

Interrelationships between Ki67, p53 with ER, PR & HER2/neu, Status and their Associations with Tumor Grade in Infiltrating Duct Carcinoma Breast

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Abstract: Introduction: Breast cancer is the most common malignancy occurring in females, accounting for 23% of all malignant tumors. Over 1 lakh new breast cancer patients are estimated to be diagnosed every year in India. It is expected to increase by 26% by 2020 in developing countries. Various predictive and prognostic factors affect tumor progression including tumor grade, ER, PR, Her2neu, Ki-67 & p53 over expression. Aims: 1) To correlate p53 and Ki 67 expression with ER PR & her2/neu expression in carcinoma breast. 2) To study p53 and Ki-67 expression in different grades of carcinoma breast. Methods and Material: A Retrospective and prospective study of 100 cases of carcinoma breast in M.Y. Hospital Indore during 3 year duration. Patient's information was extracted from hospital records regarding age, tumor size, histological grade of tumor, ER and PR, HER2/neu status. Immunohistochemistry was performed on histopathology sections to know p53 and Ki67 expression. Result: Percentage expression of different tumor markers are ER 32 %, PR 31%, HER2/neu 12%, and triple negative 65%, p53 61.2% and ki-67 is 55%. Ki-67 maximum express with luminal-A tumors. 52.3% ER PE positive tumor show p53 expression. Ki-67 and p53 expression maximum with grade III. Conclusion: Breast cancer patients with higher Ki67 expression showed more HER2/neu overexpression, and these cancers may be identified as aggressive tumors. Moreover, in young patients with breast carcinoma, the rates of Ki67 with overexpression of HER2/neu and p53. mutation were higher, and it is shown to be indicative of a more invasive tumor and a higher frequency of metastasis. However, p53 mutation was seen with higher tumor grades.

Keywords: Breast carcinoma, histologic grading, immunohistochemistry. Hormone receptor ER, PR, HER2/neu and p53 & ki67

1. Introduction

Breast cancer is the most common malignancy occurring in females, accounting for 23% of all malignant tumors⁽¹⁾. Invasive breast cancer is still the most common malignancy in female worldwide and more than 1 million women are diagnosed with breast cancer each year⁽²⁾. Over 1 lakh new breast cancer patients are estimated to be diagnosed every year in India. It is expected to increase by 26% by 2020 in developing countries⁽³⁾. Various predictive and prognostic factors affect tumor progression⁽⁴⁾. Some factors have both prognostic and predictive value, including ER and PR status and Her2neu, Ki-67 & p53 over expression⁽⁴⁾. Classification of breast carcinoma according to IHC⁽⁵⁾: Luminal A (ER +ve and PR +ve, Her2neu -ve), Luminal B (ER+ve, and/or PR +ve, Her2neu +ve or -ve), triple negative or basal like (ER-ve, PR-ve and Her2neu-ve) & Her 2 type (ER-, PR-, Her2neu+) Estrogen receptor (ER) is the most important prognostic and predictive marker and it indicates response to tamoxifen therapy in breast cancer⁽⁶⁾. Expression of estrogen receptor is present in about 70% of breast cancer cases⁽⁷⁾. Estrogen receptor (ER) status is an important predictive and prognostic factor in breast cancer⁽⁸⁾. Presence of both ER and PR is related to better prognosis and responsiveness to hormonal therapy (Wong *et al.*, 1990). Typically Her2 amplified tumors are associated with high grade and often extensive ductal carcinoma⁽⁹⁾. The expression of the nuclear proliferating antigen, Ki67 has been observed to reflect the proliferation rate of malignant tumors⁽¹⁰⁾. Ki-67 also indicate metastatic potential and prognosis of tumor⁽¹¹⁾. P53 is

associated with more aggressiveness of tumor and worse overall survival⁽¹²⁾

2. Subject and Material

A prospective and retrospective study was conducted on 50 cases of carcinoma breast whether received as MRM, Mastectomy & Lumpectomy specimen in histopathology department of M. Y. Hospital Indore for 3 year duration. Our exclusion criterion was metastatic carcinomas to the breast. Patients information was extracted from department records regarding age of patient, tumor size, histopathological grade of tumor, ER, PR & HER2/neu status. IHC was applied to know Ki-67 & p53 expression by **horseradish peroxidase-DAB chromogen method**. The Scarff-Bloom-Richardson standard grading system was used to grade the infiltrating duct carcinoma breast. The tumors were graded as 1, 2, or 3 based on the formation or nonformation of tubules, their nuclear pleomorphism, and the number of mitosis. Grade 1 is defined as a well, Grade 2 is intermediate prognosis, and grade 3 is a poorly differentiated tumor with the worst prognosis. The ER, PR & HER2/neu slides were previously immunohistochemically stained were reviewed. The current reporting format advocated for reporting ER and PR status is as follows:

Method of evaluation of ER and PR To quantify the hormonal status the Allred score was used. This takes into consideration both the proportion of marked cells and the medium intensity of the nuclear marking. The Allred score is the sum of the proportion score (proportion of marked cells)

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and the intensity score (marking intensity). Pleşan MD.,et al, Vol. 35, No. 3, 2009

Positive cell proportion	Proportion score
0	0
0-1%	1
1-10%	2
10%-1/3	3
1/3-2/3	4
2/3-100%	5

Marking intensity	Intensity score
Lack of marking	0
Low intensity	1
Moderate intensity	2
Higher intensity	3

The tumors that had an Allred score ≤ 2 were considered negative, and the ones that had an Allred > 2 score were positive.

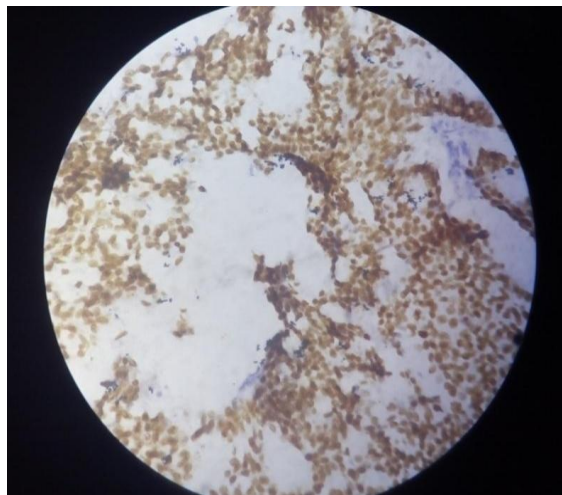


Figure 1: ER positivity 3+ in 100% of the tumour cell nuclei (Anti-ER- poly horseradish peroxidase- DAB chromogen, x40)

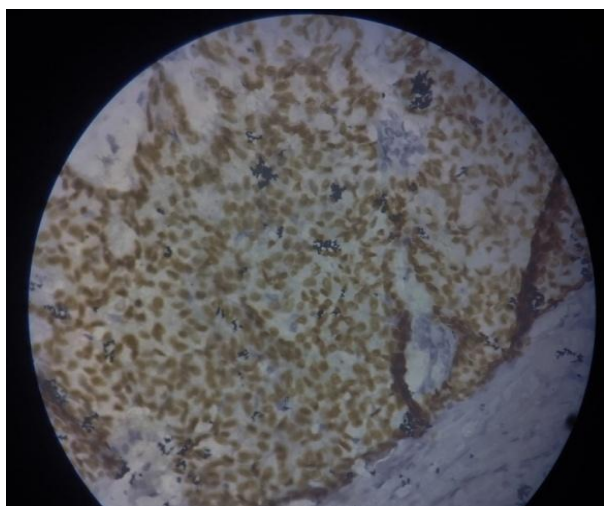


Figure 2: PR positivity 3+ in 100% of tumour cell nuclei (Anti-PR- poly horseradish peroxidase- DAB chromogen, x40)

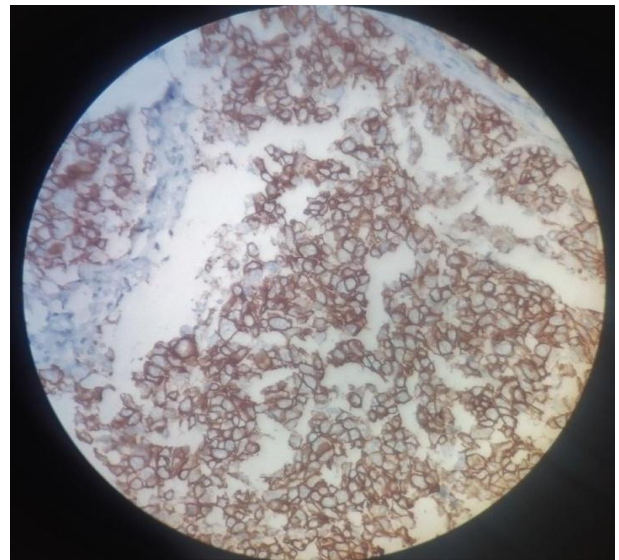


Figure 3: HER2 / neu 3+ in 100% of tumour cells with complete membrane staining (Anti-HER2/ neu- poly horseradish peroxidase- DAB chromogen, x40)

Table 2: Interpretation of HER2/neu staining:-The American society of clinical oncology and the college of American pathologists have provided guidelines for reporting HER2 immunostain (2007) which is summarized in the following:

HER2 IHC score	Staining Pattern	HER2 protein expression
0	No reactivity seen	Negative
1	Weak, incomplete membrane staining in any proportion of tumour cells	Negative
2	Non uniform or weak to moderate complete membranous reactivity in $> 10\%$ of tumour cells OR Intense complete staining of $< 30\%$ of the invasive tumour cells	Equivocal
3	Uniform , intense, complete membranous reactivity in $> 30\%$ of the invasive tumour Cells	Positive

Table 3: Immunoreactivity scoring of p53-(C.J. Fishser *et al.*, 1994: Problem with p53 immunohistochemical staining: the effect of f variation in the methods of evaluation)

Score	Staining character
Strong 4	Dark nuclear staining that is easily visible with a low power objective and involves $> 50\%$ of cells
Moderate 3	Focal darkly staining areas, ($< 50\%$ of cells) or moderate nuclear staining of $> 50\%$ of cells
Weak 2	Focal moderate staining in $< 50\%$ of cells or pale nuclear staining in any proportion of cell not easily seen under a low power
Scattered 1	Dark nuclear staining of widely scattered cell
Negative 0	Tumor that show none of above

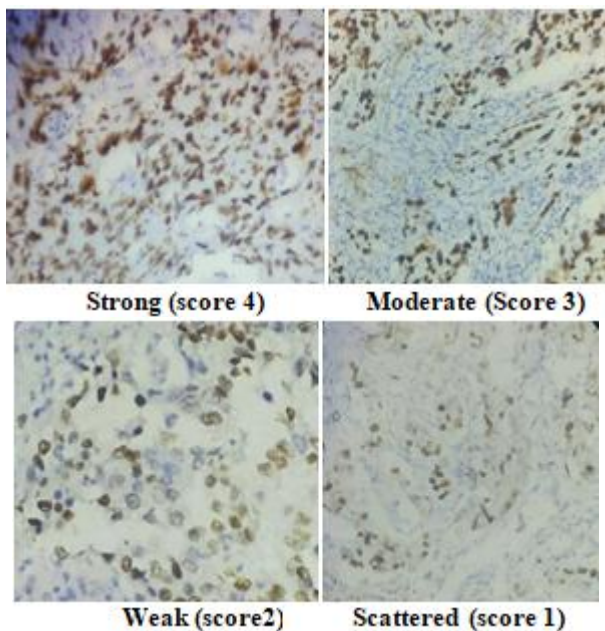


Figure 4: Anti-p53- poly horseradish peroxidase- DAB chromogen, x40

Ki67 scoring system/MIB-index Ki67 in breast cancer (13): In general, scoring systems are based on the percentage of tumour cells stained by the antibody. In this method, the pathologist examines the stained section with a standard light microscope 40x objectives, using a 10x10 graticule, and the Ki67 score is defined as the percentage of total number of tumour cells with nuclear staining. This requires counting at least 1000 tumour cells with nuclear staining in 10 high-powered fields (x40). Ki67 marker were divided into 4 groups: the nonstained as negative, those stained up to 10% as low, those stained over 10% to 20% as borderline, and >20% as high; however, the cells stained over 10% was consider as positive (9).

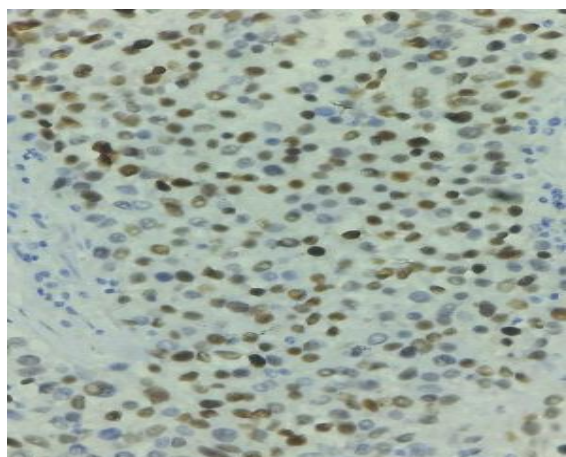
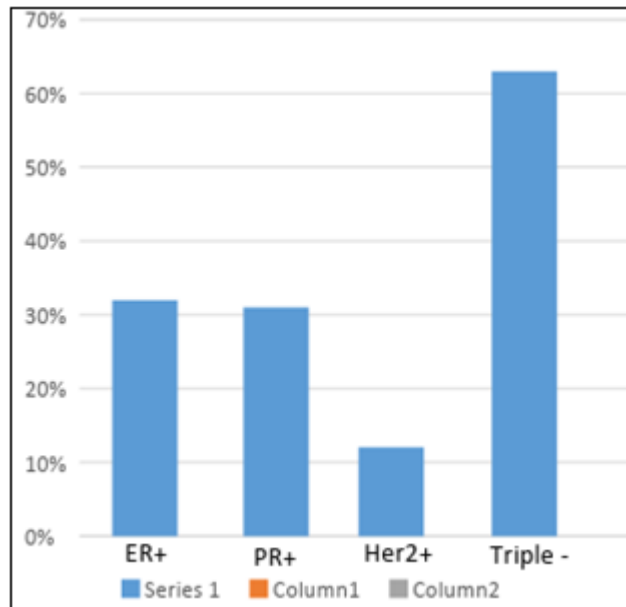


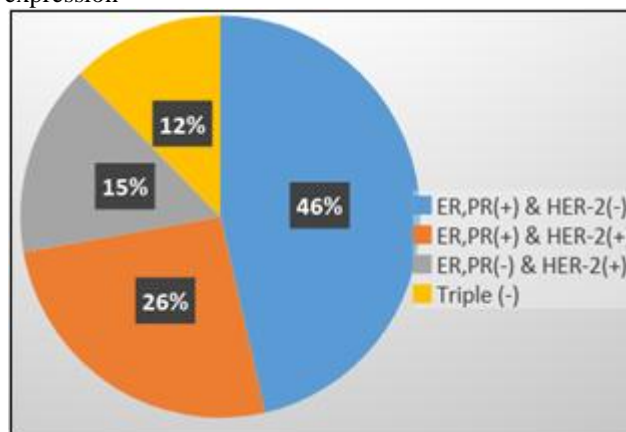
Figure 5: >20% high Ki67 score

3. Result

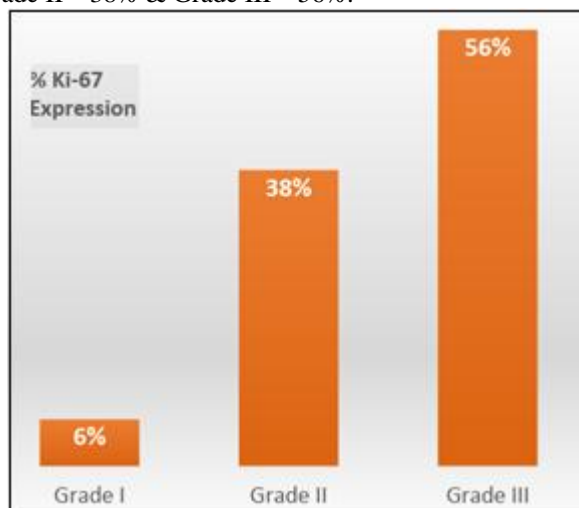
Expression of different tumor markers ER expression was 32%, PR expression was 31%, HER2/neu expression was 12%, 65% triple negative, p53 expression was 61.2% and 55% Ki-67 expression.



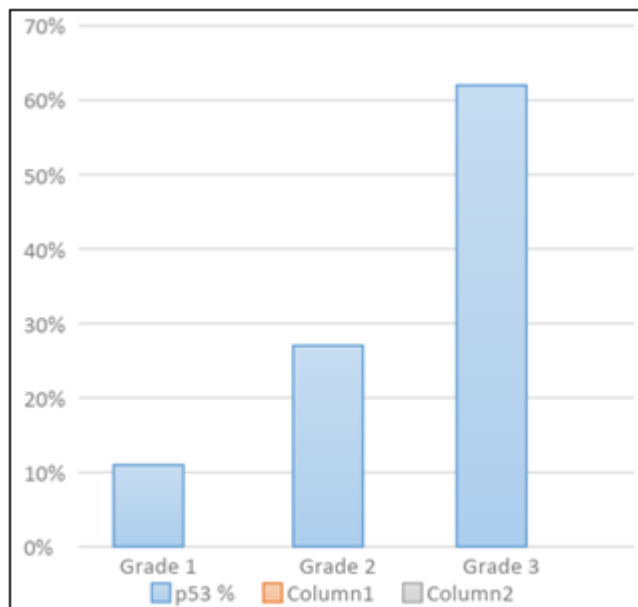
Correlation of Ki-67 expression with ER, PR, HER-2/neu expression-



Correlation of p53 expression with ER, PR, HER2/neu expression: 52.3% ER, PR(+) & 14% HER-2(+) show positivity for p53, P53 do not show any significance correlation with HER2/ne. A Significant correlation found between ki-67 & p53 expression about 55%. Association between Ki-67 expression and tumor grade- Grade I – 06%, Grade II – 38% & Grade III – 56%.



Association Between P53 expression and tumor grade Grade I – 11%, Grade II- 27% & Grade III – 62 %.



Interrelation of ER, PR, HER2/neu, p53 & Ki-67 expression with tumor grade:

Tumor marker	ER/PR+,Her2+ Luminal A	ER/PR+,Her2- Luminal B	ER/PR-,Her2+	Triple -	Ki-67	p53
Grade I	51%	48%	-	-	06%	11%
Grade II	04%	44%	11%	37%	38%	27%
Grade III	17%	08%	58%	35%	56%	62%

4. Discussion

Pawan nikhra et al., (2014) found similar correlation Grade I tumor show 50% positivity in IHC groups (ER/PR+,HER/2+) and (ER/PR+,HER/2-), while 44.4% of Grade II tumors showed (ER/PR+,HER/2-) and 58.3% Grade III tumor shows (ER/PR, HER/2+). We observed a significant correlation between histologic grade I and II and expression of ER and PR that is similar to the results of other studies^(14,15). Our study, as well as others, found a significant correlation between HER-2/neu expression and histologic grade III^(16,17,18) In present study it is 17%. Jian Yan *et al.*, (2015) found 67% ki-67 expression. In present study it is 55%. Tammim *et al.*, (2001) found p53 expression in 57.3%. In present study it is 61.2% Kim *et al.*,(2014) had found p53 expression maximum with grade III. In present study it is maximum with grade III IDC. Khanna *et al.*, (2016) found ER,PR expression is maximum with lower grades of tumors, Her2/neu and triple negative tumors are more related with higher grade of tumors and Ki-67 expression was higher with grade III tumor. It is similar to present study.

5. Conclusion

Our result showed positive correlation between ER and PR and inverse correlation of them with HER2/neu. ER and PR correlate with grade II and II IDC. HER2/neu expression maximum with grade III tumors. Ki67 and p53 expression correlation with higher tumor grades, lower ER, PR expression and higher HER2/neu expression. P53 and Ki-67 expression is a risk factor for rapid tumor recurrence & poor prognosis. That why it is considered as a new prognostic marker. Higher the Ki-67 and p53 expression higher the HER-2/neu expression. Ki-67 over expression with HER-

2/neu and p53 is indicative of more invasive tumor and a higher frequency of metastasis. Breast cancer patients with higher Ki67 and p53 expression showed more HER2/neu overexpression, and these cancers may be identified as aggressive tumors.

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