Role of p53 and Ki-67 in prognostication of carcinoma breast

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Abstract

Introduction: Tumor markers are used for the detection of risk, population screening, diagnosis, staging and prognosis. The present study was commenced out to study efficacy of p53 and Ki67 as Prognostic marker in breast carcinoma and to find correlation of ER, PR, HER2neu with p53 and Ki67 in confirmed cases of carcinoma Breast at a Tertiary Care Hospital.

Materials and Methods: This was an observational study, undertaken on 50 confirmed cases of breast carcinoma diagnosed during September 2016 to July 2018. The histopathological grading of the breast carcinoma was done according to modified Bloom Richardson score. All the cases underwent immunohistochemistry for ER, PR, HER2neu, p53 and Ki 67 expression. The results were compiled and analyzed statistically.

Result: In patient presenting with p53 overexpression,73.3% cases were <50 years of age, with tumors generally larger than 2cm (60% cases), 86.7% according to grade of tumor were high grade & nodal metastasis present in 86.7%, ER negative in 80%, PR negative 86.7% & HER2 positive in 20.0%. In Ki67 expression in relation with histo-pathological parameters 55% cases <50 years of age with tumor grade III in 80% cases with nodal metastasis present in 86.7%. Triple Antigen correlation with Ki67 overexpression shows ER Negative in 85%, PR negative in 90% & HER2 positive in 35%.

Conclusion: The present study concludes that ER, PR reveals inverse relationship while p53 and Ki 67 showed direct relationship with tumor grade; and is also inversely correlated with ER, PR positivity. In young patients with carcinoma breast, the rates of mutation of Ki67 and p53 were higher, and higher frequency of metastasis, the patients who show increased Ki 67 expression have also got increased mitotic activity and less expression of ER, PR; thus carrying a poor prognosis and requires the treatment accordingly.

Keywords: Carcinoma Breast, p53, Ki67.

Introduction

Breast carcinoma has become the most common malignancy in the female population, effecting one in eight women and is one of the leading causes of mortality among women in developing countries.[1]

Various factors like predictive and prognostic affect tumor progression.[2-4] Few factors are both prognostic and predictive, including p53 mutation status, estrogen receptor (ER), progesterone receptor (PR) status and human epidermal growth factor receptor (HER2/neu) overexpression. Prognostic factors comprise tumor type, number of involved lymph nodes at the time of diagnosis of tumor, its size, grade, Ki67 status (cellular proliferation marker), and the patient's age.[3-5] The present study was conducted for evaluation of p53 and Ki67 overexpression in known cases of carcinoma breast and to establish a logical relationship between these predictive and prognostic factors perhaps to arrive at better diagnosis and provide modalities to clinician for management so as to increase the disease free survival for patients.

Materials and Methods

Patient and Sample Collection

We studied 50 confirmed cases of breast carcinoma diagnosed during September 2016 to July 2018. The detailed history including patient's age and menopausal status taken. The paraffin sections were studied for histopathological type, grading, and lymph node metastasis.

The modified Bloom Richardson score system was used for histopathological grading of the carcinoma breast. All the cases were subjected to immunohistochemistry for p53, Ki67, ER, PR and HER2neu. Interpretation of the IHC scoring was carried out. The results were analysed statistically, the staining pattern for p53 and Ki67 evaluated respectively and elaborated in succeeding paragraph:

Evaluation of p53

In our study for p53 the tumor was considered positive when over 10% of tumor cells had a clear nuclear staining. (Fig. 1,2)

Ki67

Expression of Ki67, by immunostaining evaluation has become the gold standard, with a cutoff level of 10-14% positively-stained cells defend as high risk in terms of prognosis.[6,7]

In our study for ki67 the tumor was considered positive, if>14% of the tumor cells show positive stained nuclei.[8] The ki67 index can be calculated as -no. of positive stained tumor cells/no. of tumor cells x100. (Fig. 3,4)

Results

Table 1: p53 expression correlation with histo-pathological parameters

Characteristics	P53 overexpression	
	No. of cases= 15	%
Age [years]		
- <50	11	73.3%
- ≥50	4	26.7%
Size of tumors [cm]		
- <2 cm	6	40%
- >2 cm	9	60%
Histological grading		
- Grade II	2	13.3%
- Grade III	13	86.7%
Nodal Metastasis		
- Present	13	86.7%
- Absent	2	13.3%
Ki67		
- Positive	13	86.7%
- Negative	2	13.3%

Table 2: Biomarkers: Triple antigen correlation with p53 overexpression

Characteristics	P53 overexpression	
	No. of cases= 15	%
Estrogen receptors		
- ER positive	3	20.0%
- ER negative	12	80.0%
Progesterone receptors		
- PR positive	2	13.3%
- PR negative	13	86.7%
HER2 expression		
- HER2 positive	3	20.0%
- HER2 negative	12	80.0%

Table 3: Ki67 expression in relation with histo-pathological parameters

Characteristics	Ki67 overexpression	
	No. of cases=20	%
Age [years]		
- <50	11	55%
- ≥50	9	45%
Size of tumors [cm]		
- <2 cm	5	25%
- >2 cm	15	75%
Histological grading		
- Grade II	4	20%
- Grade III	16	80%
Nodal Metastasis		
- Present	16	86.7%
- Absent	4	13.3%

Table 4: Biomarkers: Triple antigen correlation with Ki67 overexpression

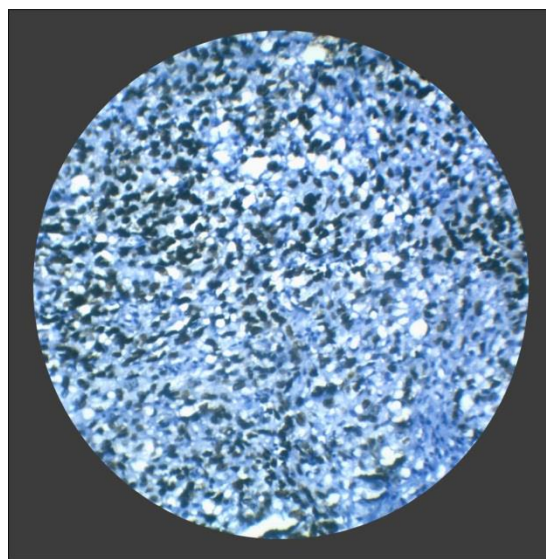| Characteristics	Ki67 overexpression	
	No. of cases=20	%
- **Estrogen receptors**		
- ER positive	3	15%
ER negative	17	85%
- **Progesterone receptors**		
- PR positive	2	10%
PR negative	18	90%
- **HER2 expression**		
- HER2 positive	7	35%
HER2 negative	13	65%

Fig. 1: Microphotograph of p53 showing nuclear positivity on 100 X
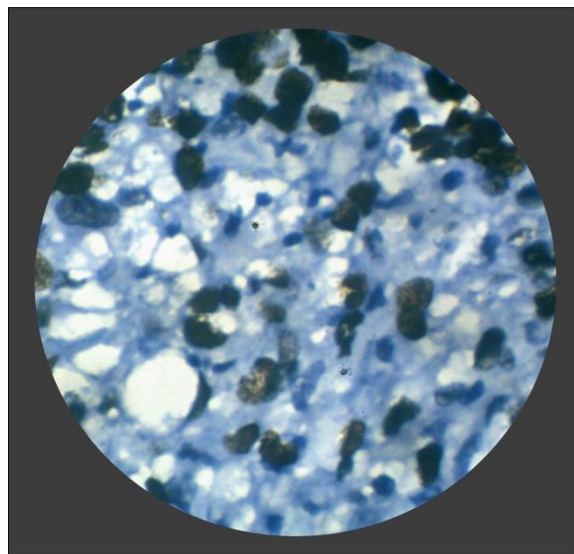

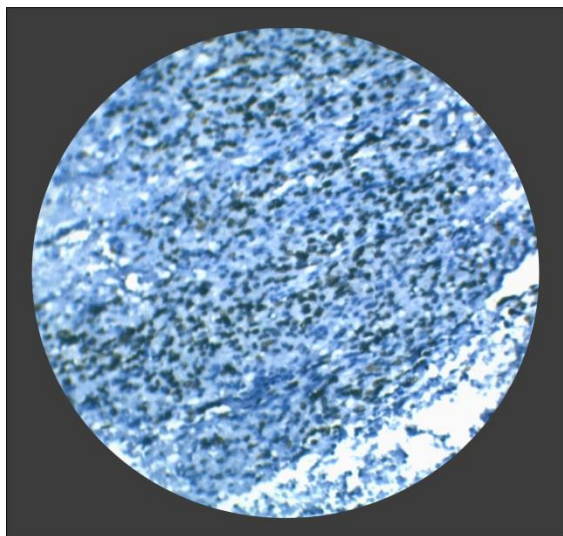**Fig. 2: Microphotograph of p53 showing nuclear positivity on 400 X**

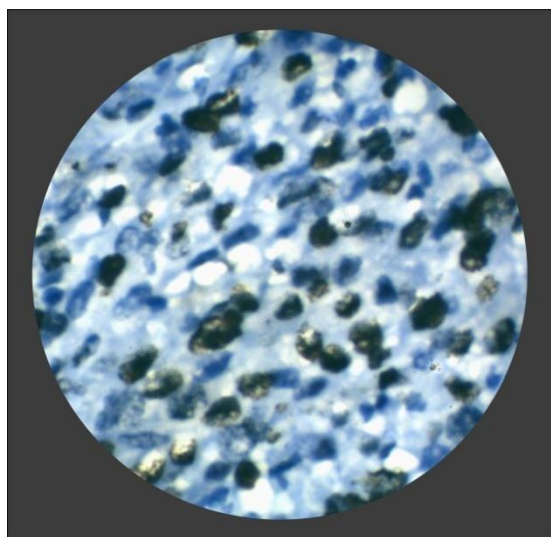**Fig. 3: Microphotograph of Ki67 showing nuclear positivity on 100 X**

Fig. 4: Microphotograph of Ki67 showing nuclear positivity on 400 X

Discussion

Breast cancer is one of the most prevalent cancers in women. It is well established fact that Proto-oncogenes and tumor suppressor genes play a central role in cell growth regulation and differentiation. Therefore in most human malignancies any alterations either in one or both genes appear to play an important role in their pathogenesis. The p53 oncoprotein is a phosphoprotein of 53 kD, encoded by the p53 gene, located on the short arm of chromosome 17. The p53 tumor suppressor gene, is a proved breast cancer progression gene that regulates the cell cycle and DNA repair.[9,10] Under normal conditions, the p53 gene act as "genome guardian", which monitor the DNA integrity during cell division. Unlike wild type (normal) p53, nonfunctional mutated p53 accumulates in the nucleus of tumor cells, and this p53 overexpression is detected by immunohistochemical analysis.

In our study, the p53 overexpression protein was encountered in 30% cases of carcinoma breast, these results are well correlated with the data from study conducted by Yamashita H. et al,[11] they found p53 overexpression in 29% cases.

We evaluated the clinical significance of p53 as a prognostic marker in relation to breast carcinoma, moreover the relationship between p53 and the clinicopathological parameters such as patient's age, size of tumor, tumor grade and ER, PR, HER2neu reactions those reflect the prognosis were investigated. (Table 1, 2)

We found that p53 overexpression was more frequently encountered in patients under 50 years of age compared to ones over age of 50 years (73% vs. 26.6%), with tumors generally larger than 2cm (60% vs. 40%), similar results were recorded by D.M Plesan et al,[12] under 50 (54.76%) compared to those over 50 years old (45.24%),. Likewise Bartley AN and Ross DW have shown, in their study,[13] that p53-positivity was detected in five out of seven patients under age 43 years.

We correlated histological grading of invasive carcinoma with p53 overexpression and found, that most cases with p53 overexpression (13 cases, 86.7% respectively) belong to high grade (G3), and only 2 cases (13.3%) had a low grade (G1 and G2). This result was consistent with the result obtained by study conducted by D.M Plesan et al[12] who found that most cases that overexpress p53 belong to high histologic grade (71.43%) and only (28.57%) cases belong to low grade. Kamal Kant Gupta et al,[14] found similar results.

The p53-immunopositivity was also correlated with the lack of estrogen and progesterone receptors: 80% cases had no receptor expression for estrogens *vs.* 20% cases having the immunoexpression of estrogen receptors. Also, 86.7% cases with p53-overexpression were negative for PR, while only 13.3% cases were PR-positive (table 2). DM Plesan et al[12] and Yamashita H.et al,[11] found similar results.

In our study we found that 20% of cases that had positive p53 had associated positivity for Her2neu, statistically it was not significant because the coexpression of p53 and HER2 was seen in 6% of all invasive mammary carcinoma cases that were included in this study. This percentage reflects the rarity of this double genetic defect, similar observation were made by DM Plesan et al,[12] they studied 100 cases from immunohistochemical point of view and found coexpression of p53 and Her2 in only 7% of all the cases of breast cancer.

Concerning the nodal metastasis most cases that had overexpression P53, 13 cases (86.7%) were lymph node positive and only 2 cases (13.3%) were lymph node negative (table1), thus a positive association found between p53 and lymphnode status, although due to small number of cases evaluated p value was not significant in our study.

We also considered Ki67, another marker of cell proliferation for prognostication of breast carcinoma. An increase of Ki-67 index shows an increase of cellular mitotic activity as well as proliferation.

It is nuclear non histone protein that is expressed in G1 through M phase of cell cycle and is not detected in resting phase of cells i.e. G0. Ki-67 increases as cells prepare to divide into new cells. The more positive cells therefore indicate, the more quick division and proliferation. Clinically, pKi67 also been shown to correlate with metastasis and the clinical stage of tumors.

In our study, Ki67 protein expression was encountered in 40% of cases of studied mammary carcinoma, the result were corresponding with the data from CH Yip et al[15] In their study, the authors observed that 43.4% cases were having Ki67 positive.

Analyzing the Ki67 expression we found that it was more frequently encountered in patients under 50 years compared to those over 50 years of age (55% vs. 45%), (Table 3), our findings coincide with the study conducted by CH YIP et al.[15]

In our study Ki67 positive tumors were larger than 2 cm out of total 20 Ki67 positive tumors, 15 are measured over 2 cm (75%), while only 5 tumors were smaller than 2 cm(25%) (Table 3), thus Ki67 expression associated with larger tumor size. This result was consistent with the result observed by Zhaoyun Liu et al,[16] Masahiro Ohara et al,[17] Taghipour Zahir Shokouh et al.[18]

As far as the histological grading is concerned, we found that 16 cases that were Ki67 positive (80%) were of high grade, thus a significant association established between ki67 and grade of tumor (Table 3), this is in concordance with various other studies like Taghipour Zahir Shokouh et al,[18] CH Yip et al,[15] Zhaoyun Liu et al.[16]

The immunopositivity of Ki67 was associated with the lack of ER and PR receptor in this study. 85% cases showed no estrogen receptor expression vs 15% cases having the immunoexpression of estrogen receptors. Also 90% Ki67 immunopositivity cases were negative for PR, while only 10% were PR positive (Table 4). Our findings perfectly correlated with the study conducted by Kiranjot Kaur et al,[19] and CH Yip et al.[15]

In our study we found that ki67 index was associated with only 35% cases of Her2neu positivity (Table 4), thus there was no statically significant relation in our study between these two parameters, similar observation was made by Reiki Nishimura et al,[20] they conducted a study on 3652 cases with breast cancer and reported that Ki67 index of her2 tumor was 40%.Similarly another study by Zhaoyun Liu et al[21] comprised of 398 cases that found no correlation between Her2neu status and Ki67.

On the contrary study conducted by CH YIP et al[15] on 450 patients reported significant association between ki67 index and her2 positivity.

Concerning the nodal metastasis 16 cases that were immunopositive for Ki67, (86.7%) were lymph node positive and only 4 cases (13.3%) were lymph node negative, thus a positive association found between ki67 and lymphnode status (Table 3), although due to small number of cases evaluated p value was not significant in our study.

Conclusion

In mammary carcinoma, the mutations of p53 are found to be associated with more aggressive behavior and lower survival rate. In our study we could prove that cases that have p53 overexpression are mostly high grade tumors, ER and PR negative, thus Breast cancer aggressiveness appears to be directly related to the percentage of p53 positive cancer cells.

Ki67 is a useful marker of cell proliferation. An increase in the Ki67 expression indicates an increase in the mitotic cell activity and proliferation.

Ki 67 showed direct relationship with the grade as it increases with higher grade and is also inversely correlated with ER, PR positivity, thus carrying a poor prognosis. In young patients with breast carcinoma, the rates of Ki67 and p53 mutation were higher, and it is shown to be indicative of a more invasive tumor and a higher frequency of metastasis

Thus we can conclude that p53 and Ki67 taken together are important predictive as well as prognostic marker for breast cancer patients.

Conflict of Interest: None

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