Utility of evaluation of P53 and BRCA1 in invasive breast cancers: An immunohistochemical study

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Abstract

Introduction: Even with the advent of effective treatment strategies for breast cancer, the prognostication and treatment of each case varies owing to the varied expression of different prognostic markers.

Aims and Objectives: In this study, there has been an attempt to study the immunohistochemical expression of p53 and BRCA1 proteins in invasive breast cancers and to find the correlation if any, between the expressions of p53 and BRCA1 with various prognostic factors like histological grading, the hormone receptors and Her-2/neu status.

Materials and Methods: The study was conducted in the Department of Pathology at S.C.B. Medical College, Cuttack. Fifty histologically proven cases of invasive carcinoma of were included in the study.

Results: P53 positivity was seen in 40% of cases in our study while BRCA1 expression was observed in 48% of the total cases. P53 positivity was found to be associated with higher histologic grades (p value =0.05), Her-2/neu expression (p value = 0.0005) and negative hormone receptors (p value = 0.0046). BRCA1 positivity was well associated with positive hormone receptors (p value = 0.03).The absence of BRCA1 staining was mostly seen in tumours of large sizes (p value = 0.01) and higher histologic grades (p value = 0.04). No significant association was seen between the expressions of BRCA1 and HER-2/neu.

Conclusion: Immunohistochemical staining of P53 can be used as a prognostic indicator in cases of invasive breast carcinoma. The immunohistochemical detection of BRCA1 may be used to generate a larger pathologic picture of breast cancers in conjunction with the traditional prognostic parameters.

Keywords: P53, BRCA1, Immunohistochemistry, Breast carcinoma.

Introduction

India continues to be overburdened by breast cancer, which is now the leading cause of cancer in Indian females with age adjusted rate as high as 25.8 per 100,000 women and mortality 12.7 per 100,000 women.[1] According to GLOBOCAN 2012, it is the most common cancer in women, surpassing cervical cancer and accounting for 25.1% of all cancers. It was estimated that 1,671,149 new cases of breast cancer were identified and 521,907 cases of deaths due to breast cancer occurred in the world in 2012. Women are the predominant sufferers from this dreaded disease while men account for only 1% of cases. Although successful treatment strategies have come up in the recent years, the need for individual consideration of each case of breast cancer still holds importance. Moreover a consensus on a definitive prognostic analysis is yet to be reached and significant progress continues to be made for identifying treatment algorithms using various prognostic markers.

Although immunohistochemistry is an inexpensive and valuable preliminary method for BRCA 1 status, but the protein localisation remains an unsolved issue. There is no consensus on the pattern of expression of this protein. So, further studies are imperative to assess the performance of various anti-BRCA1 antibodies in formalin fixed paraffin embedded tissue samples.Hence this study was undertaken to see the immunohistochemical expression of p53 and BRCA1 proteins in invasive breast cancers and to find the correlation if any, between the expressions of p53 and BRCA1 with various prognostic factors like histological grading, the hormone receptors and Her-2/neu status.

Materials and Methods

The study was conducted in the Department of Pathology at S.C.B. Medical College, Cuttack from the period of August 2011 to August 2013. Modified radical mastectomy specimens of cases that were clinically diagnosed as breast carcinoma were taken. Before proceeding for fixation of the specimens, details such as serial number, name of the patient age of the patient, sex, registration number, clinical, personal and family history, investigation findings, clinical and radiological diagnosis were noted down. This was followed by the histopathological examination of the cases after optimum processing. 50 histologically proven cases of invasive carcinoma of breast irrespective of age were included in the study. The histopathological grading assigned for the cases was according to Elston and Ellis modification of Bloom and Richardson's original classification from 1957,[2] which includes three parameters namely the architecture (considering the proportion of cells arranged in tubules), nuclear pleomorphism (size of nucleus as compared to normal duct cell nucleus, chromatin and nucleolus)) and mitotic activity(taking into account the field diameter of objective lens). Paraffin blocks which were most representative of only invasive component of tumour tissue were chosen for performing immunohistochemistry. It was done for evaluation of estrogen and progesterone receptors, Her-2/neu, P53 and BRCA1. Biogenex ready to use mouse monoclonal antibodies were utilised for this purpose. For

P53, nuclear staining in more than 10% of the tumour cells (Fig. 1, 2) was considered to be positive[3] and for BRCA 1, cytoplasmic (Fig. 3) or cytoplasmic and nuclear staining (Fig. 4) in more than 10% of tumour cells was considered positive.[4,5] The interpretation of estrogen and progesterone receptor status was done by the Alred score which includes both the proportion and intensity of staining of the tumor cells. The Her-2neu status was interpreted following the ASCO -CAP guidelines for Her-2neu test recommendations, 2013. The expression of p53 and BRCA 1 were first studied in the tumours following which they were compared with the respective histological gradings, Her-2/neu and hormone receptor status determined immunohistochemically. The results of immunohistochemistry were noted down in tabular forms. The number of cases in each category were also expressed in the form of percentages. The Chi-square test was employed to find the correlation between desired variables. The result was considered statistically significant if p value was less than 0.05.

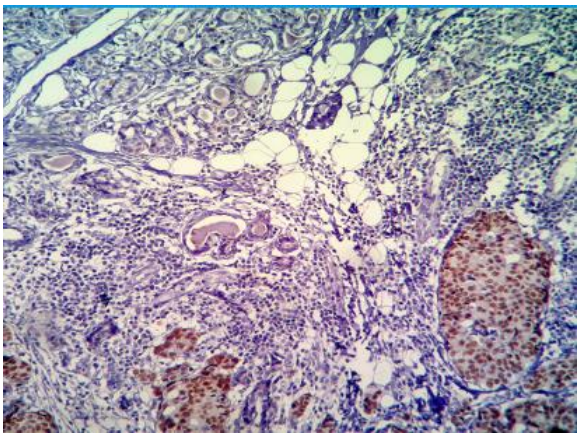

Fig. 1: (Immunohistochemical stain, 100X) Nuclear staining of the malignant cells by p53 in a case of invasive ductal carcinoma with sparing of normal breast tissue

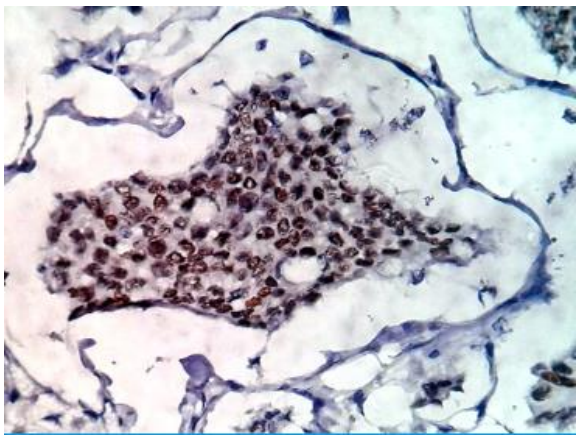

Fig. 2: (Immunohistochemical stain, 400X) Nuclear staining of malignant cells by p53 in a case of mucinous carcinoma of breast

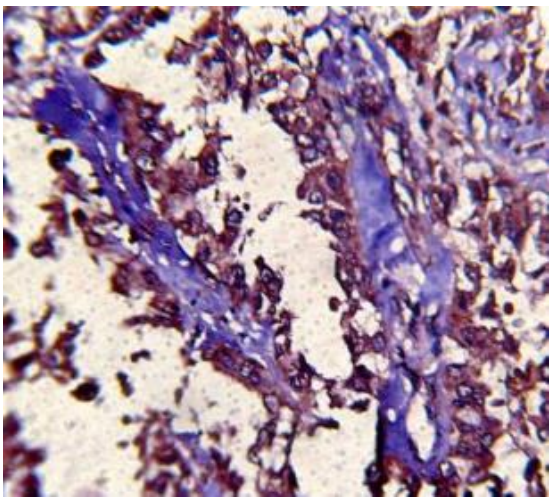

Fig. 3: (Immunohistochemical stain, 400X) Cytoplasmic staining of the malignant cells by BRCA1 in a case of papillary carcinoma of breast

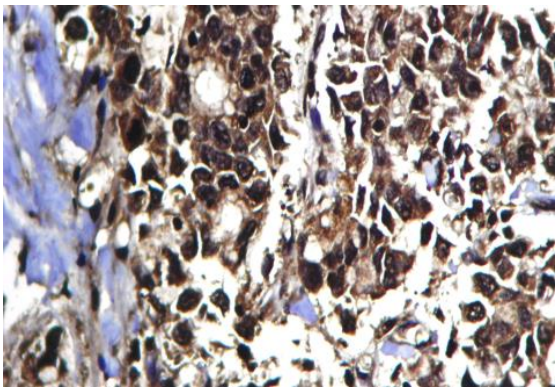

Fig. 4: (Immunohistochemical stain, 400X) Cytoplasmic and nuclear staining of the malignant cells by BRCA1 in a case of invasive ductal carcinoma

Results

The present study was done on 50 patients. The highest number of cases was found in the 5th (41-50yrs) decade (50%) followed by 6th (51-60yrs) decade (26%). The mean age was calculated to be 47.7yrs which correlated well with the international data. Amongst the 50 cases, majority cases (92%) were diagnosed as infiltrating ductal carcinoma, no special type (IDC – NST). Besides IDC-NST, there were two cases (4%) of mucinous carcinoma and 1 case (2%) each of invasive lobular carcinoma and invasive papillary carcinoma in the present study group. The commoner location for the tumours was the upper outer quadrant (44%) of left breast (27/50 cases) as compared to the right breast (23/50 cases). As far as tumoral size is concerned, 72% of the tumours belonged to T2 (ranging from 2 cm to 5 cm), 16% T3 (more than 5cm and 10% were T1(less than 2 cm). Grade II tumours accounted for most (74%) of the cases in our study while 16% were grade I and 10% grade III tumors.

P53 positivity was seen in 40% of cases in our study while BRCA1 expression was observed in 48% of the total cases. The ER, PR and HER-2/neu positivity were 76%, 74% and 30% of the cases respectively.

A statistically significant association was found between P53 expression and histologic grade, ER/PR and Her-2/neu status (Table 1). No significant association was seen between tumour size and p53 expression. P53 positivity was found to be associated with higher histologic grades (p value =0.05), Her-2/neu expression (p value = 0.0005) and negative hormone receptors (p value = 0.0046), which are adverse prognostic factors for breast carcinoma. As for BRCA1, significant associations were obtained between BRCA1 expression and tumour size, histologic grade and hormone receptor status (Table 2). BRCA1 positivity was well associated with positive hormone receptors (p value = 0.03). The absence of BRCA1 staining was mostly seen in tumours of large sizes (p value = 0.01).and higher histologic grades (p value = 0.04). No significant association was seen between the expressions of BRCA1 and HER-2/neu.

Table 1: Association of age, tumor size & grading, ER/PR status and Her2Nu expression with P53 expression

	P53 + ve (n=20)	**P53 –ve (n=30)**	**Total (n=50)**	**p value**
Age				
≤ 50yrs	14 (70)	19 (63.33)	33 (66)	0.86
> 50yrs	06 (30)	11 (36.66)	17 (34)	
Tumor Size				
T1	2(10)	4(13.3)	6(12)	0.79
T2	14(70)	22(73.4)	36(72)	
T3	4(20)	4(13.3)	8(16)	
Grading				
Grade 1	01(5.0)	07(23.3)	8(16.0)	0.049
Grade 2	15(75.0)	22(73.4)	37(74.0)	
Grade 3	04(20.0)	01(03.3)	5(10.0)	
ER/PR status				
+VE / + VE	10(50.0)	24(80.0)	34(68.0)	0.004
+VE / - VE	1(5.0)	3(10.0)	04(8.0)	
-VE / + VE	1(5.0)	2(6.7)	03(6.0)	
-VE / -VE	8(40.0)	1(3.3)	09(18.0)	
Her2nu				
+ve	12(60.0)	03(10.0)	15(30.0)	0.0005
-ve	08(40.0)	27(90.0)	35(70.0)	

Table 2: Association of age, tumor size & grading, ER/PR status and Her2Neu expression with BRCA 1 expression

	BRCA1 + ve (n=24)	**BRCA1 –ve (n=26)**	**Total (n=50)**	**P value**
Age				
≤ 50yrs	16(66.7)	17(65.4)	33(66)	0.84
> 50yrs	8(33.3)	9 (34.6)	17(34)	
Tumor Size				
T1	3(12.5)	3(16.6)	6(12)	0.36
T2	19(79.2)	17(60.3)	36(72)	
T3	2(8.3)	6(23.1)	8(16)	
Grading				
Grade-1	7(29.1)	1(3.8)	8(16.0)	
Grade-2	17(70.9)	20(76.9)	37(74.0)	0.04
Grade-3	0(0.0)	5(19.3)	5(10.0)	
ER/PR status				
+VE / + VE	20 (83.2)	14(53.8)	34(68.0)	0.03
+VE / - VE	01(4.2)	03(11.5)	04(8.0)	
-VE / + VE	02(8.4)	01(3.8)	03(6.0)	
-VE / -VE	01(4.2)	08(30.9)	09(18.0)	
Her2nu				
+ve	08(33.3)	07(26.9)	15(30.0)	0.86
-ve	16(66.7)	19(73.1)	35(70.0)	

Discussion

P53 and BRCA1 are two well known tumour suppressor genes whose mutations have been related to breast cancer. Their protein products are involved in the regulation of vital steps of cell cycle and growth. So the assessment of these markers provides information about the tumour which paves the way for prognostication and management options.

Nearly one third of breast cancers have mutations of the tumor suppressor gene *p53*.[6] Within breast cancer cohorts, p53 mutation has been independently associated with decreased survival**.** Mutation in p53 results in loss of these usual wild-p53 tumor suppressor functions. Since mutations usually result in prolonged half-life and protein accumulation, immunohistochemical detection of p53 can be used as a surrogate for mutational analysis.[7]

Pietilainen et al also found the highest percentage of p53 positivity in the 50yr or younger age group.[8] The authors suggested that the unfavourable prognosis of young breast cancer patients may be related to abnormalities in p53. In our study also (Table 1), 70% of the cases showing p53 positivity belonged to the age group of less than 50yrs. As far as BRCA1 positivity is concerned (Table 2), 66.67% of positive cases were younger than 50 yrs.

The P53 expression in our study was found to have a statistically significant association (p value =0.05) with higher grades (Table 1). Plesan et al who reported 42% p53 positivity also found that most cases that had p53 expression (30/42 or 71.43%) were higher grade tumours.[9] Yamashita et al found 30% p53 positivity which also correlated with higher histologic grades (p value= 0.0001).[3] Angela et al reported that tumours with high histologic grade frequently (12/33) showed positivity for P53 immunoexpression with intense staining while tumours of low or moderate histologic grade revealed immunopositivity in 11/82 cases.[10] An association of p53 expression with higher grades was also concluded by Hurlimann et al.[11]

In our study (Table 1), p53 expression had a negative correlation with the expression of hormone receptors (p value = 0.0046). 8 out of total 9 tumours having both ER and PR negative status, had p53 positivity while only 10 out of 34 tumours having ER and PR positivity expressed p53. Similarly Angela et al found p53 reactivity in 13 out of 23 cases with ER and PR negativity and 10 out of 90 cases of ER and PR positivity.[10] Studies by Thor et al[12] and Ferrero et al[13] also found a statistically significant correlation of p53 expression with ER/PR negativity. Plesan et al concluded that the expression of P53 was present in over half (59.37%) of the cases that had both the receptors negative and in only 21.05% of the cases with ER+/PR+ phenotype.[9]

Thor et al found a significant correlation between the expressions of p53 and Her-2/neu[12] which was also seen in case of our study(p value = 0.0005) (Table 1). 60% (12 cases) of tumours expressing p53 were also positive for Her-2/neu. Plesan et al found p53 positivity frequently in Her-2 positive cases than in Her-2/neu negative cases (46.66% vs 41.17%.[9]).

BRCA gene mutations are associated with a 40–57% lifetime risk of female breast cancer and an 18–40% lifetime risk of ovarian cancer as per a recent meta analytical study.[14] The role of BRCA1 in sporadic cancers still remains unconfirmed. There are also conflicting reports about the pattern of expression of this protein which may result from differences in the specificity of antibodies used, processing techniques and immunostaining methods, and the presence of different isoforms of BRCA1.

In the present study, BRCA1 positivity was seen in 48% of the cases, similar to Yang et al who observed 40% immunohistochemical positivity for BRCA1.[15] While Burkadze et al found 64% positivity for BRCA1 in invasive breast cancers.[16] In a study by Rakha et al complete loss of nuclear expression was observed in 223 cases (15%) and cytoplasmic expression was found in 541 breast cancers (36.6%).[17]

We found (Table 2) that BRCA1 negativity correlated with higher histologic grades (p value = 0.04). All the tumours which were assigned grade III, showed negative BRCA1 immunohistochemistry. This observation is well supported by the study of Lee et al[18] and Rakha et al[17] who concluded that the loss of BRCA1 expression correlated well with higher grades.(p value = 0.025). Wilson et al also found lack of BRCA1 immunostaining in higher grade tumours.[19] Perez et al[20] found that most grade I tumours were BRCA1 positive, which is also seen in our study where 7 out of total 8 grade I tumours are BRCA1 positive. However Burkadze et al found a positive association between BRCA1 positivity and higher grades.[16] These contradictory findings could well be the result of variable antibody specificity or the complexity regarding the subcellular localisation of BRCA1 protein.

Absent or reduced nuclear BRCA1 expression is associated with negative estrogen receptor, progesterone receptor and androgen receptor expression according to Rakha et al. Yan et al found an association between lack of BRCA1 staining and negativity of hormone receptors (p value = 0.0196).[4] Burkadze et al[16] found a significant association between BRCA1 expression and ER/PR positivity which tallied with our study (Table 2). A statistically significant association (p value = 0.03) was obtained between the expressions of BRCA1 and the hormone receptors. 20 cases out of total 34 cases of ER/PR positivity were positive for BRCA1. Lee et al concluded that the loss of BRCA1 is frequent in ER negative tumours.[18]

Regarding the expressions of BRCA1 and Her-2/neu (Table 2), 66.67% of BRCA1 positive tumours showed no reactivity for Her-2/neu. However, no significant correlation (p value = 0.86) was observed between the two markers. Yan et al also saw no correlation (p value = 0.99) between BRCA1 and Her-2/neu reactivity.[4] Burkadze et al found a negative correlation between BRCA1 positivity and Her-2/neu expression.[16] According to Yoshikawa et al, carcinomas with reduced BRCA 1 protein expression had a tendency towards an overexpression of Her-2/neu protein (p value= 0.158).[21] Interestingly, Rakha et al concluded that cytoplasmic expression of BRCA1 was associated with development of recurrence and positive HER2 expression.[17]

Conclusion

Based on the results of this study and of multiple studies reported in literature, immunostaining of P53 can be used as a prognostic indicator in cases of invasive breast carcinoma. This information will not only help physicians in the short-term care of patients but the detection of P53 expression may be particularly promising in clinical trials of new molecular therapies directed at the P53 tumour suppressor gene. The immunohistochemical detection of BRCA1 may be used to generate a larger pathologic picture of breast cancers in conjunction with the traditional prognostic parameters. Further studies with larger sample size will help to establish conclusive opinions on the expression of this marker.

The newer markers along with the existing ones will help characterise each case of invasive breast cancer individually, in a better way and thus help in dealing with the heterogenous nature of these cancers.

Conflict of Interest: None.

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