

CLINICOPATHOLOGICAL STUDY OF BREAST CANCER AND IMMUNOHISTOCHEMICAL EXPRESSION OF BRCA 1 AND BRCA2 – A PROSPECTIVE STUDY

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ABSTRACT

Objectives: The aim of this study was to study morphological features of breast cancer (BRCA), to grade according to Nottingham's modification of Bloom Richardson (BR) grading system and to detect the immunohistochemical (IHC) expression of BRCA 1 and BRCA 2 proteins among the clinicopathological parameters of BRCA as age, menopausal status, tumor size, histopathological grade, and lymphnode status.

Methods: This is a prospective and observational study conducted in the Department of Pathology from January 2015 to October 2016, Histopathological examination of 79 cases of Breast carcinoma was conducted and IHC using BRCA 1 and BRCA2 was done in 40 cases.

Results: Out of 79 invasive breast carcinoma, the most common age group for breast carcinoma was 40–49 (40.5%) years. The most common histological subtype was invasive carcinoma-NST, 77(97.4%) cases. Forty cases studied for both BRCA-1 and BRCA 2 expression, regarding BRCA-1 expression, 11 cases showed score 1, 19 cases were of score 2, and eight cases belonged to score 3. Two cases did not show any expression. A positive statistical correlation existed between the size of the tumor, modified BR grade of tumor with BRCA-1 expression. Regarding BRCA-2 expression, 12 cases showed score 1, 17 cases was of score 2, and 10 cases belonged to score 3. One case did not show any expression.

Conclusion: BRCA gene participates in the pathogenesis of breast carcinomas. It is an indicator of poor prognosis. BRCA positivity decreased with increase in age, size of tumor, lymphnodes showing metastasis, and higher grade of the tumor. The present study indicates that BRCA is a powerful predictor of prognosis.

Keywords: BRCA1, BRCA2, Immunohistochemistry, Carcinoma breast.

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INTRODUCTION

Carcinoma of the breast is the most common non-skin malignancy in women and is second only to lung cancer which is the leading cause of death from cancer for women aged 20–59 years. It accounts for 26% of all newly diagnosed cancers in females and is responsible for 15% of the cancer-related deaths in women [1,2]. Among the genes that have been associated with the genesis of breast cancer (BRCA), the BRCA1 gene) and BRCA2 genes are strongly implicated in the pathogenesis. These proteins may play an important role in mammary carcinogenesis in Indian sporadic cases and that mechanisms other than mutation may be involved in the reduced expression of BRCA1 and BRCA2 protein from the data [3].

BRCA is the commonly diagnosed cancer in females accounting for 24% of all female cancers and the leading cause of female cancer deaths worldwide [4].

As the origin of BRCA is multifactorial, most studies commonly pointed toward hormones, diet, reproductive factors, and genetics as general risk factors. BRCA is hereditary in 10% of cases and majority of cases related to mutations in the BRCA1 and BRCA2 genes. BRCA1 and BRCA2 are putative tumor suppressor genes located on chromosome 17q21 and 13q12, respectively [5].

The lifetime risk for developing breast carcinoma as a result of a BRCA1 mutation has been reported in various studies to be 56% to nearly 90% [6,7]. A slightly lower risk of breast carcinoma has been associated with BRCA2 mutations, reported to be 37–84% [7,8] BRCA1 may account for up to 45% of cases of hereditary breast carcinoma as well as nearly 90% of patients with combined breast and ovarian

carcinoma [9,10]. The lifetime risk for ovarian carcinoma attributed to BRCA1 mutations is about 45%.

Various reports indicate that BRCA1-associated breast carcinomas have distinctive pathological features, although they are not unique to these patients [11-14]. The intraductal and infiltrating duct carcinomas are typically poorly differentiated histologically (grade 3) and have a poorly differentiated nuclear grade [15]. A relatively high frequency of medullary carcinomas and of ductal carcinomas with medullary features has been reported in these patients [15]. The tumors are also characterized by high proliferative rates when studied by flow cytometry or by MIB-immunohistochemistry [11,13] BRCA1-associated breast carcinomas typically do not express estrogen receptors or HER2/neu, but they exhibit p53 nuclear reactivity. Angiogenesis may also be enhanced in BRCA1-associated carcinomas [11]. Because the risk for developing breast carcinoma associated with BRCA1 and BRCA2 most likely involves all tissue in both breasts, surgical treatment to substantially reduce or eliminate the risk requires bilateral prophylactic mastectomy [16].

This study aims to evaluate the various clinicopathological parameters of breast carcinoma, to examine their association with IHC expression of BRCA 1 and 2, and also to compare the expression of BRCA 1 and 2 in relation to BRCA carcinogenesis especially in post-menopausal women.

METHODS

A 22-month hospital-based prospective and observational study was conducted in the Department of Pathology from January 2015 to October 2016. This study was done in our tertiary care center after approval by our Institutional Ethical Committee.

Seventy-nine mastectomy specimens with BRCA were studied on light microscopy and IHC using BRCA 1 and BRCA2 was done in 40 cases.

We have included all Mastectomy specimens received in the department with the diagnosis of Invasive breast carcinoma and excluded all lumpectomy, trucut biopsy, benign, and inflammatory lesions.

All mastectomy specimens were fixed in 10% neutral buffered formalin. After adequate fixation, examination of the specimens was grossed as per protocol. Then, representative tissue bits were taken and sections were routinely stained with hematoxylin and eosin (H and E) and examined under light microscopy.

Histopathological features were studied and the tumors were typed according to the WHO classification system. The Nottingham's modification of Bloom Richardson (BR) grading system was used for grading and noted all other relevant data as per protocol. Forty cases were subjected to immunohistochemical (IHC) analyses for BRCA-1 & BRCA-2 expression using polyexcel HRP/DAB detection system. Microscopically sections were examined for the presence of a brown colored endproduct, at the site of the target antigen. The immunoreactivity was observed and recorded as cytoplasmic and membranous, 0: negative, 1+: mild, 2+: moderate and 3+: strong. or nuclear scoring done from 0 to 3 similar to Weberpals *et al.* study [17]. Statistical analysis of the collected data was done using Chi-square tests. $p < 0.05$ was considered statistically significant.

RESULTS

In the present study, a total of 79 cases of carcinoma breast were evaluated. The age of the patients with carcinoma breast ranged from 26 to 81 years. Mean age was 49.73 years. Majority of the patients (40.50%) were in the age group of 40-49 years. The size of the tumor ranged from 0.5 cm to 12 cm considering the largest dimension of the tumor. The most number of cases has a size between >2 and 5 cm about (73%).

The histological subtyping of invasive carcinoma of the breast was done according to the WHO classification. Of the 79 cases of invasive carcinoma breast studied, the majority of the cases (77 cases, 97.46%) were of invasive cancer-NST, and one case each of medullary carcinoma and mucinous carcinoma. Grossly mucinous carcinoma showed ill ill-circumscribed mass 3x3 cm with gelatinous cut surface. Microscopy showed tumor cells floating in mucinous pools.

Of the 79 cases, 43% (34 cases) of the cases had no lymphnode metastasis. (29.11%) 23 cases had metastatic deposits in 1-3 lymphnodes, 20 cases had 4-9 lymphnodes (25.31%), and 2 cases showed metastatic deposits in more than 10 lymphnodes. The largest metastatic lymph node was of 4 cm in size of the 79 cases of invasive carcinoma breast studied, 17 (21.51%) cases were of BR Grade 1 (Fig. 1a), 54 (68.35%) cases were of Grade 2, and cases 8 (10.12%) belonged to Grade 3 (Fig. 2a).

BRCA-1 expression

Of the 79 cases of invasive carcinoma breast studied, BRCA-1 IHC analysis for protein expression was studied in 40 cases. Of which

11 cases showed score 1, 19 cases were of score 2, and eight cases belonged to score 3. Two cases did not show any expression. Tumors of the higher score are having higher mean age, that is, 53.37, whereas the majority of the tumors showed 2+ expression of about 47% (19 out of 40) with a mean age of 51.7. 11 cases showed 1+ expression (Table 1).

Among the 40 cases studied, eight cases belonged BR Grade 3, 26 cases belonged BR Grade 2, and six cases belonged to BR Grade 1. Among the eight cases of Grade 3, six cases had expression of 1+, (Fig. 2b) among the 26 cases of Grade 2, 16 cases had expression of 2+, among the six cases of Grade 1, four cases had expression of 3+ (Fig. 1b and Table 2).

Of the invasive carcinoma of the breast IHC expression was studied in 40 cases, 39 cases were of Invasive carcinoma-NST, and one case of mucinous carcinoma. Of the 39 cases of Invasive carcinoma - NST 10 cases showed score 1+, 19 cases were of score 2+, and eight cases belonged to score 3+. Two cases did not show any expression. The case of mucinous carcinoma had score of 1+ (Table 3).

BRCA-2 expression

The cases studied with BRCA-1 were subsequently tested for BRCA-2 protein expression and compared with similar clinical parameters. Among BRCA 2 expression, the most common mean age group effected, was 69.16 with a larger tumor size 5.2 cm and with 52% of cases showed

Table 1: Comparison of BRCA-1 expression with mean age (n=40)

IHC Score	Cases	Mean age (years)
0	2	44
1+	11	49.63
2+	19	51.7
3+	8	53.37

Table 2: Comparison of BRCA-1 expression and histological grade of tumor (n=40)

IHC score	No. of cases	Bloom Richardson grade		
		I	II	III
0	2	0	2	0
1+	11	0	5	6
2+	19	2	16	1
3+	8	4	3	1

Table 3: Comparing BRCA-1 expression with histological subtyping of tumors (n=40)

BRCA-1 score	Invasiveca-NST	Mucinous carcinoma
0	2	-
1+	10	1
2+	19	-
3+	8	-

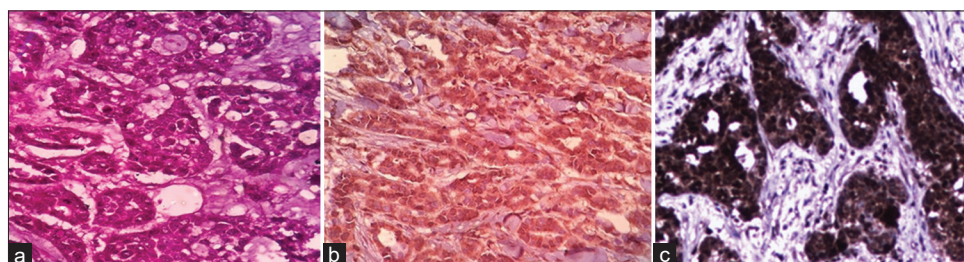


Fig. 1: (a) Photomicrograph showing Invasive carcinoma-NST, Bloom Richardson Grade 1, H&E, 100x, (b) showing BRCA-1 strong expression, (IHC) 100x, (c) showing BRCA-2 strong expression, (IHC) 100x

lymphnode metastasis (9 out of 17). The majority showed BR Grade 2 of about 76% (13 out of 17) (Table 4). Among the postmenopausal patient's 32 cases, BRCA 1 expression in 10 cases had 1+ score and 15 cases had 2+ score and six cases had 3+ score and one case did not show any expression and BRCA 2 expression in 10 cases had 1+ score and 12 cases had 2+ score and 9 cases had 3+ score (Fig. 1c and Table 5). Mucinous carcinoma showed moderate BRCA2 expression among the 40 cases BRCA-1 expression in eight cases had score 3+, whereas BRCA 2 did not show 3+ expression, the majority showed 2+ and 1+. BRCA 1 showed 2+ expression in 19 cases whereas BRCA 2 showed 2+ expression in nine cases (Table 6).

DISCUSSION

The *BRCA1* gene, which is responsible for hereditary breast and ovarian cancer, has been identified by positional cloning. The expression of BRCA2 mRNA is cell cycle regulated and associated with proliferation in normal and tumor-derived breast epithelial cells. It looks like that kinetics of BRCA2 mRNA up-regulation appeared to be similar to those of BRCA1, suggesting that two genes can be commonly controlled and regulated [18]. In the present study, the cytoplasmic, as well as nuclear expression, was taken as positive end result.

The present study is hospital-based observational study conducted on 79 mastectomy diagnosed histologically as invasive carcinomas of the breast. Of the 79 cases studied IHC analysis for BRCA-1, 2 protein expression was done on 40 cases.

In this study, majority of the patients were above 40 years of age and the mean age is 49 years. Youngest patient was 21 years old and oldest patient was 81 years old. Most of the cases, that is, 32 cases (40.50%) were of the age group 40–49 years. The majority of the patients were postmenopausal 61 cases (77%). When this was compared with Hedau *et al.* [11], 16 cases out of 40 (40%) were of premenopausal age group, which was higher than the present study. In the study by Atchley *et al.* [19], they had 30% of cases of postmenopausal age with BRCA1 mutation carriers and 53% of cases of BRCA2 mutation carriers. In the study by Pérez-Vallés *et al.* [20], who studied 45 patients with BRCA, had 25 patients up to 45 years, and 20 patients above 45 years.

The tumor size was ranging from 0.5 to 12 cm. Most of the tumors were of the size between >2 cm and 5 cm, in 58 cases (73%), 14 cases were having tumor up to 2 cm size and seven cases had tumor of >5 cm.

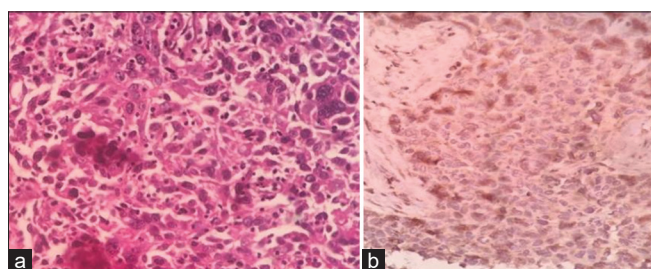


Fig. 2: (a) Photomicrograph showing Invasive carcinoma-NST, Bloom Richardson Grade 3, showing marked atypia with tumor giant cells, H&E, 100x, (b) showing BRCA-1, weak expression, (IHC) 400x

whereas study done by Pérez-Vallés *et al.* [20] showed 18 of 45 (40%) cases with 2–5 cm.

The cases were evaluated for histological grading as per modified BR grading system. Of the 79 cases of invasive carcinoma breast studied, majority of 54(68.35%) cases were of Grade 2, Results were similar to the study by Hedau *et al.* [11] and Pérez-Vallés *et al.* [20], where majority of cases belonging to Grade 2, followed by Grade 1 and Grade 3 (Table 7). Of the 79 cases studied, score 1+ was observed with a mean age of 49 years, score 2+ with a mean age of 51 years, and cases of score 3+ had a mean age of 53 years. IHC score was studied, in the cases with age 40 years onward most of the cases had 1+ or 2+ score, and fewer cases showing 3+ score. This shows that the protein expression was decreasing with increasing age. This correlated well with Hedau *et al.* [11] who had similar results with decreasing BRCA1 protein expression with increasing age (Table 8).

In the study by Atchley *et al.* [19], they had a median age of 41.5 years for BRCA1 mutation carriers. In the study by Pérez-Vallés *et al.* [20], who studied 45 patients with BRCA, 7 patients had BRCA-1 mutation. Of which five patient has less than 45 years old and two patients has more than 45 years. This shows that most of the cases which showed BRCA-1 mutation were <45 years. The present study comprised all sporadic cases without any family history of breast malignancies and the present study with IHC expression showed the results opposing that of seen in Pérez-Vallés *et al.* [20] study with decreasing expression with increasing age.

Among the postmenopausal patients – 32 cases, 10 cases had 1+ score and 15 cases had 2+ score and six cases had 3+ score, and one case did not show any expression, showing overall decrease in expression among the postmenopausal patients which in turn suggests that with increase in the age the BRCA-1 protein expression decreases. In the study by Hedau *et al.* [11] of the 24 postmenopausal patients 11 cases had 1+ score, four cases each of score 2+ and 0 expression, showing the majority patients with decreased expression in the postmenopausal age group. Similar results were observed in the present study. In the study by Atchley *et al.* [19], they had 30% of cases of postmenopausal age with BRCA1 mutation carriers.

Among the 40 cases studied, increasing size of the tumor, there is decrease in protein expression, which is in contrast to study done by Pérez-Vallés *et al.* [20] where majority showed increased expression with increasing tumor size, that is, out of seven cases with BRCA-1 mutation three were up to 2 cm size and 4 were 2–5 cm. Among the 40 cases studied, the majority belonged BR Grade 2 with 16 cases had expression of 2+. This shows that tumors with higher histological grade have reduced BRCA-1 protein expression. The results were almost similar to Hedau *et al.* [11] where the majority belonged to Grade 2 with 11 showed 1+ protein expression (Table 9).

Cases showing score 1+ had a mean age of 56 years, cases of score 2+ had a mean age of 69 years cases of score 3+ had a mean age of 39 years, which shows that more the protein expression (3+) younger were the patients, in the same way lesser, the protein expression (2+,1+) older were the patients. This shows that the protein expression was decreasing with increasing age similar to the study done by Hedau *et al.* [11]. Out of 24 postmenopausal patients six cases had 1+ score, five cases each of score 2+ and 0 expression, showing majority patients with decreased expression in the postmenopausal age group (Table 10).

Table 4: Overall comparison of BRCA-2 expression with the parameters (n=40)

IHC score	No. of cases	Mean age	Mean tumor size (cm)	L.N. metastasis	No metastasis	BR grade		
						I	II	III
0	1	66	5	1	-	-	-	1
1	12	56	3.25	9	3	7	1	4
2	17	69.16	5.2	9	8	13	3	1
3	10	39.66	2.91	6	4	6	2	2

Table 5: Comparing BRCA expression with menopausal status (n=40)

IHC score	Pre-menopausal cases		Postmenopausal cases	
	BRCA-1	BRCA-2	BRCA-1	BRCA-2
0	1	-	1	1
1+	1	2	10	10
2+	4	5	15	12
3+	2	1	6	9

Table 6: Comparing results of BRCA-1 with BRCA-2 (n=40)

BRCA1 score	No. of cases	BRCA-2 (score) 3+	2+	1+	0
3+	8	0	3	5	0
2+	19	5	9	4	1
1+	11	5	4	2	0
0	2	0	1	1	0

Table 7: Comparing histological grade with other studies

Bloom Richardson GRADE	Present study (No of cases) (%)	Hedau et al.[11] (No of cases) (%)	Pérez-Vallés et al.[20] (No of cases) (%)
I	17 (21.51)	6 (15)	14 (31)
II	54 (68.35)	22 (55)	21 (47)
III	8 (10.12)	12 (30)	10 (22)
Total	79 (100)	40 (100)	45 (100)

Table 8: Comparing age and BRCA-1 expression with other studies

Age (years)	BRCA1 score							
	Present study				Hedau et al. [11]			
	0	1+	2+	3+	0	1+	2+	3+
20-29	1	1	-	-	4	1	2	1
30-39	-	1	-	1	3	4	1	1
40-49	-	4	10	2	3	7	1	3
50-59	-	1	3	2	1	3	2	0
60-69	1	4	3	2	0	3	0	1
70-79	-	-	3	1	0	0	0	0

Table 9: Comparing histological grade and BRCA-1 expression with other studies

BRCA-1 IHC score	Present study BR grade			Hedau et al.[11] BR grade		
	I	II	III	I	II	III
	0	-	2	-	3	5
1+	0	5	6	2	11	5
2+	2	16	1	0	3	3
3+	4	3	1	1	3	3

Among the 40 cases studied, majority belonged to Grade 2 with 13 cases showing 2 + expression, that is, higher histological grade have reduced BRCA-2 protein expression compared to tumors of lower histological grade similar to study done by Hedau et al. (Table 11) [11].

Comparing BRCA-1 and BRCA 2 expression among 40 cases of invasive breast carcinoma, 39 were Invasive Ca-NST of which the majority showed score 2+ and mucinous carcinoma showed score 1+ with BRCA 1 whereas 2+ with BRCA 2. As most of the cases being invasive Ca-NST, no significant conclusion could be drawn.

Table 10: Comparing age and BRCA-2 expression with other studies

Age (years)	BRCA2 status							
	Present study				Hedau et al. [11]			
	0	1+	2+	3+	0	1+	2+	3+
20-29	-	-	2	-	0	3	3	1
30-39	-	-	2	-	0	5	3	1
40-49	-	4	5	7	3	5	4	2
50-59	-	2	2	2	1	2	0	3
60-69	1	5	3	1	0	0	1	2
70-79	-	1	3	0	0	0	0	0

Table 11: Comparing histological grade and BRCA-2 expression with other studies

BRCA-2 IHC Score	Present study BR grade			Hedau et al.[11] BR grade		
	I	II	III	I	II	III
	0	-	-	1	0	3
1+	1	7	4	2	10	4
2+	3	13	1	4	5	2
3+	2	6	2	0	5	4

According to the Findings in this study, there was loss of expression of BRCA-2 proteins in most of the cases which were showing higher expression with BRCA-1 and there was increased expression of BRCA-2 proteins in most of the cases which were showing lower expression with BRCA-1. There is almost an inverse relation to these two protein expressions.

When these expressions were compared with age both BRCA-1 and BRCA-2 were showing decreased expression with increasing age. Similar results were observed with postmenopausal age group patients In Hedau et al. [11] study, no significant correlation was observed between BRCA1 and BRCA-2 expression when compared with age.

The tumors of larger size had decreased expression of BRCA-1 protein when compared with tumors of smaller size which were showing higher expression, no significant conclusion could be drawn with BRCA-2 expression. Higher the histological grade, lower was the expression of these proteins and vice-versa. The cases which were showing regional lymph nodal metastasis had lower expression of these proteins compared to the cases without any nodal metastasis.

Statistical significance was observed when BRCA-1 expression was compared with tumor size and BR grading. Lack of statistical significance with other parameters may be due to small sample size.

The limitations of the study were smaller sample size and very few studies were available in the literature based on Immunohistochemistry for comparison of BRCA. The available studies could not specifically immunolocalize BRCA protein expression in the cell and wide differences were noticed between different studies may be due to interlaboratory, inter, and intraobserver variations.

CONCLUSION

The present study indicates that BRCA is a powerful predictor of prognosis as its expression is associated with known prognostic parameters such as size of tumor, lymph nodes showing metastatic deposits, and histological grade of tumor. Positivity decreased with an increase in age, size of tumor, lymphnodes showing metastasis, and higher grade of tumor. Although there was no significant correlation between several parameters and BRCA expression which could be due to smaller sample size. However, it is necessary to carry out studies

using other molecular markers of prognosis to evaluate the prognosis and provide better therapeutic options.

AUTHORS' CONTRIBUTION

Dr. Naveen Dr, Basumitra das contributed study conception and design and performed the work, analysis, and interpretation of results. Dr. Sunil kumar Dr., Rajani contributed manuscript preparation and statistical data analysis and corrected the manuscript.

CONFLICTS OF INTEREST

The authors declared no conflicts of interest.

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