2/neu +). 14 cases were triple negative, comprising 32.5% cases. (Figure 2)

**Figure 2: Shows distribution of cases as per IHC group results**



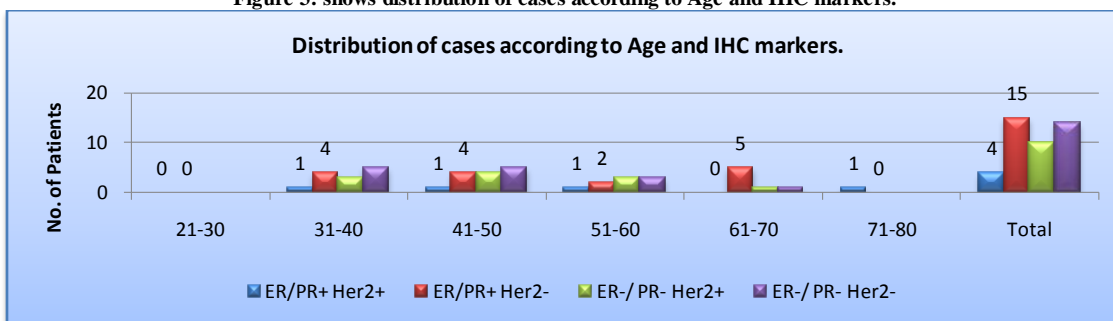
Out of 43 cases studied majority of cases (35 cases) having tumor size ranging between 2-5 cm showed more ER, PR and HER-2/neu expression than tumors having size of less than 2cm and more than 5cm.

**Table 3: Shows distribution of cases as per ER, PR, HER-2/neu status and size of tumor**

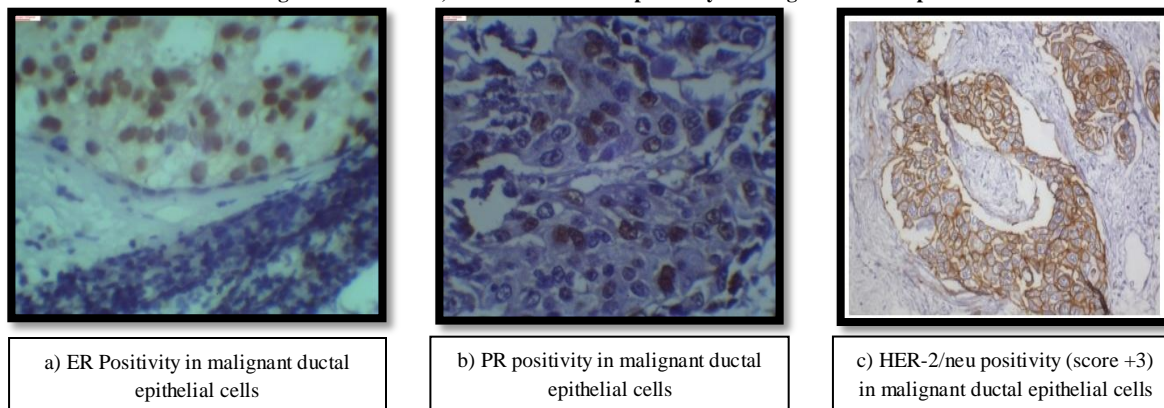
Tumor size	Cases	ER+	PR+	Her2+
<2 cm	2(4.6%)	19(50%)	2(100%)	-
2-5cm	35(81.3%)	15(42.8%)	14(40%)	13(37.1%)
>5cm	6(13.9%)	1(16.6%)	2(33.3%)	1(16.6%)

Out of 43 cases included in the study, 16 cases showed a greater presence of ER/PR+ Her 2- cases Only case was triple positive (ER/PR+ Her2+) which was in the 71-80 yrs age group implying a better predictive response to hormonal therapy. The maximum numbers of triple negative cases were seen in 31-40yrs and 41-50 yrs of age group, implying a worse prognosis.

**Figure 3: shows distribution of cases according to Age and IHC markers.**



**Figure 4: Shows ER, PR and HER-2/neu positivity in malignant ductal epithelial cells**



#### 4. Discussion

The use of IHC in breast cancer has become an integral part of a complete and comprehensive histopathology report. In terms of prognosis and prediction of response to treatment, in addition to histological grade and tumor sub type, hormone markers - ER/ PR and HER-2/neu have become the mainstay requirement for the oncologist. The present study was undertaken with the view of correlating the histopathology of the tumor by way of tumor grade, various traditional prognostic markers and its IHC profile with respect to ER, PR hormone receptors and Her2 neu. The present study was of 43 cases, all of which were confirmed cases of breast cancer.

The age of patients ranged from 31 to 75 years, with a mean age group of 49.2 years. Maximum numbers of cases were seen in the age group of 31- 40 years (30.23%) and 41-50 years (30.23%). No cases were seen in the age group of 21-30 years. As per the article published by Ghosh *et al* from TATA Memorial Hospital, Mumbai in 2008 in IJC<sup>8</sup>, the age of patients in the study ranged from 20- 99, giving a mean age of 49 yrs.

We found that out of 43 cases, 41 cases were of Infiltrating ductal carcinoma NOS type (95.34%), there was 1 case of medullary carcinoma (2.32%) , 1 case of Invasive lobular carcinoma (2.32%) and No case of any other subtypes. These findings are similar to other studies done by, Bhagat *et al*<sup>9</sup>, Adedayo *et al*<sup>10</sup> and Ghosh *et al*<sup>8</sup>. From these it was concluded that IDC (Infiltrating ductal carcinoma NOS type) is most common type as seen in all the above studies. 95.4% of cases showed more than 2 cm tumor size. Similar results were observed by Bhagat Vasudha *et al*<sup>9</sup> (91.38%), Azizun *et al*<sup>11</sup> (88%), Moses *et al*<sup>12</sup> (91.6%) and Mona *et al*<sup>13</sup> in their study. While study from western country, Adedayo *et al*<sup>10</sup> showed 71.4% cases with  $\leq 2$ cm size, this could be due to early cancer detection programs. In India owing to the lack of awareness of this disease and in absence of a breast cancer screening program, the majority of breast cancers are diagnosed at a relatively advanced stage.<sup>14</sup>

In our study ER positivity was expressed 100% (2cases) in Grade I, 42.35% (11cases) in Grade II and 23.07% (3cases) in Grade III tumors respectively While PR positivity was expressed 100% (2cases) in Grade I, 50% (13cases) in Grade II and 15.3% (2 cases) in Grade III tumors respectively while HER-2/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*<sup>11</sup> showing ER positivity in 70% cases in Grade I, 48.2% cases in Grade II and 3.5% cases in Grade III tumors respectively. 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 HER-2/neu positivity was expressed in 0%, 22.9%, 31.6% in Grade I, II and III respectively.

Significant association between increasing tumor size and HER-2/neu expression were seen in our study. Tumors larger than 2 cm size had higher rates of Her-2/neu expression than those of tumors less than 2 cm size. Similar result was observed by Bhagat *et al*<sup>9</sup> in their study. Present study showed 39.5% of cases with positive ER status while, 41.8% PR positive status. The study by Munjal *et al*<sup>15</sup> showed 41.41% positivity for ER and PR. HER-2/neu positivity in present study was 32.5%. Study by Bhagat *et al*<sup>9</sup> showed 27.58% positivity, Lal *et al*<sup>16</sup> showed 26.89% and Moses *et al*<sup>12</sup> showed 27.10% positivity respectively in their study which are correlated with our study. As already pointed out, this reaffirms that, compared to the Western population Indian subcontinent has a higher incidence of HER-2/neu positivity. It would be fruitful to have IHC group profiling of breast cancers in our country as this would generate more statistics for future research.

In the present study there is one case of Medullary carcinoma of Breast which shows 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 is one case of Invasive Lobular carcinoma of Breast which showing 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.

In the case of Invasive Lobular carcinoma there is complete loss of E-cadherin expression. Those tumors with histologically equivocal features, immunohistochemical detection of E-cadherin expression can be a useful diagnostic tool for the differentiation of ductal and lobular carcinomas of the breast.

In the present study cases between 40-60 yrs age group were associated with a higher histological grade of breast cancer. Older age group (60-80 years) was associated with lower histological grade (grade I) of breast cancer implying a better prognosis.

## 5. Conclusion

From the present study it was concluded that Infiltrating Ductal carcinoma (NOS) was the most common histopathological sub type of breast cancer and Patients of older age group (60-80 years) were associated with lower histological grade (Grade I) of breast cancer implying a better prognosis. As the tumor grade increases, ER- PR expression decreases and HER-2/neu expression increases. Prognostic accuracy improves with incorporation of IHC into the histopathology report along with the traditional TNM staging and histological grading.

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