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# Study of comparison between the grades and IHC marker status in Breast Carcinoma cases from a tertiary care hospital

Reeta Dhar[1] , Omar Bali[2], Manavv Shah[3],, Karan Sankla[4], Ameya welling[5]
*1Professor, Department of Pathology, MGM's Medical College, Navi Mumbai*
*2Assistant professor, Department of Pathology, MGM's Medical college, Navi Mumbai*
*3MBBS, MGM Medical college*
*4MBBS, MGM Medical college*
*5MBBS, MGM Medical college*

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## ABSTRACT

Introduction

The increase in the rate of mortality from breast carcinoma suggests the need for new clinical developments, preferably targeted therapy. Therefore IHC markers have gained more importance over the years. Various studies have shown prevalence of breast carcinoma with IHC markers positivity (ER/PR and HER2/neu receptors).Our study further adds to these correlational finding.

Materials and methods

This is a prospective study conducted over 2 years. The records of this study are collected from a tertiary care hospital. Biopsies were obtained after staining with 10% formalin. The sections are then stained with H&E and IHC markers.

Results

The study comprises of 50 invasive breast carcinoma cases out of which only 1 is a male patient and rest 49 are female patients. Out of 50 Samples that were tested,4 cases (8%) were of grade 1, 24 cases were of grade 2(48 %) and 22 cases of grade 3(44%). And
34(68%) were ER+ve , 28(56%) were PR +ve and 10(20%) Her2/Neu +ve in total.

Conclusion

Immunohistochemistry plays an important role in making differential diagnosis of invasive breast cancer lesions, understanding the status of IHC expression of these lesions aids in the diagnosis.

## I. INTRODUCTION

Around 2,670 breast cancer cases were seen in men and around 2,68,600 cases in women in India, with nearly 40,000 estimated deaths; this statistics is for the year 2019 alone.[1] According to cancerindia.org, India nearly has 2.25 million cases of cancer with more than 11 lakh new cases registered every year. India is likely to lodge 17 lakh new cases and report more than 8 lakh deaths due to breast cancer by 2020.In Thiruvananthapuram, crude breast cancer rates were highest i.e 43.9 , followed by Chennai (40.6), New Delhi (34.8) and Mumbai (33.6).[2]

In breast cancer patients, a routine analysis of the receptors is done for early diagnosis and prognosis. Classification based on immunohistochemistry (IHC) markers, oestrogen receptors(ER), progesterone receptors(PR) and human epidermal growth factor receptor (Her2/neu ) status gives therapeutic and prognostic information not obtained by either one alone.

An increase in the incidence of breast carcinoma mortality presents the need for a new therapeutic development preferably targeted treatment. Therefore the existence of hormone receptors can assist in the targeted therapy against the receptors present on the tumours.

Various studies have shown the prevalence of breast cancer with IHC markers positivity.By comparing the outcomes of a sample size of 50 cases at a tertiary care hospital in which histopathological as well as IHC tests were conducted, our research further adds to the correlational findings.

## II. MATERIALS AND METHODS

This is a two-year prospective study examining patients with invasive carcinoma of the breast. The records from a reputed tertiary care hospital are used to collect all the data computed and compiled here. Data collection was performed during November 2017 to November 2019. In this study, samples of 50 patients are taken. The patient

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samples were collected either from core biopsies, lumpectomy specimens, or from MRMs. Specimens were obtained with 10% of buffered formalin. The inclusion criteria for the study consists of patients of either sex above 18 years of age and surgically resected specimens and biopsies sent in fixative used for histopathological study only. Poorly fixed or unfixed specimens for histopathological diagnosis are excluded from this study. Haematoxylin and Eosin Stain (H&E) and Immunohistochemistry (IHC) markers (ER, PR and Her2/neu) are the stains used for this study. The Haematoxylin and eosin stain is the most widely used stain in histology and histological laboratories.[3] The haematoxylin stains cell nuclei blue, and eosin stains the extracellular matrix and cytoplasm pink, with other structures taking on different shades, hues, and combinations of these colours. The following steps are involved in the H&E staining of the histopathology slide: Dewaxing, dehydration, haematoxylin application, differentiation, bluing, counterstaining with eosin, dehydration, clearing, and placing a cover slip. In immunohistochemistry staining (IHC), the slide is prepared by coating in poly-l-lysine solution and dried at room temperature several times followed by cutting the specimens into 3-5microns, deparaffinizing, dipped in xylene for 10min, rehydration followed by washing in water and with phosphate buffer 3 times (with 3-3-3-minute intervals). The techniques we followed were that of antigen retrieval in tri- buffer where we used a retriever, then we blocked the endogenous peroxidase with 3% hydrogen peroxide. We then incubated them with primary mouse monoclonal antibody and for the developing chromogen we used diaminobenzidine. For counterstaining we used haematoxylin; thus, we obtained immuno-stained slides which were examined by us for nuclear staining in regards of ER, PR and membrane staining in regards of HER2/neu. [4,5,6]. We've assimilated the data and compared them to receptor outcomes, as well as the tumour grade.

## III. RESULTS

| Grades | No. Of cases | ER +ve | PR +ve | Her2/Neu +ve |
|---|---|---|---|---|
| Grade 1 | 4(8%) | 4 | 3 | 2 |
| Grade 2 | 24(48%) | 19 | 17 | 6 |
| Grade 3 | 22(44%) | 11 | 8 | 2 |
| **Total** | **50** | **34** | **28** | **10** |

This study comprised 50 cases of invasive breast carcinoma patients. Out of these 49 were female cases and 1 male case. Majority of the cases were between 48-60 years of age group.

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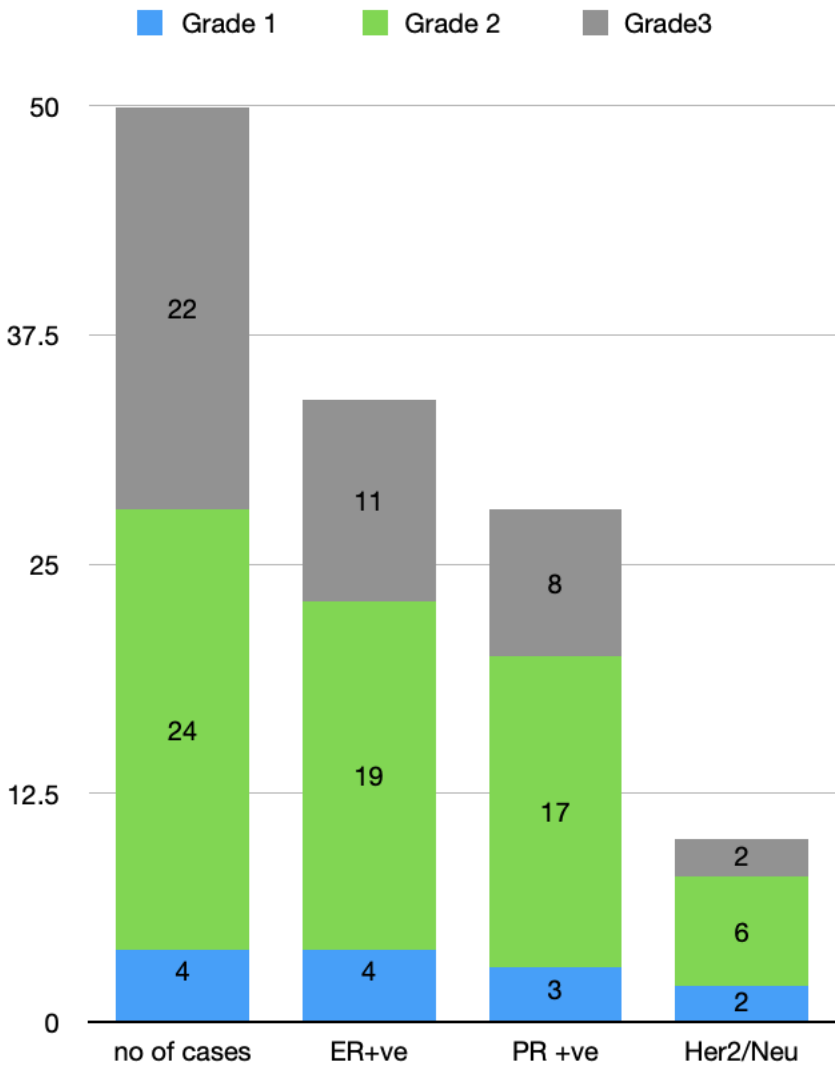


4 cases (8%) were of grade 1, 24 cases were of grade 2(48%) and 22 cases of grade 3(44%).

Out of 4 grade 1 patients; all 4 were ER+ve, only 3 were PR+ve and 1 Her2/Neu +ve. Out of 24 grade 2 patients; 19 were ER+ve , 17 were PR +ve and 6 were Her2/Neu +ve. Out of 22 grade 3 patients; 11 were ER+ve, 8 were PR+ve and 2 Her2/Neu +ve.

Out of 50 Samples that were tested, 34(68%) were ER+ve , 28(56%) were PR +ve and 10(20%) Her2/Neu +ve in total.

## IV. DISCUSSION

The most common breast cancer worldwide in females is invasive breast cancer. The mainstay of breast cancer treatment is surgery when the tumour is localised, followed by chemotherapy (when indicated), radiotherapy and for oestrogen receptor (ER) and progesterone receptor (PR) positive tumours, adjuvant hormonal therapy.[7] Breast cancer is curable if diagnosed at early stage. Traditional morphological prognostic factors include tumour size, tumour grade, and axillary lymph node metastasis. Nowadays, more importance is given to biological molecular prognostic factors. Hormone receptors (ER and PR) and human epidermal growth factor receptor-2 (HER-2) are the most relevant clinical biomarkers that are widely used in stratifying breast cancer cases management.[8]

Breasts undergo major physiological changes over the lifetime of a woman, and oestrogen is actively mediating these changes. There are two forms of ER: ER alpha and ERβ.[9] Receptor ER alpha is a well-established breast cancer prognostic and predictive factor. There is no clear description of the prognostic value of ERβ.[9,10] PR is of two

types as follows: PR-A and PR-B. Progesterone acts as a modulator of oestrogen function.[11].Over expression of Her2/Neu may lead to increased and uncontrolled cell proliferation, decreased apoptosis, increased cancer cell motility, and angiogenesis and hence worse prognosis.[12]

Comparison of ER, PR, HER2/neu receptor positivity with other studies :

| Studies | No. Of cases | ER +ve % | PR +ve % | HER2/Neu +ve % |
|---|---|---|---|---|
| Malaviya et al | 200 | 84 | 90 | - |
| Geethamala et al | 100 | 98 | 97 | 89 |
| Handa et al | 47 | 72.4 | 74.5 | - |
| Zoppi et al | 101 | 94 | 71.2 | - |
| Our study | 50 | 64 | 56.3 | 18 |

The present study showed the highest number of ER positive cases followed by PR and HER2/neu positive cases, comparable to the studies conducted by Geethamala et al and Zoppi et al[25,27]. However Malaviyaet al and Handa et al showed PR positivity more than ER expression[13,14].

Most common grade found in our study was grade 2(48%), followed by grade 3 and then grade 1.This was also seen in studies like Sinha et al, Adedayo et al, Nisa et al [15,16,17,18,19]
Within grade 2, highest occurrences were of ER+ve( 45.23%) followed by PR+ve(40.47%) and Her2/neu(14.28%)

Most common grade noticed in HER2/neu +ve cases was grade 2. This was also observed in Huang et al.[20]
HER 2/neu positivity was seen more in cases of higher grades. This is also seen in other studies like Tatjana et al, Ariga et al, Prati et al, Almasri et al and Bozcuk et al. [20-24]

On an overall evaluation of our study, 72% cases were invasive ductal carcinoma and showed the following relations, 64% ER positive 36% ER negative, 54% PR positive and 46% PR negative, 20% her2/neu positive and 80% her2/neu negative.

## V. CONCLUSION

The increase in the rate of mortality from breast carcinoma indicates the need for new clinical developments, preferably targeted therapy. The presence of hormone receptors can assist in targeted therapy against the tumour receptors. Immunohistochemistry plays an important role in making differential diagnosis of invasive breast cancer lesions; understanding the status of IHC expression of these lesions aids in the diagnosis.

Looking at all our findings we can conclude that most common grade of presentation in our study is grade 2 followed by grade 3 and grade 1. Reactivity for steroid receptors was observed to be the most with grade 2 tumours. Grade 1 tumour were more receptor status negative as compared to grade 3 and grade 2 tumour. This data correlated with other studies too.

Our study only had 1 male patient who had ER/PR +ve and Her2/neu -ve status.

ER positivity was observed to be more than PR positivity which was further higher than Her2/neu positivity.

Most common grade observed in HER2/neu +ve cases was grade 2.

Our results can be used for future references.This information can be used in a more extensive research if necessary.Clinical comparison/correlation based on the expectation of what grade to expect with the hormonal status would be beneficial, especially when it comes to staging.It's possible that a high grade indicates a high stage.

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