# Immunohistochemistry for Detection of ER (Estrogen Receptor), PR (Progesterone Receptor) and HER-2/Neu (Human Epidermal Growth Factor Receptor) Receptor Status and Correlation with Histopathological Grading in Breast Carcinoma: A Study at A Tertiary Care Centre in Ajmer Region

| KEYWORDS | Breast carcinoma;ER/PR;Her-2/neu,Immunohistochemistry. |
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**ABSTRACT** *In India, Breast Cancer is the leading cause of Cancer. Present study was aimed to study pattern of expression of ER, PR and Her-2/neu in invasive breast carcinoma and to correlate their expression with various clinicopathological aspects. The study was conducted from June 2014 to July 2015. Routine H&E staining for histopathological diagnosis and IHC analysis for ER, PR and Her-2/neu was done.Out of 56 cases studied, invasive duct carcinoma was observed in 71.43% cases. The age of patients ranged from 21 to 80 years of age. Majority of tumors were grade II (55.36%) followed by grade I (23.21%). ER, PR and Her-2/neu expression was seen in 75.0%, 60.71% and 39.28% respectively. We concluded that ER, PR and HER-2/neu status correlates well with histopathological grading and other clinico-pathological parameters. Higher number of grade II tumor showed ER, PR positivity as compared to grade III tumors*

## Introduction:
Breast carcinoma is no longer seen as a single disease but rather as a multifaceted disease. [1]

Breast cancer is the most common carcinoma accounts for 22% of all female cancers [2]. In India, Breast Cancer is second to cancer of the Cervix among women. [3]

Risk factors for breast carcinoma includes age at menarche, first-degree relative, mutation in BRCA 1and 2, radiation exposure, exogenous estrogen. [4]

Approximately 50% carcinomas arise at the upper outer quadrant. [5]

These assays have the advantage of allowing only tumor cells to be assessed for receptor status on routinely processed tissue sections. [6]

Determination of ER, PR status on biopsy specimen prior to therapeutic intervention is advocated as standard practice.[7]

## Material and method:
Undertaking and permission was obtained from ethical committee for conducting the study and collecting information.In one year prospective study, 56 breast cancer specimens ( including Biopsy, Lumpectomy, MRM (Modified Radical Mastectomy) were received, from June 2014 to July 2015 in the Department of Pathology, J.L.N. Medical College and Associated Group of Hospitals, Ajmer.

After carrying out the detailed gross examination, multiple sections were taken from the tumor and its margins according to "whole slice-multiple block method" [8] and fixed in 10% buffered formalin (pH 7.2-7.4) for 24-28 hours. Histopathological study of the specimen was done by Haematoxylin and Eosin staining and as per standard protocol. The histopathological sections were diagnosed based on WHO classification 2004, and graded adopting Modified Blooms Richardson grading system. Immunohistochemistry(IHC) for ER, PR and Her-2/neu was performed on representative neoplastic tissue blocks (paraffin embedded), cut at 3.0µ on Poly-L-Lysine coated slides. The following antibodies were used for staining: for ER, a mouse monoclonal (clone 1d5) from dako; for PR, a mouse monoclonal (pr 636) from dako; and for HER -2/NEU, polyclonal *rabbit* anti-human HER-2/NEU oncoprotein.[9]

The Peroxidase antiperoxidase (PAP) method was followed. Biocare reagents were used for the antigen retrieval and IHC staining process. Two heating cycles of 10 minutes at 95 °c and 5 minutes at 98 °c were carried out in temperature controlled microwave. The normal epithelial component present in the tissue section served as internal control for ER/PR.

The immunohistochemical analysis was done using Allred Scoring system for ER/PR and ASCO scoring guidelines for HER-2/neu,.

## METHOD OF REPORTING BY IHC:
The ER/PR scoring system and criteria-Allred method. [10-14]
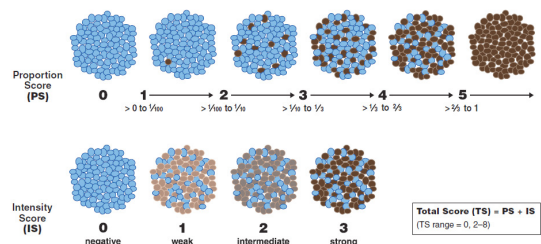


Total Score = Sum of Proportion score and Intensity score ie. PS +IS:

| 0-2 | Negative |
|---|---|

| 3-8 | Positive |
|---|---|

HER-2/Neu Scoring (ASCO Guidelines) [15]

| Nature of Staining | Score |
|---|---|
| No staining or membranous staining in <10% of cells | 0 |
| Incomplete membranous staining in >10% of cells | 1 |
| Complete membranous staining in >10% of Cells of weak to moderate intensity | 2 |
| Complete membranous staining in >10% cells of strong intensity | 3 |

**Statistical Analysis:**
Data analysis was done through the SPSS for Windows (version 12.0). p value <0.05 was regarded as significant.

**Results:**
The present study was done for prospective histopathological evaluation of 56 cases of breast cancers. The predominant histopathologic subtype was Infiltrating ductal carcinoma(NOS) had 40 cases (71.43%) , 07 cases (12.50%) were lobular carcinomas and 06 cases (10.70%) were DCIS,01case (1.79%) each of papillary, metaplastic and medullary breast carcinomas. (Table 1)

**TABLE 1: Distribution of histological types of Breast carcinoma**

| Histological types of breast cancers | Number of patients (n=56) | % |
|---|---|---|
| DCIS | 06 | 10.70% |
| IDC (NOS) | 40 | 71.43% |
| Lobular Carcinoma | 07 | 12.50% |
| Papillary Carcinoma | 01 | 1.79% |
| Metaplastic Carcinoma | 01 | 1.79% |
| Medullary Carcinoma | 01 | 1.79% |
| Total | 56 | 100% |

Chi-Square=124.89; $P$ 0.00001(Significant at p Value<0.05)

Majority, 21cases (37.52%) belonged to 51-60 years of age followed by 14 (25%)cases belonging to 41-50yrs,07 (12.5%) cases belonged to 61-70yrs, 06 (10.70%) cases belonged 21-30yrs,less common 4 cases each in 31-40 years & 71-80years of age. (Table 2)

**Table 2: Age wise distribution of Breast carcinoma**

| Age in years | Number of patients (n=56) | % |
|---|---|---|
| 21-30 | 6 | 10.70 |
| 31-40 | 4 | 7.14 |
| 41-50 | 14 | 25.0 |
| 51-60 | 21 | 37.52 |
| 61-70 | 7 | 12.5 |
| 71-80 | 4 | 7.14 |
| Total | 56 | 100 |

Mean ±SD: 51.36 ±13.25

Chi-square=24.78, $P$ 0.00015(Significant)

23 cases (41.07%) were ER/PR+ and HER-2-, 03(5.37%) were ER/PR-and HER-2+, 01 (1.79%) was ER/PR+ and HER-2+ and remaining 01 (1.79%) was triple negative (Table 3).

**Table 3: Immunohistochemical sub-types of breast carcinoma**

| ER/PR and HER-2 Sub-types | Number of patients | % | 95%CI* |
|---|---|---|---|
| ER/PR+ HER2- | 23 | 41.07 % | 64.41-92.12 |
| ER/PR+ HER2+ | 01 | 1.79 % | 0.63-17.71 |
| ER/PR- HER2- | 01 | 1.79 % | 0.63-17.71 |
| ER/PR- HER2+ | 03 | 5.37 % | 3.71-27.19 |

*: Confidence Interval

The most common histopathologic sub-type in which ER, PR and HER-2 positivity were noted, was of Ductal Carcinoma subtype. 35/42(83.33%) of ER positive cases, 29/34(85.29%) of PR positive cases, and 21/22(95.45%) of HER-2/neu positive cases were Ductal Carcinoma. There were 5/42(11.91%) of ER positive cases, 4/34(11.77%) of PR positive cases and 1/22(4.55%) of HER-2/neu positive cases of lobular carcinoma. 01 papillary carcinoma studied was positive for both ER & PR and negative for HER-2/neu.01 metaplastic carcinoma studies was positive for ER, and negative for both PR and HER-2. 01medullary carcinoma was encountered in this study which was triple negative for ER,PR and HER-2/neu. (Table 4)

**Table 4: Relationship between histopathologic subtypes and ER,PR and HER-2/neu positivity in breast carcinoma**

| Histologic Sub-type | ER+ (n=42) | PR+ (n=34) | HER-2+ (n=22) |
|---|---|---|---|
| Ductal Carcinoma | 35 (83.33%) | 29 (85.29%) | 21 (95.45%) |
| Lobular Carcinoma | 5 (11.91%) | 4 (11.77%) | 1 (4.55%) |
| Papillary Carcinoma | 1 (2.38%) | 1 (2.94%) | 0 (0%) |
| Metaplastic Carcinoma | 1 (2.38%) | 0 (0%) | 0 (0%) |
| Medullary Carcinoma | 0 (0%) | 0 (0%) | 0 (0%) |

Chi square=5.01, $P$ 0.542 (NS)

Subjects with ER/PR+, HER2- were 13 in number and younger compared to the other subtypes, with more number of grade II cancer. 5/23(21.73%) cases of grade I, 13/23(56.52%) cases of grade II and 5/23(21.73%) cases of grade III tumors were ER/PR+& HER2-, 01/01(100%) case of grade II tumors was triple positive, 01/01(100%) case of grade II tumors was triple negative, and 03/03(100%) cases of grade II tumors were ER/PR-, HER-2+.

Most of the cases with ER/PR+, HER2- had tumor size 2-5cms and showed LVI in 11 out of 23 cases (47.82% ). Majority14/23 (60.86%) of them were IDC (NOS).

There was one triple positive case, which was between 2-5cms tumor in size, and it was IDC (NOS) subtype and one was metaplastic carcinoma subtype.

The triple negative case was only 01 in number and was between 2- 5cms, tumor in size and was medullary carcinoma sub-type.

There were 03 cases of ER/PR-, HER-2+ subtype, the patients were older in this subtype, and all of them were MBR grade II and 03 cases were IDC (NOS)( Table 5).

Table 5: Clinicopathological correlation with immunohistochemical subtypes in Breast carcinoma

| Clinical variables | ER/PR+ & HER2neu- (n=23) | ER/PR+ & HER2neu+ (n=01) | ER/PR- HER2neu- (n=01) | ER/PR-& HER2neu+ (n=03) | p value |
|---|---|---|---|---|---|
| Age (Min-Max) in yrs | 21-75 | 55 | 60 | 38-65 | - |
| Age(Mean ±SD) in yrs | 51.04±14.61 | 55±0 | 60±0 | 51±13.52 | - |
| Duration(Mean ±SD) in months | 9.91±9.88 | 12±0 | 10±0 | 4.33±2.51 | - |
| Histopathologic MBR Grade | | | | | P 0.759 (NS ) |
| • I | 5 | 0 | 0 | 0 | |
| • II | 13 | 01 | 01 | 03 | |
| • III | 05 | 0 | 0 | 0 | |
| Tumor Size | | | | | P 0.815 (NS ) |
| <2 cms | 06 | 0 | 0 | 01 | |
| 2-5 cms | 09 | 01 | 01 | 01 | |
| >5 cms | 08 | 0 | 0 | 01 | |
| Lymphovascular Invasion(LVI) | | | | | P 0.497 (NS ) |
| • Present | 12 | 01 | 0 | 01 | |
| • Absent | 11 | 0 | 01 | 02 | |
| Histopathological sub- type | | | | | P 0.183 (NS ) |
| • IDC (NOS)* | 14 | 01 | 0 | 03 | |
| . DCIS** | 04 | 0 | 0 | 0 | |
| • Lobular Carcinoma | 03 | 0 | 0 | 0 | |
| • Papillary Carcinoma | 01 | 0 | 0 | 0 | |
| • Metaplastic Carcinoma | 01 | 0 | 0 | 0 | |
| • Medullary Carcinoma | 0 | 0 | 01 | 0 | |

* :Infiltrating Duct Carcinoma; Not Otherwise Specified **: Ductal Carcinoma In-Situ
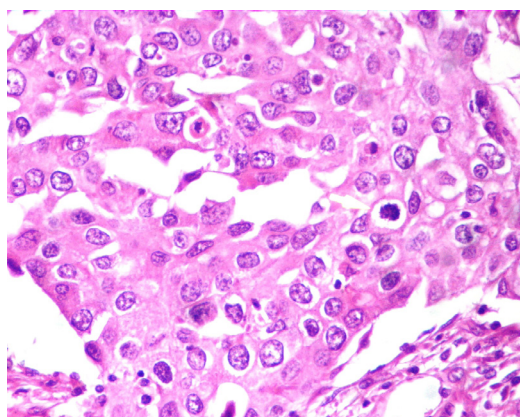


Fig.1 IDC (NOS): Moderately differentiated carcinoma with tubule formation, nuclear pleomorphism and contain mitotic figures (Arrow mark) with moderate amount of eosinophilic cytoplasm. MBR Score, 2+2+3=7; Grade=II (H&E,400X)
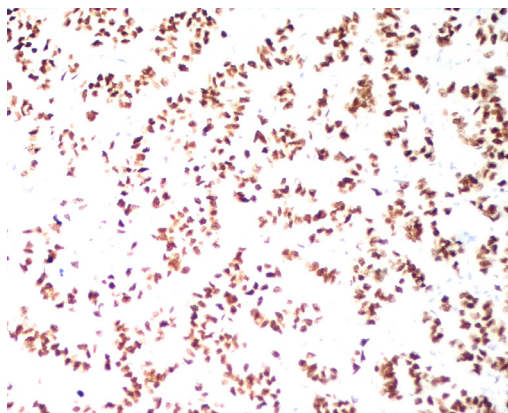


Fig.2 IDC (NOS): Nuclear immunostaining for ER ,showed strong nuclear ER positivity of score-8 (200x)
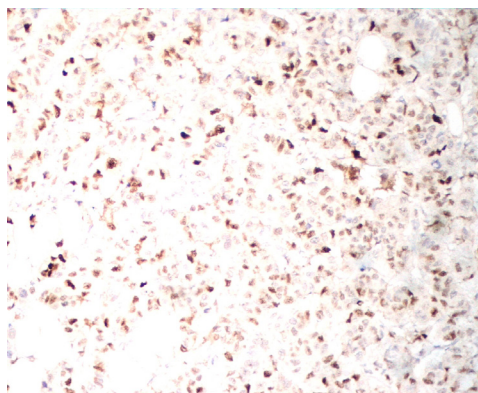


Fig 3 IDC (NOS): Nuclear immunostaining for PR, showed intermediate nuclear PR positivity of score-7 (200x)
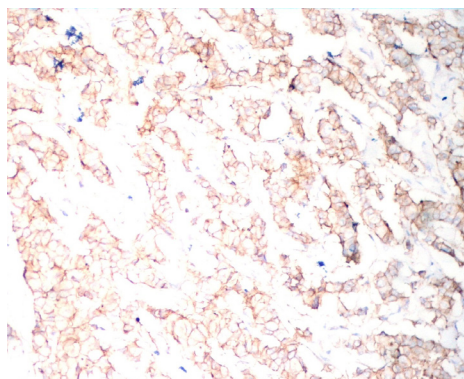


Fig. 4 IDC (NOS): Membranous immunostaining for HER-2/neu,showed complete membranous HER-2 positivity of score-3(200x)

Discussion:

The present study comprised of 56 confirmed cases of breast cancer, age at presentation ranged from 21-80 years with a mean age of 51.36 years. Similar observation was made by Ayadi L et al (2008), [16] the age of patients in the study ranged from 22- 89, giving a mean age of 51.5 years.

The majority of cases 40 (71.43%) were Invasive ductal carcinoma (NOS) and least common 07 (12.5%) were Invasive lobular carcinoma. 09 (16.07%) cases were other types of carcinomas. All similar observation was made by Jacob TW (2000)[17], showing 70% cases of IDC (NOS),11% cases of

Invasive lobular carcinoma and 17% cases of other types of carcinoma. Dissimilar observations were made by Malley PO et al (2001), [15] and  Raina V et al(2005).[18]

The majority of 24(42.86%) cases had tumor size in between 2-5cms and least common 15 (26.78%) cases were tumor size less than 2cms .Similar observation was made by Ayadi L et al (2008),[16] showing 63.2% cases of tumor size in between 2-5cms and  12.9% cases of tumor size less than 2cms and differed from Bernard Pc et al(2014)[19] and An-qi Li et al(2015).[20]

histological MBR grading was done using Modified Bloom Richardson grading system, majority of 31 (55.36%) cases were MBR grade II tumors, similar observation was made by Sofi GN et al(2012),[21]   Patel D et al(2014),[3] Patnayak R et al (2015),[22] showing 46% cases, 52.1% cases, 60.9% cases respectively, in their studies. Least 12(21.43%) cases were found in MBR Grade III tumors , similar observations were made by Patel D et al(2014),[3] Patnayak R et al(2015),[23] showing 26% cases, 35.2% cases respectively in their studies.

Present study showed 41.07% cases with ER/PR+, HER2-status,   are consistent with those of other published studies like those of  Onitilo AA et al (2009)[06] and Pavani M et al (2015),[02] showing 68.9% cases, 60.8% cases respectively, in that ER and or PR expression is generally correlated inversely with HER-2 over expression. However, a substantial number of HER-2+ tumors still expressed ER and or PR in our study they constitute 01/56 (1.79%) cases which are inconsistent with above studies.

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 and 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 Papillary Adenocarcinoma 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.

## Conclusion:
Breast cancer is one of the most common female malignancies and cause of death among women worldwide. Breast cancer is a major public health problem in India. Invasive ductal carcinoma was the most common histologic type of breast cancer in both pre and post menopausal females in the present study followed by Invasive lobular carcinoma and DCIS. ER, PR and Her-2/neu expression in breast carcinoma by IHC method indicates higher rates of positive expression correlated with various clinicopathological aspects. Higher number of grade II tumors showed ER, PR positivity as compared to grade III tumors.

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