Original Article

Breast cancer susceptibility gene 1 expression in breast cancer patients and its correlation with histopathological features using the immunohistochemical method in the Indian population

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Abstract

Background: Pathogenic mutations in breast cancer susceptibility gene 1 (BRCA1) are the common causes for germline as well as sporadic aggressive subtypes of breast cancer. This study was conducted to know BRCA1 expression in breast cancer by immunohistochemistry (IHC) testing.

Methods: This was a cross-sectional study conducted in the Department of Medical Oncology, Vardhman Mahavir Medical College and Safdarjung hospital, New Delhi, from July 2016 to April 2018. The patients with confirmed diagnosis if breast cancer and aged >18 years were included in the study. BRCA1 expression, estrogen/progesterone receptor (ER/PR) status, and Human epidermal growth factor receptor 2 (Her 2-neu) were evaluated by IHC in all patients.

Results: Of the 50 patients enrolled, the mean (± standard deviation) age was 53 (±11.76) years; 17 (34%) were in the range of 41–50 years, while 13 (26%) were in the range of 51–60 years. The distribution of patients with breast cancer according to risk factors showed that family history of cancer was present in 8 (16%), prebenign breast disease in 5 (10%), use of hormone replacement therapy/oral contraceptives pills in 31 (62%), exposure to radiation in 2 (4%) and history of smoking in 15 (30%) patients. Metastasis was seen in 43 (86%) patients and the prevalence of BRCA1 was 8% (n = 4); three patients in the age group of 41–50 years and one patient in the age group of 31–40 years. A negative BRCA1 expression was observed in 46 (92%) patients; 40/46 patients had >40 years and 39/46 patients had no family history of cancer. Similarly, of the 17 and 33 pre- and post-menopausal patients, 16 and 30 patients had negative BRCA1 expression, respectively. BRCA1 expression was negative in all the ER/PR receptor and Her-2-neu-positive patients. Histological Grade II tumors were observed in 26 (52%) patients, of whom 22 patients showed negative BRCA1 expression.

Conclusion: The prevalence of BRCA1 among breast cancer patients was 8%. Studies with larger sample size are needed to further assess BRCA1 gene mutations and determine clinical usefulness as a potential biomarker by IHC.

Keywords: Breast cancer susceptibility gene 1, breast cancer, histopathology, immunohistochemistry, Indian population

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INTRODUCTION

Breast cancer is the second-most common cancer overall, with 2.0 million diagnosed cases worldwide according to the recent data from GLOBOCAN. Furthermore, of all the cancer deaths worldwide, 6.6% are due to breast cancer. New cases and deaths due to breast cancer are more common in less developed countries than in more developed countries.[1,2] In India, breast cancer ranks first with 162,468 new cases (14%) and 87,090 (11.1%) related deaths in both male and female population according to GLOBOCAN 2018 statistics.[3]

Pathogenic mutations in the tumor suppressor gene or breast cancer susceptibility gene 1 (BRCA1) protein are responsible for the increased risk of breast cancer. Furthermore, BRCA1 mutations are the most common cause for germline as well as sporadic aggressive breast cancer. Carriers of BRCA1 have nearly 80% lifetime risk of developing breast cancer.[4] Tumors of BRCA1 mutation carriers are more likely to be high-grade with greater metastatic potential.[5] Germline mutations account for 5%–10% of all breast cancers, while the majority occur sporadically and are attributed to somatic genetic alterations.[6] A number of immunohistochemical (IHC) markers have been shown to be of value in assessing BRCA1 tumor phenotype in female patients, including estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (Her-2), p53 cytokeratin 5/6 (CK5/6), CK14, CK17 and epidermal growth factor receptor.[7-10] Over-expression of BRCA1 is associated with aggressive disease and poor response to chemotherapy and poor prognosis.[11] The molecular method of BRCA1 testing by gene sequencing is the gold standard but is very expensive and not available to most of the patients. In contrast, BRCA1 testing by IHC is a highly reproducible and accurate way of detecting germline, somatic, or epigenetic mechanisms of BRCA1 expression and is also relatively simple, cheap, and available.[12-14] The present study was conducted to know BRCA1 expression in breast cancer patients by IHC testing.

METHODS

This was a cross-sectional study conducted in the Department of Medical Oncology, Vardhman Mahavir Medical College and Safdarjung hospital, New Delhi, from July 2016 to April 2018. Ethical clearance from the ethical committee before conducting the study, written and informed consent from all the subjects participating in the study was taken as per standard protocol. This study included patients with confirmed diagnosis of breast cancer from the outpatients and indoor units of the Department of Medical Oncology. All patients attending the hospital during the study period with diagnosed cases of breast cancer and >18 years of age, irrespective of tumor stage/pathological characteristics, were included in the study. Various risk factors were noted from the patients' history that included age at menarche, parity, late age at first birth, age at onset menopause, family history, previous benign breast disease, use of hormone replacement therapy (HRT)/oral contraceptives (OC), body weight/obesity/body mass index, alcohol consumption, smoking, diet and exposure to radiation. Routine investigation of blood samples and urine samples was conducted for complete blood count, urine R/E, RBS/F/PP, kidney function test, liver function test, serum electrolytes, serum lactate dehydrogenase, serum uric acid, serum calcium/phosphate, total serum proteins/albumin/globulin, viral markers (Hbs Ag/Anti-hepatitis C virus/human immunodeficiency virus I and II), tumor marker (CA 15.3). Electrocardiogram, chest X-ray, 2D–Echo, contrast-enhanced computed tomography of brain/neck/chest/abdomen/pelvis, mammogram (bilateral breast), bone scan (if required) were also performed. BRCA1 expression and ER/PR status, and human epidermal growth factor (Her 2-neu) were evaluated by IHC in all patients. Biopsy of tumor tissue was obtained by core needle biopsy or surgical biopsy for routine histopathological examination. The specimens were processed and fixed in 10% formalin and were examined grossly. Paraffin-embedded sections were stained with the hematoxylin and eosin stain. The tumors were classified and graded according to the World Health Organization and Nottingham modification of the Scarff-Bloom–Richardson system, respectively.

Immunohistochemistry analysis was performed on 3-μm thick paraffin sections in the moist and humid container. The section slide was marked using a diamond pencil. The 3 μm section was deparaffinized by putting them on a hot plate and by dipping them in xylene. They were then hydrated with graded ethanol and brought to water. The section was then placed in 3% hydrogen peroxide in methanol (hydrogen peroxide block) for 30 min. To unmask the antibody binding epitopes (masked during formalin fixation), the slides were then put in a Coplin jar filled with 10 mM citrate buffer (pH 6.0) covered with a lid, placed in a pressure cooker till one whistle or 9–10 min. The slides (along with buffer) were cooled and washed thrice with Tris buffer. Nonspecific proteins were blocked with 5% milk block followed by three washes with Tris buffer. The tissue was then incubated with primary antibody overnight at 4°C followed by again Tris buffer washing thrice. The slides were washed with biotinylated goat anti-polyvalent antibody for 20 min and with Tris

buffer thrice each. Tertiary antibody (peroxidase-labeled Streptavidin peroxidase complex) was added for another 20 min. Di-amino-benzidine saline was applied on the slides and the reaction was monitored under microscope. The slides were immersed in water as soon as crisp golden brown nuclear membranous staining is seen and then were counterstained with Hematoxylin. They were then dehydrated in graded alcohol solutions. After cleaning in xylene, mounting was done in dibutyl phthalate in xylene.

Categorical variables were presented in number and percentage (%), and continuous variables were presented as mean ± standard deviation (SD) and median. Qualitative variables were correlated using the Chi-square test/ Fisher's exact test. A value of $P < 0.05$ was considered statistically significant. The data were entered in the MS Excel spreadsheet, and analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 21.0.

RESULTS

A total of 50 breast cancer patients were enrolled for this cross-sectional study. The mean (±SD) age of the patients was 53 (±11.76) years with more number of patients (n = 17) in the range of 41–50 years of age [Table 1].

Table 1: Distribution of patients with breast cancer according to various clinicopathological factors

Variables	*n* (%) of patients (*n*=50)
Age group (years)	
18-30	2 (4)
31-40	5 (10)
41-50	17 (34)
51-60	13 (26)
61-70	10 (20)
>70	3 (6)
Family history of cancer	8 (16)
Prebenign breast disease	5 (10)
Use of HRT/OC pills	31 (62)
Exposure to radiation	2 (4)
History of smoking	15 (30)
Pre-menopausal	17 (34)
Postmenopausal	33 (66)
Histopathology type	
Infiltrating ductal carcinoma	45 (90)
Lobular carcinoma	4 (8)
Ductal carcinoma *in situ*	1 (2)
Histological Grade I	6 (12)
Histological Grade II	26 (52)
Histological Grade III	18 (36)
Estrogen receptor positive	27 (54)
Estrogen receptor negative	23 (46)
Progesterone receptor positive	11 (22)
Progesterone receptor negative	39 (78)
Her-2-Neu positive	10 (20)
Her-2-Neu negative	40 (80)

Her-2-Neu: Human epidermal growth factor, HRT/OC: Hormone replacement therapy/oral contraceptives

Table 1 represents the distribution of breast cancer patients according to risk factors and clinicopathological factors. This distribution showed that 62% were using HRT/OC pills while 30% had a history of smoking. Metastasis (advanced stage) was seen in 43 (86%) patients. Furthermore, it was observed that the prevalence of BRCA1 was 8% (n = 4) among breast cancer patients; three patients were in the age group of 41–50 years and one patient in the group of 31–40 years of age. All the patients showing BRCA1-positive expression had +1 expression.

The correlation of clinicopathologic and immunohistochemical profile of BRCA1 expression is represented in Table 2. A larger number of patients showed a negative BRCA1 expression (n = 46 [92%]) in the patients with breast cancer; 40/46 patients aged >40 years, and this correlation between age and BRCA1 expression was statistically nonsignificant. Similarly, of the 17 and 33 pre -and post-menopausal patients, 16 and 30 patients had statistically nonsignificant negative BRCA1 expression, respectively. BRCA1 expression was negative in 22 histological Grade II tumors. BRCA1 expression was negative in all the ER/PR receptor and Her 2-neu positive patients. The correlation of BRCA1 expression and patients' age, family history of cancer, menopausal status, histopathology type, histological grade, PR and Her-2-Neu were all statistically nonsignificant ($P > 0.05$; [Table 2]), whereas a was statistically significant ($P = 0.02$) correlation was observed between BRCA1 expression and ER.

DISCUSSION

According to the National Cancer Registry Project, the average age of onset of breast cancer in Indian women is earlier compared to Western populations. The average age of patients was found to be from 44.2 to 49.6 years, while it is 61.0 years among Americans, which is a decade earlier than the Western population.[15] In line with these results, our study also demonstrated that 34% of patients with breast cancer were in the range of 41–50 years of age. The present study demonstrated from the distribution of patients according to risk factors that 62% of patients who had breast cancer were using HRT/OC pills. In previous studies by Mørch *et al*. and Rosenberg it was reported that the use of hormonal contraception increased the risk of breast cancer by 20% and this risk increases with the duration of use.[16,17] Our study observed that 66% of patients were postmenopausal. Although this alone cannot infer as an independent risk factor, breast cancer risk increases in patients who had early onset of menarche and late menopause irrespective of their endocrine profile.[18] According to literature, the most

Table 2: Correlation of clinicopathological factors and immunohistochemical profile of BRCA1 in breast cancer patients

Variables	BRCA1 expression		*P*
	Positive (*n*=4)	Negative (*n*=46)	
Age group (years)			
≤40	1	6	0.51†
>40	3	40	
Family history of cancer			
Yes	1	7	0.61†
No	3	39	
Menopausal status			0.29†
Premenopausal	1	16	
Postmenopausal	3	30	
Histopathology type			
Infiltrating ductal carcinoma	4	41	0.49†
Lobular carcinoma	0	4	
Ductal carcinoma *in situ*	0	1	
Histological grade			
Grade I	0	6	0.13†
Grade II	4	22	
Grade III	0	18	
Estrogen receptor sensitivity			
Positive	0	27	0.02*
Negative	4	19	
Progesterone receptor sensitivity			
Positive	0	11	0.27†
Negative	4	35	
Her-2-Neu			
Positive	0	10	0.30†
Negative	4	36	

*Statistically significant ($P<0.05$), †Statistically nonsignificant ($P>0.05$). Her-2-Neu: Human epidermal growth factor

common histological type of breast cancer is infiltrating ductal carcinoma with an incidence of 55% by Makki and 70%–80% by Malhotra *et al.*[19,20] The study also reveals that 90% of patients were of infiltrating ductal carcinoma type on histopathological examination. Of the 50 patients from our study, 52% were classified as Grade II tumors. A similar pattern of 71% of patients with Grade II tumors was reported in a 10-year retrospective study in the African population.[21] This study also reported that nearly 90% incidence of infiltrating ductal carcinoma standing in line with our study observations. Here, we observed 86% of patients had advanced-stage breast cancer. Lymph node metastasis depends on several factors like the type of breast cancer (hormone-receptor and/or HER2-positive or triple-negative breast cancer), the stage of cancer and tumor growth rate at the time of diagnosis.

BRCA1 positivity was seen in 8% of patients in our study. The correlation between BRCA1 expression and various clinic-pathological factors have been studied using the IHC method of analysis in the present study. The correlation between family history of breast cancer and BRCA1 expression, although nonsignificant, revealed that most of the patients had no family history and showed negative BRCA1 expression. Furthermore, there were more patients in infiltrating ductal carcinoma with BRCA1 negativity, and all Grade III tumors were BRCA1 negative. With the similar findings as from our study, Wilson *et al.* reported that BRCA1 mutations are rare in sporadic cancers, and its expression was reduced or undetected in the majority of high grade, ductal carcinomas. This suggests that BRCA1 absence may contribute to the pathogenesis of sporadic cancers.[22] Hedau *et al.* also reported a decline in BRCA1 expression prominently in Grade III disease.[23] From the findings of our study, all the ER-positive patients were negative for BRCA1. On the other side, of all the BRCA1 negative patients, 50% were ER negative. This correlation between BRCA1 expression and ER was statistically significant. These results are in track with those of Amirrad *et al.* and Niwa *et al.*, where a decreased expression of BRCA1 in breast cancer is associated with a negative ER status.[24,25] Burkadze *et al.* stated that BRCA1 expression was positively associated with PR positivity and negatively associated with HER-2-neu expression.[26] The study also reported that most of the patients were PR negative and BRCA1 negative, and all BRCA1 positive tumors were Her-2-Neu negative.

BRCA1 positive tumors 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 there is a 50% risk of inheritance of breast cancer and 72% lifetime risk of developing breast cancer.[27,28]

Limitations of the present study were nonsignificant findings of the correlation between BRCA1 expression and various risk factors, clinicopathological factors, and hormone receptor sensitivity, which may be attributed due to a small number of population.

CONCLUSION

The prevalence of BRCA1 among breast cancer patients was 8%. Breast cancer patients with high-risk factors, including a family history of breast or first-degree ovarian cancer, bilateral cancer, male breast cancer, multiple organ cancer, and earlier age at onset, who have a higher prevalence of BRCA mutations, should be tested for BRCA mutations.

The clinical benefits of establishing BRCA1 expression status are helpful for breast cancer treatment, prophylaxis, and prognosis. Thus, IHC can be a valuable preliminary test for detecting the reduction in BRCA1 protein expression and much cheaper than reverse transcription-polymerase

chain reaction. The study with a larger number sample size may establish BRCA1 to be used as a potential marker in population-based screening for risk assessment.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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