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Original article:

p53 expression and its correlation with other molecular markers in breast cancer

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Abstract:

Introduction: In light of surgical advances leading to breast-conserving therapy, it has become necessary to more accurately stratify patients based on relative risk of recurrence or progression. These demands have led to the generation of several newer classification systems that incorporate molecular markers such as estrogen and progesterone receptor(ER,PR) and Human epidermal growth factor receptor 2(Her2/neu or ErbB2). Status of these markers helps determine which patients are likely to respond to targeted therapies .Recently p53, a tumor suppressor protein has been recognized as an important predictive and prognostic marker in many studies.

Methods: 50 histologically confirmed female breast cancer patients in the age group of 18-80 years were recruited. Information on patient's age, menopausal status, disease stage, clinical nodes were noted from the case files.

The histopathological grading of the breast carcinoma was done according to the Nottingham modification of the Bloom Richardson grading system. IHC (Immunohistochemistry) was performed by using antibodies against the ER, PR, Her2neu, and p53.

Results: Overall positivity/overexpression of p53 was seen in 38 cases (76%).Of these 22 were of grade II and 15 were of grade III tumors. An increase in expression of p53 was found with an increase in histological grade. No significant correlation was observed between p53 and ER, PR, Her2/neu overexpression.

Keywords: Breast cancer, p53, Her2/neu, biomarkers, tumor markers

Introduction:

An estimated 1.38 million women worldwide are diagnosed annually with breast cancer [1]. A study of breast cancer in India revealed that 1 in 28 women develop breast cancer during her lifetime (1 in 22 in urban areas). Breast cancer is a complex disease including very distinct clinical, morphological and molecular entities[2].

Biomarkers are useful for early detection of cancer by screening and for monitoring cancer status in patients during and after anticancer treatment as tumor markers. According to US National Institutes of Health's (NIH) Working Group and Biomarkers Consortium, a molecular marker is a characteristic that is objectively measured as an indicator of pathogenic or normal biological processes, or a pharmacological response to a therapeutic intervention.

The p53 gene is located on the short arm of chromosome 17 and encodes a 375 amino acid nuclear phosphoprotein that prevents propagation of genetically altered cells[3]. In normal cells, p53 is expressed in minutes and has a very short half-life by virtue of ubiquitylation and proteasome degradation mediated by MDM2 (Mouse double minute 2

homolog)[4,5]. However, missense mutations within the p53 gene result in protein that is stabilized through posttranscriptional modification and accumulation within the cell nucleus.

Over half of human cancers acquire p53 mutations during malignant transformation resulting from either loss of function or gain of function mutations. Gain of function mutations in p53 result from a change in a single amino acid in the DNA binding domain, leading to inactivity, increased stability, and expression of high levels of p53 protein detectable by immunohistochemistry[6]. Immunohistochemical staining for the p53 protein, performed on tissue sections, is a proxy for gain of function mutations in p53 that result in inactivity. Mutations in the tumor suppressor gene p53 are present in 18%–25% of primary breast carcinomas[7].

Knowledge about p53 is important because breast carcinoma is a clinically diverse and heterogeneous disease, and the clinical course of these patients varies greatly. Unlike Estrogen receptor (ER), Progesterone receptor (PR), and Human epidermal growth factor receptor 2(Her2/neu or ErbB2), the predictive and prognostic value of p53 in breast cancer, is still under discussion.

We aimed to study the expression of p53 in breast cancer patients and its correlation with histopathological grade and other molecular markers (ER, PR, Her2/neu).

Material and methods:

The study was conducted on 50 patients attending the Cancer Research Institute, Swami Ram Himalayan University, Dehradun during the study period of one year (2015-2016), after the approval by the Ethics Committee. Histologically confirmed female patients in the group 18-80 years were included in the study. Histologically benign, previously treated or patients suffering from other cancers as well were excluded.

The tissue after proper labeling was processed by standard processing methods using Automated Tissue Processor, formalin, paraffin embedded and was then stained for Haematoxylin and Eosin for histopathological typing and grading. The histopathological grading of the breast carcinoma was done according to the Nottingham modification of the Bloom Richardson grading system.

IHC was performed by using antibodies against ER, PR (Diagnostic Biosystem) and Her-2/neu, p53 (Biogenex).Microwave treatment was used as an effective method for the antigen retrieval with 10mmol citrate buffer at pH 6. Tris buffer was used as the wash buffer and diaminobenzenetetrahydrochloride (DAB) was used as the chromogen. The endogenous activity was blocked by using hydrogen peroxide. After protein blocking, the slides were incubated overnight with the available ER, PR, Her2neu and p53 primary antibodies and were conjugated with streptavidin Horse Radish Peroxidase (HRP). The slides were counterstained with hematoxylin and were examined by light microscopy.

ER & PR was assessed semiquantitatively using Mc Carty H scoring system..

For the HER2/neu overexpression, the data were classified from 0 to 3 based on the criteria provided by Dako (Glostrup); the scores 0 and 1+ were considered as negative, 2+ as borderline, and 3+ as positive. All the cases with a 2+ score were sent to another center for the FISH (Flurescent In situ Hybridisation) test, and all the cases with a positive FISH were identified as HER2/neu overexpression positive.

Staining pattern - nucleus of malignant cells.

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(A) To assess the p53 staining positivity, the number of p53 positive nuclei out of 200 nuclei were counted and was expressed in percentage and score as follows:

0 - Negative

1 - <10% (positive staining)

2 - 10-50% (positive staining)

3 - >50%

The score was taken as A.

(B)The intensity of staining in p53 positive nuclei was compared with the positive control and scored as follows.

0 - negative

1 - cleared stained but intensity < positive control

2 - intensity = positive control

3 - intensity > positive control

Total score of 200 cells was taken as B.

The final scoring was average of two observations.

Overall p53 expression was the sum total of scores of stain positivity.

(A) Stain intensity (B) Overall positivity = (A) + (B)

Results:

The maximum number of patients were in the age group of 31-40 years (n=14, 28%), followed by 41-50 years (n=11, 22 %), 8(16%) patients were seen in 71-80 years of age. The youngest patient was 25 years old. The oldest patient was 74 years old..26 (52%) patients were pre menopausal whereas 24 (48%) patients were post menopausal**.** Out of the 50 patients, 29 (58%) patients had Invasive Ductal Carcinoma (IDC) Grade II, followed by 20 (40%) patients who had IDC grade III and 1 (2%) patient was IDC grade I.

26 patients belonged to the molecular class Luminal A and were ER+ve and Her-2/neu negative, 11 patients belonged to the molecular class Luminal B and were ER +ve and showed overexpression of Her-2/neu. 8 patients were triple negative and did not express ER, PR and Her-2/neu. 5 patients overexpressed Her-2/neu and did not express ER (Fig 1).

Of the 50 patients, 21 (42%) patients showed strong ER positivity, followed by 16 (32%) patients which showed negative staining for ER.Of the 50 patients , 19 (38%) patients showed negative expression of PR, 18 (36%) patients showed strong positivity for PR, followed by 11 (22%) patients showing moderate positivity for PR There were nearly equal number of cases were ER and PR positive (n=18, 36%) and ER and PR negative (n=16, 32%).

Of the 50 patients, the maximum cases i.e. n=29 (58%) showed no staining for Her-2/neu ; followed by 13 (26%) patients which showed strong membrane staining for Her-2/neu. Of the 29 IDC grade II patients,17(58.6%) patients showed negative staining for Her-2/neu, followed by 8 (27.5%) patients showing strong positivity. Of the 20 IDC grade III, 12 (60%) patients showed negative staining for Her-2/neu, followed by 5 (25%) patients showing

strong positivity.there was no significant correlation between ER and Her-2/neu (p= PR and Her-2/neu expression (p=0.246).

Of the 50 patients, 22 (44%) patients out of 29 of IDC grade II showed strong p53 positivity and 15 (30%) cases of IDC grade III, also showed strong positivity for p53. An increase in expression of p53 was found with an increase in histological grade (Table 2).

Out of 12 (24%) patients negative for p53, 8 were positive for ER. Of the 38 cases positive for p53, 26 were positive for ER. No significant correlation was found between p53 and ER expression .The proportion of percentage was found to be statistically independent (p=0.910)

Of the 50 patients, 14 (28%) patients each showed negative and severe PR positivity; followed by 5 patients (10%) which showed negative staining for p53 as well as for PR score. No significant correlation between expression of p53 and PR staining was found. The proportion of percentage was found to be statistically independent (p=0.764)

Of the 50 patients, 40 % of p53 positive patients showed negative staining for He-2/neu. Out of 12 (24%) p53 negative patients, only 3 patients showed strong Her-2/neu positivity. The proportion of percentage was found to be statistically independent (p=0.928)

Out of 8 Triple negative breast cancer patients, 5 patients showed positive staining for p53; followed by 3 patients which showed negative staining (Table 3)

Figure 1:

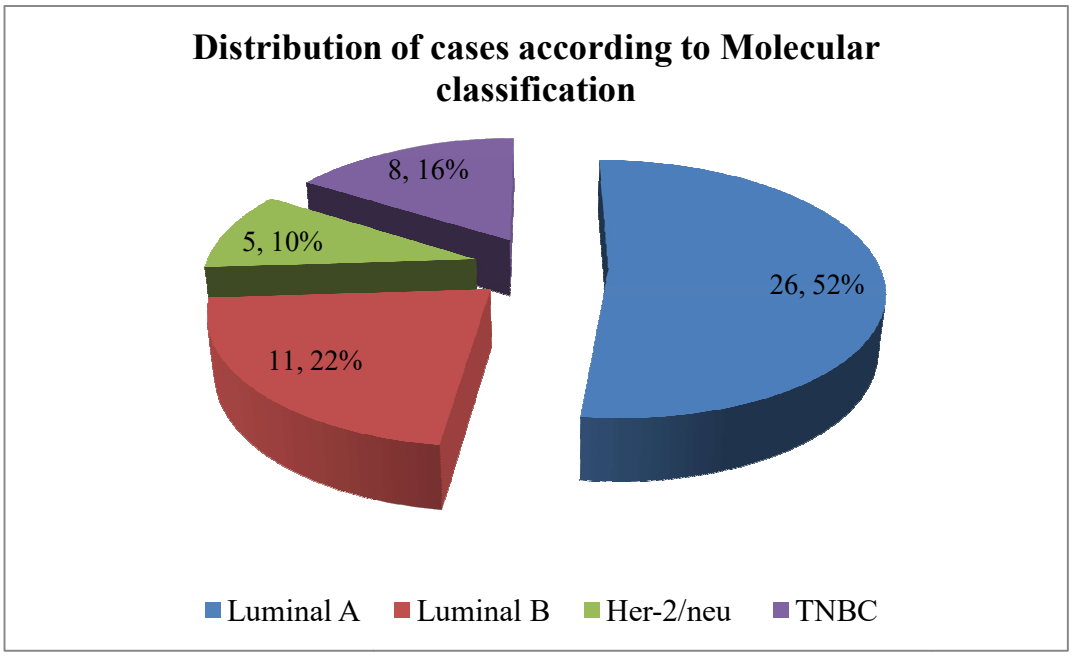

Table 1: Expression of p53 and its correlation to histological grading

Grade	p53 Score							Total	Percentage
	0	1	2	3	4	5	6		
I	0	0	0	0	0	0	1	1	2%
II	4	2	1	2	5	9	6	29	58%
III	2	1	2	4	2	3	6	20	40%
Total	**6**	**3**	**3**	**6**	**7**	**12**	**13**	**50**	**100%**

Table 2: Correlation between p53 and ER score, PR score, Her2/neu

p53	ER score				P value
	Negative (0-50)	Weakly+ (51-100)	Moderately+ (101-200)	Strongly+ (201- 300)	
Negative (n=12)	4 (8%)	1 (2%)	2 (4%)	5 (10%)	Chi square = 0.12 d.f=1 p=0.910
Positive (n=38)	12 (24%)	4 (8%)	6 (12%)	16 (32%)	

p53	PR score				P value
	Negative (0-50)	Weakly+ (51-100)	Moderately+ (101-200)	Strongly+ (201- 300)	
Negative (n=12)	5 (10%)	0 (0%)	3 (6%)	4 (8%)	Chi square = 0.90 d.f=1 p=0.764
Positive (n=38)	14 (28%)	2 (4%)	8 (16%)	14 (28%)	

p53	Her-2-neu				P value
	0	1+	2+	3+	
Negative (n=12)	9 (18%)	0 (0%)	0 (0%)	3 (6%)	Chi square = 0.82 d.f=1 p=0.928
Positive (n=38)	20 (40%)	3 (6%)	5 (10%)	10 (20%)	

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Table 3: Correlation between p53 immunostaining and Triple Negative breast cancers

Molecular classification	p53	
TNBC (n=8)	Negative	Positive
	3	5

Discussion:

In our study maximum number of cases were from 35-50 years which corroborates the findings in studies conducted in Indian subcontinent which is a decade earlier than results observed in European countries .Histologically, IDC is the most common type of invasive carcinoma.. In the present study all 50 breast cancer patients had Invasive ductal carcinoma. In a study done by Korkolis et al, 84% of breast cancers were ductal infiltrative type [8]. In another study conducted by Tan et al the tumor subtype was infiltrative ductal carcinoma in all but one case which was an invasive lobular carcinoma [9].

ER expression in breast cancer patients indicates better prognosis because ER in primary tumor identifies patients with lower risk of relapse and better response to endocrine therapy. Of the 50 patients, 34 patients (68%) showed ER positivity, followed by 16 patients (32%) which showed negative staining for ER.

PR assessment is valuable in breast cancer patients as it indicates the disease free survival in these patients.Of the 50 cases studied, 19 cases (38%) showed negative expression of PR and 31 cases (62%) showed positivity for PR. ER and PR values were co-related with each other in most of the cases.

Overall positivity for Her-2-neu (2+/3+) in our study was 36%. Ross et al in their study reported over expression of Her-2-neu protein to be in the range of 10-34% of breast cancer cases[10]. Korkolis et al and Sharif et al showed Her-2/neu positivity to be 46% and 31% respectively[8,11]

The p53 tumor suppressor protein, encoded by the TP53 gene, is a transcription factor that, when activated as part of the cellular stress response, regulates genes involved in cellular processes including the cell cycle, apoptosis, and senescence. In this study, p53 protein accumulation was assessed by immunohistochemistry (IHC). IHC was ideal because wild-type p53 protein is rapidly degraded, whereas TP53 mutations are often associated with the production of a stable

protein that is detectable by IHC of the cancer cells32. Currently, IHC is the most commonly used modality for the evaluation of p53 mutations because sequencing of the p53 gene in all breast cancers would be expensive and time consuming for daily practice[12]

In this present study, out of 50, 38 (76%) patients showed p53 positivity and 12 (24%) patients showed negative p53 staining. Similar results were seen in a study done by Lu et al who stated p53 overexpression to be 74.38% [13]. However, in a study done by Korkolis et al p53 overexpression was seen in 31% cases[8]. Another study by Byung Joo Chae et al IHC for p53 was positive in 42.2% % breast cancer cases [14]. According to Dong-Soo Lee et al p53 overexpression was detected in 37.1% cases +15. These differences may attribute to the demographic genetic variations and sample size used by different investigators [16]

The correlation of p53 expression and grade of tumors were analyzed. Out of 29 patients of IDC grade II, 22 patients (75%) showed strong p53 positivity and 15 cases (75%) out of 20 cases of IDC grade III, also showed strong positivity for p53. These observations show a correlation between p53 expression and histological grade of tumors. However, a study done by Ahmed et al showed statistically significant association between p53 expression and tumor grade [17]. Another study done by Miller et al also showed correlation between p53 overexpression and high tumor grade [18].

The correlation between p53 expression and ER score was also studied. Out of 12 patients (24%) negative for p53, 8 were positive for ER. Of the 38 cases positive for p53, 26 were positive for ER. Thus, as can be seen from the above observations no significant correlation was found between p53 and ER expression. Similar results were seen in study done by Ozer et al who also did not find any correlation between p53 mutation and ER expression [19]. However, a study done by Abdollahi et al showed that p53 expression was inversely correlated with ER score [20] Another study done by Ahmed et al also showed a tendency of ER hormone receptor positive tumors to be negative when associated with p53 expression [17].

The correlation between p53 expression and PR score was also studied. Of the 50 patients, 14(28%) patients each showed negative and severe PR positivity; followed by 5 patients (10%) which showed negative staining for p53 as well as for PR score. No significant correlation between expression of p53 and PR staining was found. Study done by Ozer et al also showed similar observations and did not find any correlation between p53 mutations and PR expression[19]. Whereas, studies done by Abdollahi et al and Ahmed et al showed that p53 expression was inversely correlated with ER score [17,20].

The correlation between p53 expression and Her-2/neu was analyzed. Of the 50 patients, 40 % of p53 positive patients showed negative staining for He-2/neu. Out of 12 (24%) p53negative patients, only 3 patients showed strong Her-2/neu positivity. In a study done by Abdollahi et al no significant relationship was found between Her-2/neu and p53 expression [20] . Ahmed et al also did not find any association between Her-2/neu overexpression and p53[17]

Out of 8 Triple negative breast cancer patients, 5 patients showed positive staining for p53; followed by 3 patients which showed negative staining. The results of this study show that there is no correlation between p53 and TNBC. However, study conducted by Turner et al showed a definite correlation between the two [21].

This study had several limitations. First, the small sample size may have limited the ability to derive statistically significant results and correct for differences in subgroup characteristics. Our studies did provide more information in the evaluation of p53's significance in metastatic breast cancer (MBC).

Conclusion:

These results indicate, by IHC, that patients with p53 protein accumulation appear to have higher grade of cancer than those without p53 expression. However, presence or absence of p53alone is not a clinically useful tool to diagnose or predict overall survival (OS). Larger prospective studies will be needed to confirm these findings.

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