

## BRCA1 expression and its association with histological typing, grade, ER, PR, HER2 in carcinoma of breast

Kush Juneja<sup>1</sup>, Anupam Varshney<sup>2\*</sup>, Rajnish Kumar<sup>3</sup>, Mamta Singla<sup>4</sup>, Purva Sharma<sup>5</sup>, Veena K. Sharma<sup>6</sup>

<sup>1</sup>Resident, <sup>2</sup>Professor, <sup>3</sup>Associate Professor, <sup>4,6</sup>Professor and Head, <sup>5</sup>Assistant Professor, <sup>1-3,5,6</sup>Dept. of Pathology, <sup>4</sup>Dept. of Surgery, <sup>1-4,6</sup>Muzaffarnagar Medical College, Muzaffarnagar, Uttar Pradesh, <sup>5</sup>Mulayam Singh Medical College, Meerut, Uttar Pradesh, India

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### Abstract

Breast cancer is the most commonly diagnosed cancer in female worldwide. BRCA 1 is located predominantly in nucleus. BRCA 1 is associated with breast cancer.

**Materials and Methods:** This was retrospective study, 50 known cases of breast carcinoma were analysed in Muzaffarnagar Medical College, Muzaffarnagar.

**Observation and Results:** The age range of patients was from 28-70 years and mean age of patients was 50.24 years. Majority of breast carcinoma were above 45 years of age (64%) and of upper outer quadrant (52%) and of grade II (48%). Invasive carcinoma of no special type (NST) formed the majority of cases (82%) followed by Invasive lobular carcinoma (6%). Altered BRCA 1 expression was seen in ER, PR and HER 2 negative cases.

**Conclusions:** Invasive Carcinoma of no special type was the commonest morphological type. Majority of cases of breast carcinoma were of grade II.

### Introduction

Breast Cancer is the leading cause of death in women. It is most commonly diagnosed cancer nowadays, showing a rapid rise especially in urban areas of India. Among all other cancers, breast and uterine cervix cancer together account for about 50% cases.<sup>1</sup>

According to the recent data of nation, 80,000 new cases of breast cancer were reported and approximately 35,000 deaths due to breast cancer.<sup>2</sup>

Breast cancer development is also associated with several genetic and non-genetic factors. Non-genetic factors include lifestyle modifications and reproductive risk factors. Prolonged exposure to estrogen, late menopause, early age of menarche, use of oral contraceptives, sedentary lifestyle and diet rich in fats play an important role in breast cancer development.<sup>3</sup>

BRCA 1 protein expression was seen exclusively in nuclei of normal epithelial and myoepithelial cells of ductal and lobular region.<sup>1</sup>

BRCA1 are tumor suppressor genes located on chromosomes 17q21.<sup>1</sup>

Estrogen has an effect on target tissues by binding to the fraction of cells called estrogen receptors. Estrogen receptor play an important role in growth and development.<sup>4</sup>

Estrogen and progesterone receptors are codependent variables, progesterone receptor (PR) being a weaker predictor of response to endocrine therapy than estrogen receptor (ER).<sup>5</sup>

HER2 is an oncogene that encodes a transmembrane glycoprotein tyrosine kinase activity known as p185, which

belongs to the family of epidermal growth factor receptors.<sup>5</sup>

Non-BRCA 1 was characterized by lower grade, more tubule formation, less nuclear pleomorphism and fewer mitosis than combined group of BRCA 1 or BRCA 2 associated breast carcinomas. Non-BRCA 1 mutation expression had significantly more frequent expression of ER, PR and BCL 2 and less frequent expression of p53 and lower proliferation index of Ki 67.<sup>6</sup>

### Aims and Objectives

#### Aim

BRCA1 protein expression and its association with ER, PR, HER2 status in carcinoma breast in a tertiary care hospital.

#### Objective

1. Expression of BRCA 1 protein in breast cancer patients was observed by immunohistochemistry.
2. Association of BRCA 1 protein with clinicopathological features such as age, menopausal status, side, grade and histological typing.

### Materials and Methods

Haematoxylin-Eosin (H&E) stained sections from randomly selected 50 breast cancer specimens (Mastectomy and lumpectomy) which was formalin-fixed received in Department of Pathology, Muzaffarnagar Medical College and Hospital, Muzaffarnagar. Tumors were graded from grade I to grade III with method of Nottingham modification of Bloom Richardson method. Histological typing of tumors were done.

IHC was performed by using different antibodies of

\*Corresponding Author: Anupam Varshney, Professor, Dept. of Pathology, Muzaffarnagar Medical College, Muzaffarnagar, Uttar Pradesh, India

Email: [anupam\\_path@yahoo.co.in](mailto:anupam_path@yahoo.co.in)

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estrogen receptor(ER), progesterone receptor (PR), HER 2(Bio SB) and BRCA 1 (Biocare Medical). Antigen retrieval was done with the help of pressure cooker method by 10 mmol citrate buffer at PH 6. Diaminobenzene tetrahydrochloride (DAB) was used as a chromogen and tris buffer was used as wash buffer. Hydrogen peroxide blocks the endogenous activity. After protein blocking, incubation of slides were done overnight with available ER, PR, HER 2 and BRCA 1 primary antibodies and conjugation was done with the help of streptavidin Horse Radish peroxidase. Slides counterstaining was done with haematoxylin and was examined by light microscopy.<sup>7</sup>

Allred scoring system is used for ER, PR.<sup>25</sup>

For HER 2 neu ASCO/CAP guidelines 2013 was followed.<sup>8</sup>

Scoring for breast cancer type 1 (BRCA1).<sup>9</sup>

BRCA 1 staining is observed as brown nuclear staining in parenchymal cells with no cytoplasmic or membranous staining.

Percentage of Nuclear Staining	Intensity Score (Nuclear)
Score 0: 0% nuclear staining	0: no nuclear staining
Score + 1: ≤10% nuclear staining	1: weak nuclear staining
Score +2: 11-50% nuclear staining	2: moderate nuclear staining
Score+3:51-80% nuclear staining	3: strong nuclear staining
Score +4:>80% nuclear staining	

**Final score= Stained cells X Intensity:**

Negative 0

Weakly positive 1

Moderately positive 2

Strong positive 3

Score 0 and 1- Altered

Score 2 and 3 – Positive

**Table 1**

Variables	Total	Altered	Positive
<b>1. Age Range (years)</b>			
20-29	2(4%)	2(100%)	0
30-39	7(14%)	5(71%)	2(29%)
40-49	16(32%)	11(69%)	5(31%)
50-59	10(20%)	6(60%)	4(40%)
60-69	15(30%)	13(87%)	2(13%)
<b>2.Menopausal status</b>			
≤45 years	18(36%)	16(89%)	2(11%)
>45 years	32(64%)	21(66%)	11(34%)
<b>3. Side</b>			
Left	28(56%)	20(71%)	8(29%)
Right	22(44%)	17(77%)	5(23%)
<b>4. Histological type</b>	50(100%)	37(74%)	13(26%)
IC-NST	41(82%)	30(73%)	11(27%)
Infiltrating Lobular carcinoma	3(6%)	2(67%)	1(33%)
IC with neuroendocrine differentiation	2(4%)	1(50%)	1(50%)
Metaplastic carcinoma	2(4%)	2(100%)	0
Mucinous carcinoma	1(2%)	1(100%)	0
Medullary carcinoma	1(2%)	1(100%)	0
<b>5.Grade(Score)</b>			
I(3-5)	15(30%)	11(73%)	4(27%)
II(6-7)	24(48%)	18(75%)	6(25%)
III(8-9)	11(22%)	8(73%)	3(27%)
<b>6.Tumor size</b>			
≤2cm	6(12%)	4(67%)	2(33%)
2-5 cm	31(62%)	26(84%)	5(16%)
>5cm	13(26%)	7(54%)	6(46%)

Table 2

BRCA1 Expression	ER +	ER-	PR+	PR-	HER 2 Positive	HER2 EQUIVO-CAL	HER 2 Negative
No. of cases (Percentage)	20 (40%)	30 (60%)	12 (24%)	38 (76%)	12 (24%)	3 (6%)	35 (70%)
Altered (37)	13 (35%)	24 (65%)	9 (24%)	28 (76%)	10 (27%)	3 (8%)	24 (65%)
Positive (13)	7 (54%)	6 (46%)	3 (23%)	10 (77%)	2 (15%)	0	11 (85%)

## Results

### Age Range

The age of the patients range from 20-70 years. Majority of cases were seen in age group of 40-49 years. (32%) with mean age of 50.24 years.

Maximum number of cases with altered BRCA1 were seen in seventh decade 13 cases followed by 11 cases in fifth decade. BRCA 1 positive expression was seen in sixth decade (40%) followed by fifth decade (31%). On statistically analysis, a significant association of altered BRCA 1 expression was seen with decade ( $p < 0.05$ ).

### Menopausal Status

Majority of breast carcinoma cases were above 45 years (64%) in the present study.

Altered BRCA 1 was more in number of patients with  $\leq 45$  years of age (89%) while BRCA 1 positive expression was seen more in  $>45$  years of age (34%). On statistically analysis, insignificant association of altered BRCA1 expression was seen with menopausal status ( $p > 0.05$ ).

### Side

Cases of breast carcinoma were more on left side (56%).

Altered BRCA-1 score were more in right side (77%). BRCA-1 positive expression (score 2 & 3) was more common in left side (29%). On statistical analysis no significant correlation of altered BRCA 1 expression was seen with side ( $p > 0.05$ ).

### Histological Type

Invasive Carcinoma –NST formed the bulk of the cases (82%) which was followed by invasive lobular carcinoma (06%), carcinoma with neuroendocrine differentiation and metaplastic carcinoma(04%), medullary and mucinous carcinoma (02%).

Altered BRCA1 expression were mostly in metaplastic carcinoma, mucinous carcinoma and medullary carcinoma of breast each (100%) followed by Invasive Carcinoma NST (73%). IC with neuroendocrine differentiation showed altered BRCA1 expression in 50% of cases. On statistically analysis, a significant association of histological typing was seen with altered BRCA 1 expression ( $p < 0.05$ ).

### Grade

Majority of breast carcinoma cases were of grade II (48%) which was followed by grade I (30%) and grade III (22%). Altered BRCA 1 expression was seen in grade II (75%) which was followed by grade I and grade III each (73%). On Statistical analysis no significant correlation of altered expression was seen with grade ( $p < 0.05$ ).

### Tumor Size

The size of tumor varied from 1.5 to 11 cm. Majority of cases had tumor size of  $> 2-5$ cm (62%) which was followed by tumor size  $> 5$  cm (50%)

Altered BRCA1 expression score was seen more in cases of tumor size  $>2-5$  cm (84%) which was followed by cases of tumor size  $\leq 2$  cm (67%). On statistically analysis, a significant correlation of altered BRCA 1 expression was seen with tumor size ( $p < 0.05$ ).

### ER and PR Status

Out of 50 cases, 20 cases (40%) were ER positive and 30 cases (60%) were ER negative, 12 cases (24%) were PR positive and 38 cases (76%) were PR negative. On comparison of BRCA 1 with ER, PR, altered BRCA 1 expression was seen in ER negative cases (65%) in comparison to ER positive cases (35%).

Altered BRCA 1 expression was seen more in PR negative cases (76%) in comparison to PR positive cases (24%). On Statistical analysis no significant association of altered BRCA1 expression was seen with ER, PR status. ( $p > 0.05$ ).

### HER 2 Status

In the present study, out of 50 cases, 35 cases with HER 2 negative, 3 cases with equifocal and 12 cases positive were found. Altered BRCA 1 expression was seen in 65% of HER 2 negative cases which was followed by 27% of HER 2 positive cases.

## Discussion

### Breast Carcinoma and Clinical Parameters

Breast cancer, the most common malignancy in women, is highly heterogenous with a wide range of biological, pathological and clinical characteristics of these hormone receptors and HER 2 status have a great influence on the clinical role in proliferation and progression of breast cancer. BRCA 1 is located predominantly in nucleus, it contains an NH2 terminal nuclear export signal and can undergo dynamic shunting between nucleus and cytoplasm.<sup>24</sup>

BRCA 1 expression by immunohistochemistry is reduced or absent in familial and sporadic breast cancer.

Majority of breast carcinoma cases (32%) were seen in age group of 40-49 years. Majority of patients (64%) were above 45 years of age. On statistically analysis, a significant association of altered BRCA1 expression was seen with decade.

In the present study, Invasive Carcinoma of NST formed the majority of the cases (82%). Similar results have been reported in the past by various workers Dixon et al, 1985; Ayadi, Khabir, Amoursi et al, 2008; Hameed; Hedau et al 2015 and Puvithav & Shifa, 2016.<sup>10,11,12</sup> Altered BRCA 1 expression was seen in all cases of metaplastic carcinoma, mucinous carcinoma and medullary carcinoma of breast followed by invasive carcinoma of NST. On statistically analysis, a significant association of altered BRCA 1 expression was seen with histological typing.

In the present study, 56% tumor were on the left side. None of the case shows bilaterality. Geethamala et al 2015 also found more cases on left side than right side while Shrivastava et al 2016 found 55.7% of cases on right side.<sup>13,14</sup>

Maximum cases of breast carcinoma were of size >2-5 cm (62%). Similar to present study, Sharma M et al 2016, Vasudha B et al 2012, Ansquer Y et al 2005, Nisa A et al 2008, Verma et al 2018 in their studies demonstrated that maximum cases of breast carcinoma were of tumor size more than 2 cm.<sup>15-19</sup> On statistically analysis, a significant correlation of altered BRCA 1 expression was seen with tumor size (p value<0.05).

Altered BRCA 1 expression was seen in grade II (75%). Altered expression was seen in low grade tumors. Similar to our study, Hedau et al (2015), Pellegrino B et al (2016) shows altered expression in 83% of cases. There is no significant correlation seen in altered BRCA 1 expression with grade.<sup>1,20</sup>

In present study, it was found that altered BRCA1 expression was seen more in ER-negative cases (65%) Similar to the present study Amirrad M(2005), Tulchin N (2013), Pellegrino B (2016), Ansquer et al(1998), Sharma et al(2016) in their study demonstrated that altered BRCA 1 expression was seen more in estrogen receptor-negative breast tumor tissues.<sup>22,23,20,17,15</sup>

In present study altered BRCA 1 expression was seen more in PR negative cases (76%). Similar to the present study Amirrad M (2005), Sharma et al 2016 in their study demonstrated that BRCA 1 mutation was progesterone receptor negative.<sup>22,15</sup>

In present study, altered BRCA 1 expression was seen in HER 2 negative (65%). In contrast to the present study, Pellegrino B 2016 in their study demonstrated that BRCA 1 expression were HER 2 negative.<sup>21</sup>

In contrast to present study, Ansquer et al 1998 in their study demonstrated that BRCA 1 negativity is seen in HER 2 positive cases (9/37,24%).<sup>17</sup>

## Conclusion

Mean age of patient was 50.24 years in breast carcinoma. Invasive carcinoma of no special type (NST) was the commonest histological type. Majority of cases of breast carcinoma were of grade II and of a moderate prognostic group. A statistically significant association of altered BRCA 1 expression was seen with histological type of tumor (p-value<0.05). No statistically significant association of altered BRCA 1 expression was found with

grade, ER, PR, HER 2 (p value>0.05). Altered BRCA 1 expression was seen in cases of ER, PR and HER 2 negative cases. The present study highlights the importance of BRCA 1 expression by Immunohistochemistry as a screening test in sporadic breast cancer. BRCA 1 protein may play a crucial role in the development and progression of sporadic breast carcinoma to select a more targeted and effective chemotherapeutic regime to patients.

**Conflict of Interest:** None.

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