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Original Research Article

# Morphological Profile and Hormone Receptor Status in Breast Carcinomas

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**Abstract:** The data on histological and hormone receptor status in breast cancer in an Indian population is limited as receptor status is not routinely carried out for these patients. In our study, receptor status was analyzed and it was correlated with morphological prognostic parameters. The objective is to analyze the morphological prognostic parameters and its correlation with receptor status in Indian women. The sample consisted of 66 specimens of invasive breast cancer received in Department Of Pathology of SVS Medical College and Hospital, Mahabubnagar between year 2012 and 2014. ER, PR and HER-2 status correlates well with histopathological grading. Higher the tumor grade, the more likely that ductal carcinoma will be Her2 + and ER/PR negative or triple negative. Follow up study of these patients is needed to assess the prognostic significance.
**Keywords:** Breast cancer, estrogen receptor, HER-2/neu, progesterone receptor

## INTRODUCTION

Breast carcinoma is the most common malignant tumor and the leading cause of carcinoma death in women with an estimated life time risk of 13% [1]. Fifty eight percent of patients were 50 years or younger. Morphological classification of breast carcinomas divide these tumours into a number of subtypes . Approximately three fourth of mammary carcinoma are invasive ductal or lobular type. Within these, and also among other morphologic categories , tumors display marked heterogeneity in many of their biologic properties . One is the expression of steroid receptors in concert with the oncogene ErbB2/Human epidermal growth factor receptors 2 (HER2). This has important clinical implications, such as selection of patients for endocrine therapy [2, 3].

A large number studies have correlated the presence or absence of tumour estrogen receptor (ER) or Progesterone Receptor (PR) with ultimate clinical outcomes . In addition to pathological grade and stage , we set out to evaluate tumour ER/PR status and HER2/neu expression by Immunohistochemistry (IHC) as a prognostic and predictive factors in breast carcinoma [1].

## AIM AND OBJECTIVES

1. To assess ER , PR status and HER2/neu overexpression by IHC of malignant tumors
2. To correlate the ER/PR and HER2/neu status with modified Bloom - Richardson histopathological grading .
3. To study the relationship between clinicopathological parameters, IHC subtypes and histopathological grading.

## METHODOLOGY

This is a prospective study of 66 cases conducted in Department Of Pathology, S.V.S Medical College from 2012 to 2014. This study included lumpectomies, mastectomy, modified radical mastectomy cases. The specimens were thoroughly examined and clinical details were analyzed. The Specimen sent in formalin was sliced at 1 cm interval and fixed immediately in 10% NBF. One dedicated block from the tumor not fixed for more than 24 hours in formalin was used for IHC. Four µ thickness sections were cut and taken on poly-L-Lysine coated slides and stained for evaluating ER, PR receptors and HER2/ new expression. And also sections were routinely stained with H & E and histological grading of tumor was done on H&E stained sections according to Modified Bloom –Richardson grading.

Procedure followed for IHC staining is according to guidelines given in Dako manual. ER or PR was considered positive if finding of more than 1% tumor cell nuclei are immunoreactive. Negative for ER

or PR if finding of less than 1% of tumor cell nuclei are immunoreactive [4] (Figure 1A and 1B)

HER-2/neu status was assessed by a score that includes the intensity and the percentage of positive tumor cells. HER2 testing results fall into three categories; positive, equivocal and negative [5] [Table 1] (Figure: 2). For the purpose of this study equivocal results were considered negative.

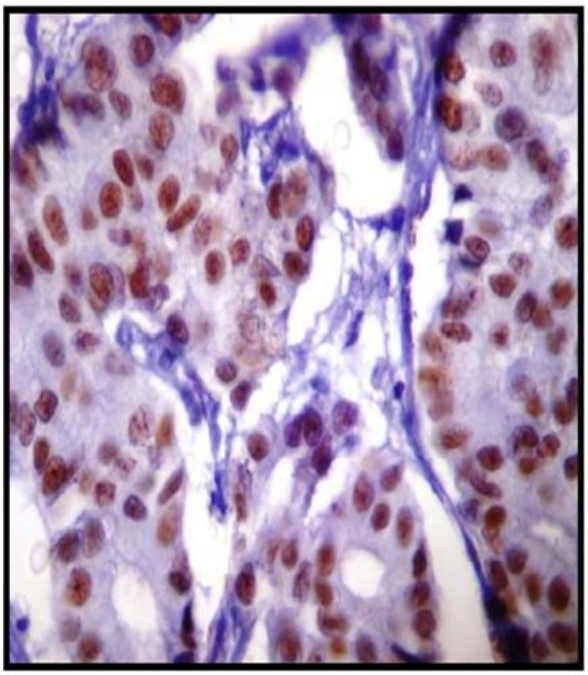

**Fig 1A: Showing ER positivity of score 8 in the above tumour.**

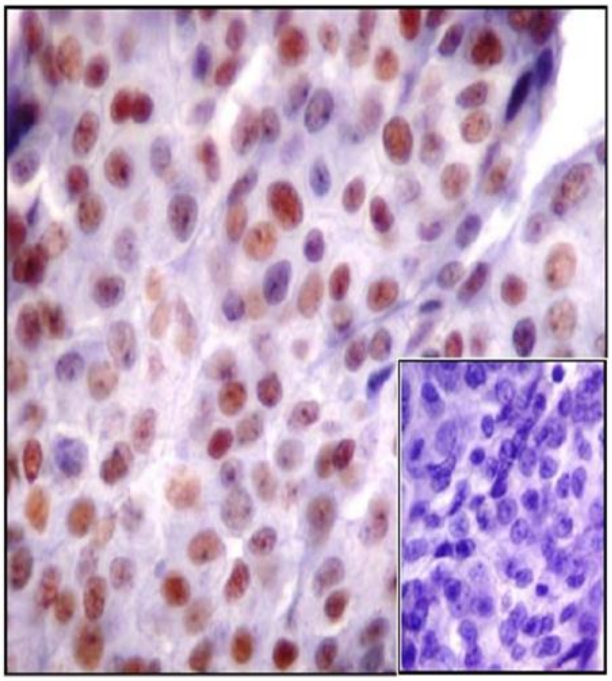

**Fig 1B: Showing PR positivity of score 7 in the above tumour. Inset shows HER2 negative staining**

**Table 1: HER2 Testing by Validated Immunohistochemistry Assay**

| Status | Score | Significance |
|---|---|---|
| Positive | 3+ | Uniform intense membrane staining of >30% of invasive tumor cells |
| Equivocal | 2+ | Complete membrane staining ,non-uniform or weak in intensity ,in at least 10% of the cells or intense complete membrane staining in 30% or less of tumor cells |
| Negative | 1+ | Weak or incomplete membrane staining in any proportion of tumor cells |
| Negative | 0 | No staining |

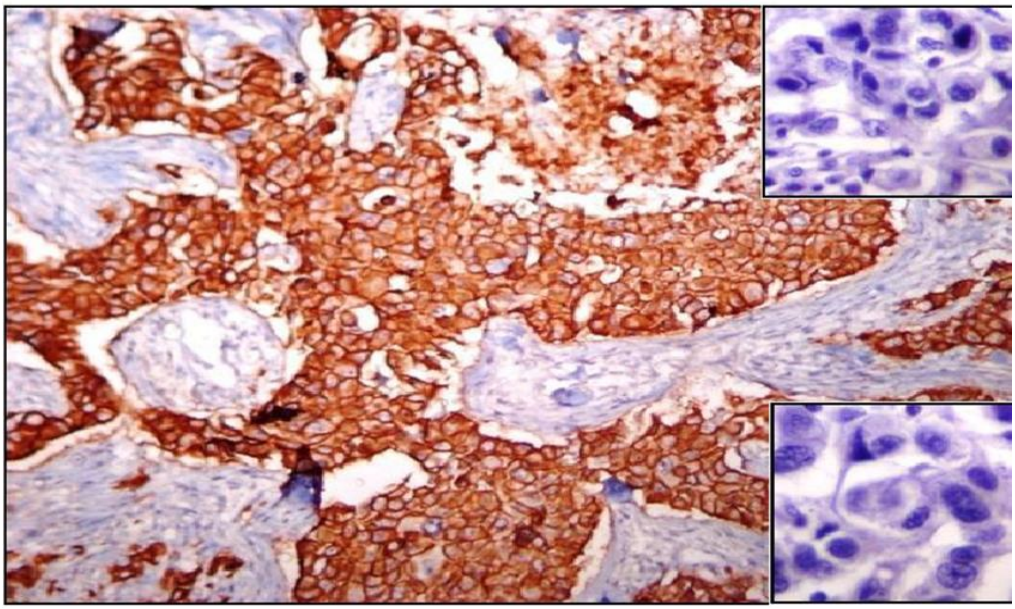

**Fig 2: Grade III IDC .Showing Strong HER 2 membranous staining of score3 (x Inset shows ER & PR negativity**

**Statistical analysis:**

Statistical analysis was done using spss software version 17.Te relationship between clinicopathological parameters, IHC subtypes and histopathological grading was done using chi- square test.

**RESULTS:**

There were 66 cases of microscopically confirmed invasive breast carcinoma in women. Most women (28 of 66; 42.42%) were between age group of 41 and 50 years. Eighteen (27.27%) were 40 years and younger at diagnosis [Table 2].

**Table 2: Age at diagnosis of invasive breast carcinoma**

| Age in years | Frequency | Percentage |
|---|---|---|
| <40 | 18 | 27.27 |
| 41-50 | 28 | 42.42 |
| <50 | 20 | 30.30 |

Most (47 of 66; 71.21%) were invasive ductal carcinoma, not otherwise specified (IDC NOS). [Figure 3, 4] There were other subtypes of invasive carcinoma, including (12 of 66; 18.18%) of medullary carcinoma , IDC with Mucinous Type (3 of 66 ; 4.54%),invasive lobular carcinoma(ILC) (2 of 66 ; 3.03%) intracystic papillary carcinoma (1 of 66; 1.51%) ,metaplastic carcinoma(1 of 66 ; 1.51%) .

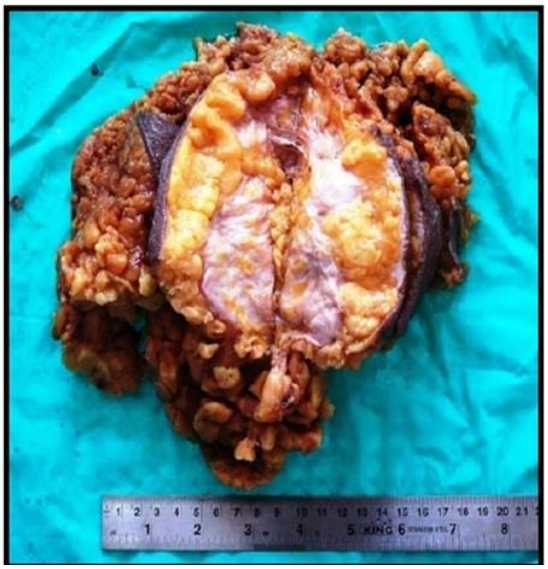

**Fig 3: IDC (NOS): Gross specimen**

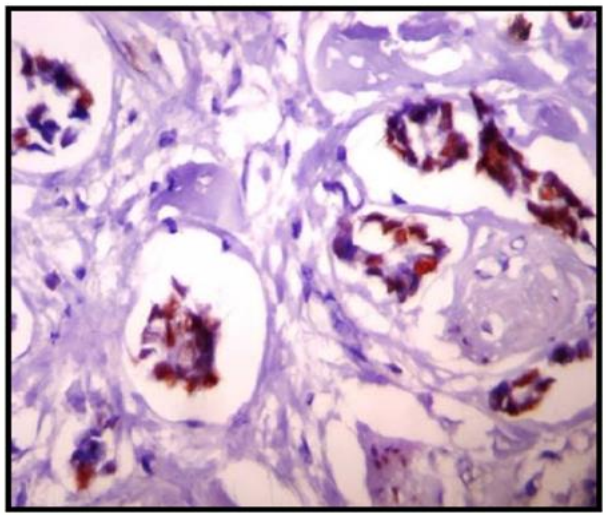

**Fig-5: Grade II IDC showing ER positivity(x400) PR Positivity Of score 8**

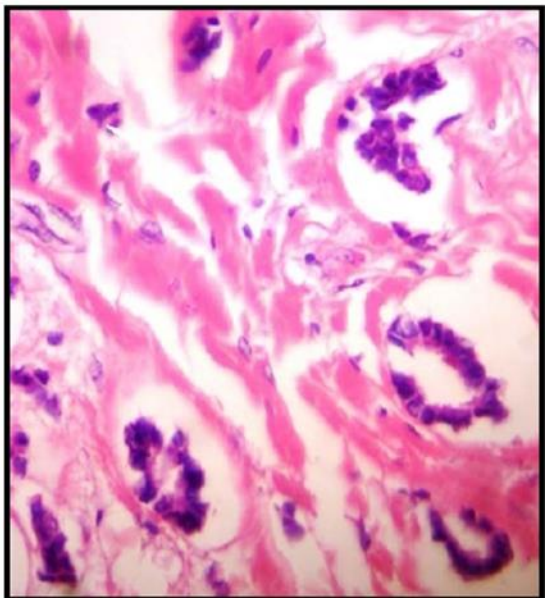

**Fig 4: Grade I IDC (NOS) with tubular pattern**

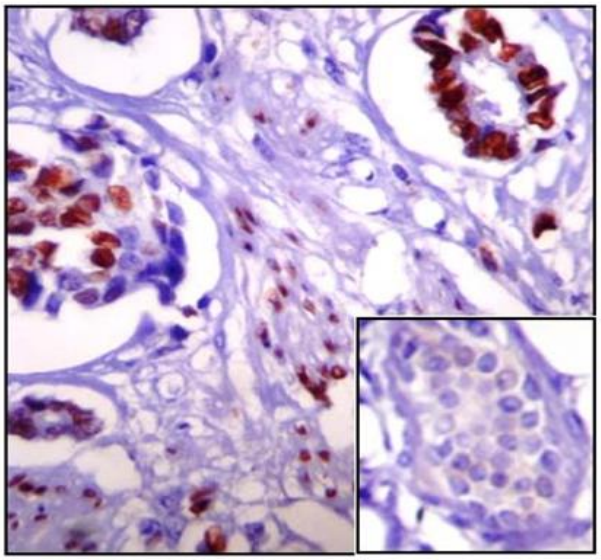

**Fig-6: Grade I IDC showing nuclear of score 8 nuclear Inset Shows HER 2 Negativity (x400)**

**Table 3: Correlation Of Hormone Receptor Status With Histological Subtypes (Figure 5, 6)**

| Histologic Subtype | Frequency | Percentage (%) | ER | | PR | | HER-2 | |
|---|---|---|---|---|---|---|---|---|
| | | | Pos | Neg | Pos | Neg | Pos | Neg |
| IDC (NOS) | 47 | 71.21 | 31 | 16 | 29 | 18 | 14 | 33 |
| Medullary carcinoma | 12 | 18.18 | 00 | 12 | 00 | 12 | 00 | 12 |
| Invasive lobular carcinoma | 02 | 3.03 | 01 | 01 | 01 | 01 | 01 | 01 |
| IDC with Intracystic Papillary Carcinoma | 01 | 1.51 | 01 | 00 | 00 | 01 | 00 | 01 |
| IDC with mucinous type | 03 | 4.54 | 00 | 03 | 00 | 03 | 00 | 03 |
| Metaplastic Carcinoma | 01 | 1.51 | 00 | 01 | 00 | 01 | 00 | 01 |

**Table 4: Correlation of ER, PR and HER2 status with clinicopathological data**

| | ER + n (%) | p value | PR+ n (%) | P value | HER2+ n (%) | P value |
|---|---|---|---|---|---|---|
| **Age (years)** | | | | | | |
| < -50 (38) | 20 (52.63 ) | 0.80 | 18(47.37) | 0.90 | 7(18.42) | 0.49 |
| >50 (28) | 13 (46.43 ) | | 12(42.86) | | 8(28.57) | |
| **Tumor size (Cms)** | | | | | | |
| <_5 (41) | 20(48.78) | 1 | 18(43.90) | 0.94 | 11(26.82) | 0.47 |
| >5 (25) | 13(52) | | 12(48) | | 4(16) | |
| **Histologic type** | | | | | | |
| **Infiltrating ductal (47)** | 31(65.96%) | 0.72 | 29(61.70%) | 0.64 | 14(29.79%) | 0.87 |
| **Infiltrating lobular(2)** | 1(50%) | | 1(50%) | | 1(50%) | |
| **Lymph node** | | | | | | |
| **Positive (27)** | 17(62.96%) | 0.52 | 15(55.56%) | 0.83 | 5(18.52%) | 0.87 |
| **Negative(11)** | 5(45.45%) | | 5(45.45%) | | 3(27.27%) | |
| **Tumour grade** | | | | | | |
| Grade1-2 (41) | 24(58.54%) | 0.12 | 23(56.10%) | 0.048 | 12(29.27%) | 0.18 |
| Grade 3 (25) | 9(36%) | | 7(28.0%) | | 3(12%) | |

**Table 5: ER, PR and HER2/neu status in various types of breast carcinoma**

| | ER | PR | HER2/new |
|---|---|---|---|
| Positive | 33(50%) | 30(45.45%) | 15 (22.73%) |
| Negative | 33(50%) | 36(54.55%) | 49 (74.24%) |
| Equivocal | - | - | 2 (3.03%) |
| **Total** | **66** | **66** | **66** |

**Table 6: Immunohistochemical subtypes**

| ER/PR and HER2 | No.of cases(n=66) | % |
|---|---|---|
| ER/PR + HER2 - | 15 | 22.73 |
| ER/PR + HER2 + | 13 | 19.70 |
| ER/PR - HER2 - | 31 | 46.97 |
| ER/PR - HER2+ | 2 | 3.03 |
| ER+PR - HER2- | 3 | 4.55 |
| Er/PR +HER2- Equivocal | 2 | 3.03 |

## DISCUSSION

Neoplasms of the breast are one of the common lesions of the breast which though virtually limited to females can occur in males as rare exceptions. Breast cancer is a heterogeneous disease composed of growing number of recognized biological subtypes. Prognostic indicators based on currently available clinical and histopathologic variables such as tumor size, tumor grade, lymph node status and hormone receptor status already exist and are used to predict a patient's clinical outcome in certain situations [6]. It is well known that ER, PR and HER-2 represent the most acceptable factors for predicting prognosis response or resistance to treatment and the potential use of newer drugs [7]. Assessment of ER/PR and HER2 in breast cancer is mandatory in clinical practice [8].

So we undertook this study to evaluate for estrogen and progesterone receptors and HER2/neu expression in various types of carcinoma breast and correlated with tumor type, histological grade and lymphnode status. Our study comprised of 66 cases of malignant tumours of breast tumors.

**Table 7: Comparison of ER, PR and HER2/neu status in breast carcinoma with other studies**

| **ER,PR and HER2 status** | **Authors** | | |
|---|---|---|---|
| | Ayadi L *et al.;* [7] (n= 155)% | Hung HJ *et al.;* [9] (n=1362)% | **Present Study (n=50)%** |
| **ER** | | | |
| Positive | 59.4 | 81.1 | 50 |
| Negative | 40.6 | 18.9 | 50 |
| **PR** | | | |
| Positive | 52.3 | 64.2 | 45.45 |
| Negative | 47.1 | 35.8 | 54.55 |
| **HER 2** | | | |
| Positive | 18.1 | 10.9 | 22.73 |
| Negative | 81.9 | 89.1 | 74.24 |
| Equivocal | - | - | 3.03 |

In our study, there were equal number of ER+ and ER- cases. PR + cases were less in number than PR- cases. Ayadi *et al.;* and Huang HJ *et al.;* Observed more number of ER and PR Positive cases. In the present study, number of HER2+ (22.73%) and HER2- (74.24%) cases observed were in consistent with the observation made by Ayadi *et al.;* which showed 18.1% positively and 81.9% negatively. (Table-7).

**Table 8: Immunohistochemical Subtypes.**

| **Immunohistochemical Subtypes.** | Satti MB *et al.;* [8] (%) | Onitilo AA *et al.;* [10] (%) | Hung HJ *et al.;* [9] (%) | **Present Study (%)** |
|---|---|---|---|---|
| ER/PR+, HER 2- | 53 | 68.9 | 66.4 | 22.73 |
| ER/PR -, HER 2+ | 11 | 10.2 | 30.9 | 19.70 |
| ER/PR -, HER 2- | 24 | 13.4 | 13.8 | 46.97 |
| ER/PR -, HER 2+ | 12 | 7.5 | 45.6 | 3.03 |
| ER/PR+,/HER 2- | - | - | - | 4.55 |
| ER/PR+, HER 2 equivocal | - | - | - | 3.03 |

In our study, triple negative cases were more accounting for 46.97% when compared to other studies. Special variants like medullary carcinoma (18.18%), and Metaplastic carcinoma (1.51%) which showed triple negativity were included in present study.

Frequency of other subtypes observed was consistent with the observation made by Satti MB *et al.;* Apart from these subtypes in present study 3 cases of ER+PR-, HER2- type, one was IDC with intracystic papillary type of histological grade 2 and 2 cases were IDC (NOS) of histological grade 3 and also 2 cases of ER+PR+ and HER2 equivocal, which included IDC (NOS) of grade II and grade III (Table-8).

**Table 9: Correlation of ER status in various types of carcinoma breast with clinicopathological data**

| | **Ayadi L *et al.;*[7]** | | **Present Study** | |
|---|---|---|---|---|
| | **ER + %** | **P Value** | **ER +%** | **P Value** |
| **Age (years)** | | | | |
| < 50 | 47.5 | 0.002 | 52.63 | 0.80 |
| >50 | 72 | | 46.43 | |
| **Tumour Size(cms)** | | | | |
| < 5 | 62.7 | 0.129 | 48.78 | 1 |
| >5 | 48.5 | | 52 | |
| **Histologic type** | | | | |
| Infiltrating Ductal | 61.1 | 0.31 | 65.96 | 0.72 |
| Infiltrating Lobular | 50 | | 50 | |
| **Lymph node** | | | | |
| Positive | 60 | 0.88 | 62.96 | 0.52 |
| Negative | 58.9 | | 45.45 | |
| **Tumour grade** | | | | |
| Grade-1-2 | 72.2 | 0.000 | 58.54 | 0.12 |
| Grade 3 | 22.5 | | 36 | |

In our study, ER + tumors were more in the age group of < 50 years. Whereas in the study done by Ayadi et al, ER + tumours were more in the age group > 50 years and Satti MB *et al.;* had similar proportion of ER + cases above and below the age group of 50 years. A negative correlation between ER expression and histological grade was noted. No association was found between ER expression and tumor size, histologic type and lyrnphnode involvement which were similar to the observation made by Ayadi L *et al.;* (Table-9).

**Table 10: Correlation of PR status in various types of carcinoma breast with clinicopathological data**

| | Ayadi L *et al.;* [7] | | **Present Study** | |
|---|---|---|---|---|
| | PR +% | P Value | PR+ % | P Value |
| **Age ( years)** | | | | |
| < 50 | 53.8 | 0.76 | 47.37 | 0.90 |
| >50 | 51.4 | | 42.86 | |
| **Tumor Size (cms)** | | | | |
| < 5 | 53.4 | 0.72 | 43.9 | 0.94 |
| >5 | 50 | | 48 | |
| **Histologic type** | | | | |
| Infiltrating ductal | 53.8 | | 61.7 | |
| Infiltrating Lobular | 45.8 | 0.47 | 50 | 0.64 |
| Other | | | 0 | |
| **Lymph node** | | | | |
| Positive | 54.7 | | 55.56 | |
| Negative | 51.1 | 0.66 | 45.45 | 0.83 |
| **Tumor grade** | | | | |
| Grade 1-2 | 61.4 | | 56.10 | |
| Grade 3 | 27.5 | 0.000 | 28 | 0.048 |

In our study, PR + tumors were more in the age group of < 50 years which was similar to the observation made by Ayadi L *et al.;* A negative correlation between PR expression and histological grade was noted. No association was found between PR expression and tumor size, histologic type and lymphnode involvement which were similar to the observation made by *et al.;* (Table-10).

**Table 11: Correlation of HER2 status in various types of carcinoma breast with clinicopathological data Ayadi L *et al.;* 62 Huang HJ *et al.;***

| | Ayadi L *et al.;* [7] | | Hunjg HJ *et al.;* [9] | | **Present study** | |
|---|---|---|---|---|---|---|
| | HER2+ n(%) | P value | HER2+ (%) | P Value | HER2 + | P Value |
| **Age ( years)** | | | | | | |
| < 50 | 21.3 | 0.28 | 12.1 | 0.344 | 18.42 | 0.49 |
| >50 | 14.7 | | 10.4 | | 28.57 | |
| **Tumor Size (cms)** | | | | | | |
| < 5 | 15.3 | 0.104 | 10.5(<2) | 0.617 | 26,82 | 0.47 |
| >5 | 27 | | 11.4(>2) | | 16 | |
| **Histologic type** | | | | | | |
| Infiltrating ductal | 16.8 | 0.33 | - | - | 29.79 | 0.87 |
| Infiltrating Lobular | 25 | | - | - | 50 | |
| **Lymph node** | | | | | | |
| Positive | 36.9 | 0.000 | 12.2 | 0.255 | 18.52 | 0.87 |
| Negative | 4.4 | | 10.2 | | 27.27 | |
| **Tumor grade** | | | | | | |
| Grade 1-2 | 14.8 | 0.072 | 4.6 | <0.001 | 29.27 | 0.180 |
| Grade 3 | 27.5 | | 20.8 | | 12 | |

In our study, HER2/neu overexpression was seen in the age group of < 50 (18.42%) similar observation was made by Ayadi *et al.;* . There was no correlation between HER2 status and tumor size, axillary lymph node, type and age at diagnosis in the present study and correlated with the study done by Huang HJ *et al.;* Ayadi *et al.;* observed significant

correlation between lymph node status and HER2 overexpression (Table-11).

**Table 12: Comparison of Immunohistochemical subtypes with Histologic grading**

| **Immunohistochemical Subtypes (%)** | Onitilo AA *et al.;* [10] | | | | **Present study** | | | |
|---|---|---|---|---|---|---|---|---|
| | Histologic Grades | | | | | | | |
| | Grade I | Grade II | Grade III | P Value | Grade I | Grade II | Grade III | P Value |
| ER/PR, +HER2- | 28.9 | 44.9 | 21.5 | < 0.001 | 37.5 | 27.27 | 12 | 0.057 |
| ER/PR, +HER2+ | 6.0 | 41.4 | 49.1 | | 0 | 30.3 | 12 | |
| ER/PR, - HER2- | 4.0 | 12.5 | 76.3 | | 62.5 | 30.3 | 64 | |
| ER/PR, -HER2+ | 1.2 | 20.0 | 77.7 | | 0 | 6.06 | 0 | |

In our study Observed close correlation between tumor grade and immunohistochemical subtypes which was similar to the study done by Onitilo AA *et al.;* HER2+ and basal like subtypes were associated with poorly differentiated tumors (p=0.003) (Table-12).

**Correlation between ER and PR status:**

In our study positive correlation observed between ER and PR (p = 0.000) which was similar to the study done by Ayadi L *et al.;* [7] and Eisenberg ALA *et al.;* [11]. According to Rosen, most PR positive tumors are also positive for ER [11].

**Correlation between HER2 overexpression and hormonal receptors status:**

In the study done by Ayadi L *et al.;* [7] the percentage of HER-2 over-expression was sharply weaker in ER positive tumors: 7.6%, compared with 33.3% for ER negative tumors (p:0.000). Over-expression of HER-2 was also inversely related to PR status (p: 0.048).

In our study inverse relationship between HER2/neu and ER/PR expression was observed, similar observation was made by Ambroise *et al.;* [12] and Eisenberg ALA *et al.;* [11]. The inverse association between HER-2/neu and hormone receptors to lower or absent hormone receptors in women with HER-2/neu positive cancers. This is one of the reasons why women who overexpress HER-may be resistant to tamoxifen [13].

## CONCLUSION

Our study highlights the importance of histopathological examination in breast lumps but also in predicting the prognosis by typing, staging and grading malignant neoplasms of breast. The interrelationship between ER, PR and HER-2/neu has an important role in the management of breast cancer. Endocrine therapy (tamoxifen) is recommended for tumors expressing ER/PR. Patients with breast carcinoma overexpressing HER-2 do not respond to tamoxifen therapy. Recently anti-HER-2 antibodies (Herceptin) have been shown to be effective against HER-2 overexpressing breast carcinomas. In our study an attempt was made to understand the correlation of ER, PR & HER-2 status with histopathological grading and clinicopathological parameters.

In conclusion, ER, PR and HER-2 status correlates well with histopathological grading. Higher the tumor grade, the more likely that ductal carcinoma will be Her2 + and ER/PR negative or triple negative. Hence, our study support IHC classification as a clinical tool as ER/PR and HER 2 testing is widely available at a reasonable cost, is a clinically-used, therapeutically informative classification of breast cancer based on immunophenotype / biologic phenotypes, and is prognostic as well as somewhat predictive. Follow up study of these patients is needed to assess the prognostic significance.

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