

Immunohistochemical Characteristics of Breast Cancer Patients with the Comparative Study of BRCA1, ER, PR, BCL2, P53 and Ki-67 Immunohistochemical Markers: A Population Based Study

Manisha Sharma*, Menka Khanna, Mridu Manjari, Manas Madan, Taranveer Singh and Tania Garg

Dept of Pathology, SGRDIMSR Amritsar, India

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ABSTRACT

Background: To correlate clinicopathological and immunohistochemical profile of BRCA1 positive and non BRCA1 breast cancer patients with ER, PR, BCL2, P53 and Ki-67 to gain more insight into biological characteristics of breast cancer to emphasise the need of genetic testing for BRCA1 in blood relatives of patients with BRCA1 mutation.

Methods: The study was conducted in 70 randomly selected cases of breast carcinomas received in the Department of Pathology, SGRDIMSR, Amritsar. Clinical History was taken as per proforma and formalin fixed paraffin embedded tissue was studied for histopathological typing and grading after staining with haematoxylin-eosin. All cases were subjected to immunohistochemistry for BRCA1, ER, PR, P53, BCL2 and Ki-67 expression.

Results: Grade II Tumors constituted the maximum (67%). The most common age group was 41-60 years (62%). BRCA1 positivity was seen in 27/70 cases (38%). BRCA1 positive cases tend to present at higher stage than BRCA1 negative cases showing significantly greater tumor size ($p < 0.001$) and lymph node involvement ($p = 0.001$). Similarly BRCA1 positivity was associated with poor prognostic factors significantly as with high grade of tumor ($p = 0.015$), hormonal receptors negativity (81.5% vs 18.5%, $p < 0.001$) and high proliferative index (71% vs 29%, $p < 0.007$), BRCA1 related cases had significantly high P53 positivity (67% vs 33%, $p < 0.008$) and lower BCL2 expression (78% vs 2.2%, $p < 0.005$)

Conclusion: Our study proves that BRCA1 positive tumors have a higher grade and are associated with poor clinicopathological and immunohistochemical prognostic markers. Further studies are needed to justify more aggressive treatment in BRCA1 positive cases.

***Corresponding author:**

Dr Manisha Sharma, Associate Professor, Dept of Pathology, SGRDIMSR Amritsar, Punjab India

Phone: +91 9876842942

Email: manisha_salwan@yahoo.com



Introduction

Breast Cancer is the leading cause of death among women suffering from cancer. In the year 2000, there were about 796,000 new breast cancers diagnosed and about 314,000 deaths due to breast cancer around the world.^[1,2] Earlier BRCA1 and BRCA2 were estimated to be responsible for 75% of familial breast cancers. However, recent data shows this percentage to be much less and depends upon the population studied.^[2] It has been analysed that the cancer arising in carriers of mutation in BRCA1 differs from non BRCA1 mutation carriers. BRCA1 mutation positive carriers have poorly differentiated morphology with higher mitotic count and pleomorphism.^[3] Immunophenotypically BRCA1 tumours are more frequently ER, PR negative, BCL2 negative, P53 positive and have high proliferation index (Ki-67 positivity).^[4]

Most familial breast cancers are not associated with BRCA1 mutation, so in an attempt to better define the clinical features and outcome of such patients it is important to define the immunohistochemical features and their relation to BRCA1 positivity to carry out genetic testing more effectively and to know the biological character of such tumours better.

This study in 70 breast cancer patients has been done to attempt to gain insight better relationships of BRCA1 with other immunohistochemical markers like ER, PR, BCL2, P53 and Ki-67.

Material and Methods

Haematoxylin–Eosin (H&E) stained sections from randomly selected 70 formalin fixed breast cancer specimens received as mastectomy and lumpectomy specimens were taken and studied among all the breast cancer cases received over the period of one year (Nov 2013 –Oct 2014) in the Department of Pathology, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar. The tumours were graded from grade I to grade III according to Nottingham Modification of Bloom-Richardson method taking into account the parameters – tubule formation, nuclear grading and number of mitosis/HPF. The tumours were evaluated for the histological types, lymphocytic stromal response, nuclear chromatin pattern and nucleoli. Lymph nodes recovered were evaluated for the presence of metastatic deposits.

IHC was performed by using antibodies against the estrogen receptors (ER), the progesterone receptors (PR) (Diagnostic Biosystem) and BRCA1 (Biocare Medical), P53, BCL2, Ki-67 (Diagnostic Biosystem). The antigen retrieval was done by using pressure cooker method with 10mmol citrate buffer at pH 6. Tris buffer was used as

the wash buffer and Diaminobenzene tetrahydrochloride (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, BRCA1, BCL2, P53 and Ki-67 primary antibodies and were conjugated with streptavidin Horse Radish Peroxidase (HRP). The slides were counterstained with hematoxylin and were examined by light microscopy. $\geq 10\%$ nuclei stained brown were taken positive for ER and even 1% stained were taken positive for PR as taken by other researchers in their studies.^[5,6] For BRCA1 this value for positive stained nuclei was $\geq 30\%$ as per other studies.^[7,8] For BCL2 cytoplasmic staining in $>1\%$ cells was taken as positive. P53 immunostaining was characterized by the percentage of immunostained nuclei. $>5\%$ of nuclei stained were taken positive. Ki-67 was evaluated by the percentage of immunostained nuclei, $<10\%$ staining was scored low, 10-20% intermediate and $>20\%$ as high as done in the study by Jaramillo et al.^[9]

Results

The patients age varied from 28-70 years with the maximum number of cases belonging to group 41-60 years (62% of patients). The size varied from 1-5 cms with maximum cases with the size >2 cms (68%). All the tumours diagnosed were infiltrating ductal carcinoma NOS (not otherwise specified). Grade II cases were maximum consisting of 67% of cases followed by Grade III (28.5%) and then Grade I (4.5%). Lymph node involvement was seen in 39 cases out of 60 cases in which lymph nodes were recovered. BRCA1 positivity was significantly higher in grade II and grade III tumour ($p=0.013$). Similarly BRCA1 positive cases showed significant lymph node involvement as compared to BRCA1 negative cases ($p=0.001$). BRCA1 positivity was higher in cases where tumour size was >2 cms ($p<0.001$) (Table 1). 22 cases were ER+ and PR+ and 3 cases were ER+ and PR-. They were taken together as positive for ER PR (25 cases-36%). BRCA1 positivity was seen in 27/70 cases (38%). Out of 27 BRCA1 positive only 5 cases showed positivity for ER PR. Rest 20 positive ER PR cases were BRCA1 negative. Correlating ER PR expression with BRCA1 it was concluded that BRCA1 expression is significantly correlated with lower ER PR expression ($p<0.001$).

BCL2 positivity was observed in 37% (26/70) of the cases with percentage of cell positivity varying from 26-71%. While correlating BCL2 expression with BRCA1 expression it was found that out of 27 BRCA1 positive only 6 cases were immunopositive for BCL2, therefore BRCA1 expression is associated with low BCL2 expression significantly ($p<0.005$).

P53 positivity was observed in 40% cases (28/70). P53 expression was found to be directly correlated to BRCA1 expression. Out of 27 positive BRCA1 cases 18 were also positive for P53. BRCA1 expression was associated with increased P53 expression significantly ($p < 0.008$).

Ki-67 positivity was seen in 28 cases (40%). Out of 28 positive cases 19 cases of high and intermediate

proliferation rate were BRCA1 positive. So BRCA1 positivity was associated with increased proliferation index significantly. ($p < 0.007$) (Table 2)

Discussion

Breast cancer is becoming number one cancer in Indian population like western world thus making cervical cancer as second common.^[10] Breast cancer diagnosis,

Table 1: Pathological characteristics of the breast cancer patients tested for BRCA1 mutation analysis.

Parameter		No mutation	Mutation
Histological Grade	Grade I	03	00
	Grade II	36	11
	Grade III	04	16
Lymph Node Status	N0	20	01
	N1(1-3)	03	07
	N2(4-9)	07	14
	N3(>10)	03	05
Size	<2cm	17	05
	>2cm	26	22

Table 2: Immunohistochemical characteristics of the breast cancer patients tested for BRCA1 mutation analysis.

Parameter		No mutation	Mutation	p-value
Estrogen And Progesterone Receptor Status	Positive	20(46.5%)	05(18.5%)	<0.001
	Negative	23(53.5%)	22(81.5%)	
BCL2 receptor status	Positive	20(46.5%)	06(22%)	<0.005
	Negative	23(53.5%)	21(78%)	
P53 receptor status	Positive	10(23%)	18(67%)	<0.008
	Negative	33(77%)	9(33%)	
Ki-67 receptor status	Intermediate/ High	9(21%)	19(71%)	<0.007
	Low proliferation/Negative	34(79%)	08(29%)	

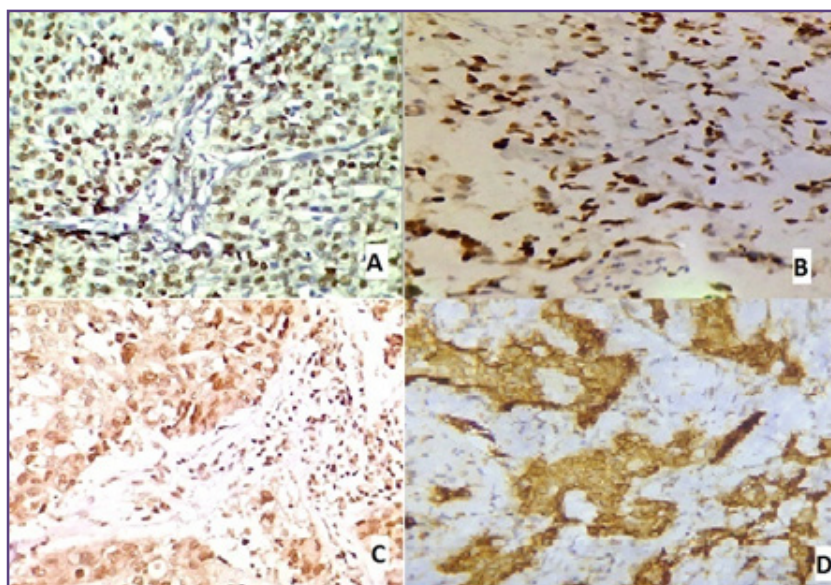


Fig. 1: Immunohistochemistry showing IHC (400X) (A) ER positivity (Nuclear) (B) : P53 positivity (Nuclear) (C): BRCA1 positivity (Nuclear), (D): BCL2 positivity (Cytoplasmic).

treatment and prognosis have been found to be associated with various morphological immunohistochemical and genetic variables.

The morphological findings in BRCA1 positive cases were correlated to various studies conducted in past. 16/20 (80%) of grade III cases were BRCA1 positive as compared to 4/20 (20%) of grade III cases which showed BRCA1 negativity. This result is similar to previous studies which have shown that BRCA1 related cancers were of higher histological grade.^[3,11] In a study conducted in Jewish women, 76.5% of BRCA1 related tumours had a higher nuclear grade as compared to only 23.5% of BRCA1 negative tumours.^[11] The individuals with BRCA1 mutation were significantly less likely to present with stage I disease. BRCA1 positive cases had higher number of lymph nodes involvement ($p=0.001$) and tumour size >2 cms at the time of presentation ($p<0.001$). These results are in concordance with the results reported previously in the literature.^[11,12]

As in this study, morphological differences were significant among BRCA1 positive and BRCA1 negative cases, this study was an appropriate sample to compare various immunohistochemical variables as well.

In our study BRCA1 positive cancers were less frequently ER PR positive as (22/27-81.5%) cases were ER PR negative. Thus BRCA1 expression was associated with lower ER PR expression significantly ($p<0.001$) correlating with various previous studies conducted where 60-85% of BRCA1 mutations were diagnosed ER PR negative as compared to 20-40% of BRCA1 non mutations.^[12,13,14]

BRCA1 positivity was associated with lower BCL2 expression as out of 27 BRCA1 positive cases only 6 had BCL2 expression as well ($p<0.005$). This finding is in accordance with the results of other studies conducted where BRCA1 was found to be associated with decreased expression of BCL2.^[11,15,16]

Intermediate and higher proliferation rate (Ki-67 score) was observed in 19/27 (71%) cases of BRCA1 related tumours ($p<0.007$). Similar results were observed by others researchers in their study where this percentage varied from 60-78%.^[16,17]

Our study revealed a higher positivity for P53 immunostaining in BRCA1 positive cases. Out of 27 positive cases 18 cases had positivity for P53, so associated with P53 expression significantly ($p<0.008$). Most previous studies have demonstrated a higher positivity of P53 in tumours in BRCA1 mutation carriers with significant correlation between two varying from 0.005 to 0.001.^[11,15,16,17]

It was proposed that there is some correlation between BRCA1 and other markers at molecular level. BRCA1 mutation is followed by P53 dysfunction and cancer cells to be ER PR negative, therefore favoring some mechanism of interaction among BRCA1 and other molecular markers.^[17,18,19,20]

Several studies have proved the BRCA1 positivity relation with poor prognostic markers but have not demonstrated worse clinical outcome in the terms of five year relapse free survival, five year event free survival and five year overall survival. But the association of BRCA1 mutation with younger age of presentation and increased chances of development of contralateral breast cancer and ovarian cancer was proved.^[21]

In the above study, BRCA1 positivity showed a statistically significant association with poor prognostic and clinical variables such as high histological grade, higher stage at presentation, P53 positivity, Ki-67 proliferation index and ER PR and BCL2 negativity. Thus, proving that although BRCA1 positive tumours are heterogeneous from a genetic point of view, but they share common characteristics. Blood relatives of these patients should be screened for BRCA1 gene mutation as they may show 50% increased chances of its expression and 87% of life time risk for developing breast cancer.^[22]

Conclusion

Our study proves that BRCA1 positive tumors have a higher grade and are associated with poor clinicopathological and immunohistochemical prognostic markers showing ER, PR and BCL2 negativity with high proliferation index (Ki-67 expression) and increased P53 expression. Whether the selection and delineation based upon morphological, immunohistochemical and molecular features not only justifies the aggressive treatment approach but also selection of candidate patient and blood relatives for BRCA gene studies is still a matter of debate. Further studies should address the treatment outcome difference in familial breast cancer cases (BRCA1 carriers than non BRCA1 carriers).

Abbreviations

ER - Estrogen Receptor
 HPF - High Power Field
 HRP - Horse Radish Peroxidase
 PR - Progesterone Receptor

Funding

None

Competing Interests

None Declared

Reference

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