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Original Research Article

DETERMINATION OF BRCA1 AND BRCA2 PROTEIN EXPRESSION IN BREAST CANCER BY IMMUNOHISTOCHEMISTRY AND TO STUDY THEIR CLINICAL CORRELATION: A CROSS SECTIONAL STUDY

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ABSTRACT

Background: Breast cancer is the most frequently diagnosed malignancy and the leading cause of cancer-related mortality among women worldwide. Mutations and altered expression of the tumor suppressor genes BRCA1 and BRCA2 play a crucial role in breast carcinogenesis by impairing DNA repair mechanisms. Immunohistochemical assessment of BRCA protein expression provides a practical approach for evaluating their clinicopathological significance, particularly in resource-limited settings. The aim is to determine the immunohistochemical expression of BRCA1 and BRCA2 proteins in breast carcinoma and to correlate their expression with clinicopathological parameters.
Materials and Methods: This hospital-based cross-sectional study was conducted in the Department of Pathology, Mandya Institute of Medical Sciences, Mandya, over 18 months. A total of 68 histopathologically confirmed breast carcinoma cases from biopsy and mastectomy specimens were included. Formalin-fixed, paraffin-embedded tissues were stained with hematoxylin and eosin for histopathological evaluation and subjected to immunohistochemistry using the avidin-biotin complex technique for BRCA1 and BRCA2 protein expression. Nuclear staining was assessed semi-quantitatively using a five-point scoring system based on the percentage of positively stained tumor cells. Associations between BRCA expression and clinicopathological variables were analyzed using the Chi-square test or Fisher's exact test, with $p<0.05$ considered statistically significant.
Results: Moderate BRCA1 (2+, 29.4%) and BRCA2 (3+, 26.5%) expression patterns were the most frequent. BRCA1 expression demonstrated a significant association with age ($p=0.016$), whereas BRCA2 expression showed no significant age-related association ($p=0.091$). Neither BRCA1 nor BRCA2 expression correlated significantly with gender, family history, or lymph node status. A highly significant inverse association was observed between tumor grade and BRCA expression. Grade I tumors predominantly exhibited strong BRCA1 and BRCA2 expression, whereas Grade III tumors showed weak or absent expression ($p<0.01$ for both), suggesting progressive loss of BRCA protein expression with increasing tumor grade.
Conclusion: Immunohistochemical expression of BRCA1 and BRCA2 proteins is significantly associated with tumor differentiation in breast carcinoma. Reduced BRCA1 and BRCA2 expression correlates with higher histological grade, indicating their potential value as prognostic biomarkers.

Immunohistochemical assessment of BRCA proteins may serve as a useful adjunct in the pathological evaluation of breast cancer and assist in identifying patients who may benefit from further molecular testing and personalized therapeutic strategies.
Keywords: Breast carcinoma, BRCA1, BRCA2, Immunohistochemistry, Tumor grade, Clinicopathological correlation, Prognostic biomarker.

INTRODUCTION

Breast cancer is the most commonly diagnosed cancer and the leading cause of cancer death among females worldwide.[1] It accounts for 11.7% of all new cancer cases and 6.9% of all cancer deaths.[2] The development of breast cancer is linked to various genetic and non-genetic factors. Non-genetic factors include reproductive risks such as prolonged estrogen exposure from early menarche, late menopause, and oral contraceptive use, along with lifestyle factors like a high-fat diet and sedentary lifestyle.[1] Genetic factors prominently involve mutations in the BReast CAncer 1 (BRCA1) and BReast CAncer 2 (BRCA2) genes, which are crucial for DNA repair.

Globally, breast cancer affects approximately 13% of women at some point in their lives. However, this risk increases dramatically for women with BRCA1 and BRCA2 mutations. By the age of 70-80, about 55%-72% of women with a BRCA1 mutation and 45%-69% with a BRCA2 mutation will develop breast cancer. These genes, located on chromosomes 17q21 and 13q12 respectively, produce proteins essential for repairing damaged DNA. When mutated, these tumor suppressor genes lose their ability to repair DNA, significantly increasing the risk of breast cancer development.[3]

Despite extensive research, several gaps remain in the understanding of BRCA1 and BRCA2 protein expression and their role in breast carcinogenesis. Previous studies have noted reduced or absent BRCA1 protein expression in familial and sporadic breast cancer cases through immunohistochemical analysis. Mechanisms such as loss of heterozygosity, decreased mRNA and protein expression, and methylation of the BRCA1 promoter region contribute to these reduced levels.[1] However, comprehensive analysis correlating BRCA1 and BRCA2 protein expressions with clinical outcomes in breast cancer patients is limited. This gap necessitates further investigation to better understand the molecular underpinnings of breast cancer and improve diagnostic and therapeutic strategies.

This study aims to analyze the expression of BRCA1 and BRCA2 proteins in breast cancer cases and correlate these expressions with clinical outcomes. Given the significant risk posed by BRCA mutations, understanding the precise role of BRCA1 and BRCA2 protein expression in breast cancer can lead to improved screening, early detection, and targeted therapies. By focusing on the molecular pathways involving these genes, the study seeks to uncover critical insights into mammary carcinogenesis.[4] This research is essential for developing more effective strategies to manage and treat breast cancer, ultimately reducing morbidity and mortality associated with this prevalent disease. Through meticulous analysis and correlation with clinical data, the study hopes to contribute valuable knowledge to the ongoing battle against breast cancer.[5]

AIM & OBJECTIVES OF THE STUDY

- To determine the level of expression of BRCA1 and BRCA2 proteins in breast cancer cases.
- To correlate clinically the expression of BRCA1 and BRCA2 proteins in breast cancer cases.

MATERIALS AND METHODS

Study Design:

This hospital-based cross-sectional study was conducted in the Department of Pathology, Mandya Institute of Medical Sciences, Mandya, Karnataka, over a period of 18 months. The study was undertaken to determine the immunohistochemical expression of BRCA1 and BRCA2 proteins in breast carcinoma and to evaluate their clinicopathological correlation.

Study Setting:

The study was carried out in the Histopathology and Immunohistochemistry Laboratory, Department of Pathology, Mandya Institute of Medical Sciences, Mandya. Histopathological examination and immunohistochemical analysis were performed on breast carcinoma specimens received during the study period.

Study Population

The study included 68 histopathologically confirmed cases of breast carcinoma obtained from biopsy and mastectomy specimens. Relevant clinical and pathological details were collected from hospital records and pathology requisition forms.

Sample Size:

A total of 68 breast carcinoma cases were included in the study based on the predefined eligibility criteria during the study period.

Inclusion Criteria

- Histopathologically confirmed cases of breast carcinoma.
- Breast biopsy and mastectomy specimens received during the study period.
- Adequately preserved tissue suitable for histopathological examination and immunohistochemistry.

Exclusion Criteria

- Recurrent breast carcinoma.
- Metastatic breast carcinoma.
- Inadequate or poorly preserved tissue specimens unsuitable for immunohistochemical analysis.

Specimen Collection and Histopathological Examination:

All specimens were fixed in 10% neutral buffered formalin immediately after surgical excision. Following fixation, routine tissue processing was carried out, and paraffin blocks were prepared. Sections measuring 4–5 µm were cut from the paraffin blocks and stained with hematoxylin and eosin (H&E). Histopathological examination was performed to establish the diagnosis and evaluate tumor type, histological grade, lymph node status, and pathological stage according to standard pathological criteria.

Immunohistochemistry:

For immunohistochemical analysis, 3 µm-thick sections were obtained from formalin-fixed paraffin-embedded tissue blocks. Immunostaining was performed using the avidin-biotin complex (ABC) technique.

The sections were mounted on poly-L-lysine-coated slides, deparaffinized with xylene, and rehydrated through graded alcohols. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide in phosphate-buffered saline (PBS) for 30 minutes, followed by washing in PBS.

Antigen retrieval was performed using 1 mmol/L citrate-phosphate buffer (pH 6.0) in a microwave oven at 100 watts for approximately 15 minutes. After cooling, the sections were incubated with normal horse serum for one hour to minimize non-specific staining.

The slides were incubated overnight at 4°C in a humidified chamber with primary antibodies against BRCA1 and BRCA2 proteins. After washing with PBS, sections were treated with biotinylated secondary antibody, followed by avidin-biotinylated horseradish peroxidase (HRP). 3,3′-Diaminobenzidine (DAB) was used as the chromogen to visualize antigen-antibody complexes. Finally, the slides were counterstained with Mayer's hematoxylin, dehydrated in graded alcohols, cleared in xylene, and mounted using DPX.

Evaluation of BRCA1 and BRCA2 Expression:

Immunohistochemical staining was evaluated independently under light microscopy. Only nuclear staining of tumor cells was considered positive for BRCA1 and BRCA2 protein expression.

For each case, 100–200 tumor cells were counted in 10 randomly selected high-power fields (×400 magnification), and the percentage of positively stained nuclei was determined. Protein expression was scored according to the proportion of positively stained tumor cells as follows:

Percentage of Positive Tumor Cells	Score
0%	1
1–25%	2
26–50%	3
51–75%	4
>75%	5

The immunohistochemical findings were subsequently correlated with clinicopathological parameters, including age, gender, family history, histological grade, lymph node involvement, and histopathological diagnosis.

Data Collection: Clinical information and pathological findings were recorded using a structured proforma. Variables studied included:

- Age
- Gender
- Family history of breast cancer
- Histopathological diagnosis
- Tumor grade
- Number of lymph nodes involved
- BRCA1 protein expression
- BRCA2 protein expression

Outcome Measures: The primary outcome was the expression of BRCA1 and BRCA2 proteins by immunohistochemistry. Secondary outcomes included the association of BRCA1 and BRCA2 expression with various clinicopathological parameters.

Statistical Analysis: The collected data were entered into Microsoft Excel and analyzed using appropriate statistical software. Categorical variables were expressed as frequencies and percentages. Associations between BRCA1/BRCA2 protein expression and clinicopathological variables were assessed using the Chi-square test or Fisher's exact test, wherever appropriate. A p-value <0.05 was considered statistically significant.

Ethical Considerations: The study was conducted after obtaining approval from the Institutional Ethics Committee of Mandya Institute of Medical Sciences, Mandya. Patient confidentiality was strictly maintained throughout the study, and all procedures complied with the ethical principles of biomedical research.

RESULTS

Table 1: Correlation of BRCA1 with important parameters of the subjects

Subjects (N=68)		BRCA1 Score												p-value#
		0		1+		2+		3+		4+		5+		
		N=5	%	N=16	%	N=20	%	N=17	%	N=7	%	N=3	%	
Age group	<30 years	1	20.0%	0	0.0%	0	0.0%	2	11.8%	1	14.3%	1	33.3%	0.016*
	31 to 40 years	0	0.0%	4	25.0%	5	25.0%	2	11.8%	1	14.3%	0	0.0%	
	41 to 50 years	1	20.0%	2	12.5%	7	35.0%	4	23.5%	2	28.6%	1	33.3%	
	51 to 60 years	1	20.0%	2	12.5%	7	35.0%	5	29.4%	1	14.3%	1	33.3%	
	61 to 70 years	0	0.0%	8	50.0%	0	0.0%	2	11.8%	0	0.0%	0	0.0%	
	>70 years	2	40.0%	0	0.0%	1	5.0%	2	11.8%	2	28.6%	0	0.0%	
Gender	Male	0	0.0%	1	6.3%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	
	Female	5	100.0%	15	93.8%	20	100.0%	17	100.0%	7	100.0%	3	100.0%	0.654
Family History	Yes	5	100.0%	14	87.5%	15	75.0%	14	82.4%	7	100.0%	3	100.0%	0.486
	No	0	0.0%	2	12.5%	5	25.0%	3	17.6%	0	0.0%	0	0.0%	
	Nil	5	100.0%	13	81.3%	12	60.0%	9	52.9%	5	71.4%	2	66.7%	0.368
Lymph node Status	1 to 5	0	0.0%	1	6.3%	3	15.0%	6	35.3%	2	28.6%	1	33.3%	
	6 to 10	0	0.0%	2	12.5%	1	5.9%	1	5.9%	0	0.0%	0	0.0%	
	>10	0	0.0%	0	0.0%	4	20%	1	5.9%	0	0.0%	0	0.0%	

Chi-square test * Statistically significant

The majority of subjects fall within the BRCA1 scores of 1+, 2+, and 3+, with 16 (23.5%), 20 (29.4%), and 17 (25.0%) individuals, respectively, showing these levels of expression. Age group analysis reveals a significant correlation (p=0.016), with themajority of subjects in the 41-50 and 51-60 age ranges displaying BRCA1 scores of 2+ and 3+. Interestingly, the no expression of BRCA1 are more prevalent in older age groups, particularly those aged over 70. Gender analysis indicates that BRCA1 is expressed even in male breast cancers similar to female breast cancers and hence no significant variation. Family history did not show significant variation across BRCA1 scores, although there was a higher expression among those with a family history of the condition. Lymph node (LN) status displayed some variability; most subjects with had nil to moderate lymph node involvement showed BRCA1 expression, suggesting an inverse relationship between BRCA1 expression and lymph node involvement. Inference from this data suggests that BRCA1 expression is significantly associated with age, potentially reflecting underlying biological mechanisms influenced by these demographic factors.

Table 2: Correlation of BRCA2 with important parameters of the subjects

Subjects (N=68)		BRCA2 Score												p-value#
		0		1+		2+		3+		4+		5+		
		N=6	%	N=15	%	N=15	%	N=18	%	N=12	%	N=2	%	
Age group	<30 years	0	0.0%	1	6.7%	0	0.0%	2	11.1%	2	16.7%	0	0.0%	0.091
	31 to 40 years	0	0.0%	3	20.0%	5	33.3%	2	11.1%	2	16.7%	0	0.0%	
	41 to 50 years	1	16.7%	1	6.7%	3	20.0%	5	27.8%	5	41.7%	2	100.0%	
	51 to 60 years	0	0.0%	6	40.0%	4	26.7%	5	27.8%	2	16.7%	0	0.0%	

	61 to 70 years	3	50.0%	4	26.7%	2	13.3%	1	5.6%	0	0.0%	0	0.0%	
	>70 years	2	33.3%	0	0.0%	1	6.7%	3	16.7%	1	8.3%	0	0.0%	
Gender	Male	0	0.0%	0	0.0%	0	0.0%	1	5.6%	0	0.0%	0	0.0%	0.728
	Female	6	100.0%	15	100.0%	15	100.0%	17	94.4%	12	100.0%	2	100.0%	
Family History	Yes	6	100.0%	12	80.0%	12	80.0%	18	100.0%	8	66.7%	2	100.0%	0.132
	No	0	0.0%	3	20.0%	3	20.0%	0	0.0%	4	33.3%	0	0.0%	
LN Status	Nil	4	66.7%	12	80.0%	9	60.0%	12	66.7%	9	75.0%	0	0.0%	0.295
	1 to 5	1	16.7%	1	6.7%	5	33.3%	4	22.2%	1	8.3%	1	50.0%	
	6 to 10	1	16.7%	1	6.7%	0	0.0%	0	0.0%	1	8.3%	1	50.0%	
	>10	0	0.0%	1	6.7%	1	6.7%	2	11.1%	1	8.3%	0	0.0%	

Chi-square test

The majority of subjects fall within the BRCA2 scores of 1+, 2+, and 3+, with 15 (22.1%), 15 (22.1%), and 18 (26.5%) individuals, respectively, showing these levels of expression. In terms of age, the majority of subjects with BRCA2 scores of 2+, 3+, and 4+ fall within the 41 to 50years and 51 to 60 years age group, representing a high concentration of higher BRCA2 scores in middle-aged individuals. Notably, those over 70 show fewer high scores, with only one individual in the 2+ and 4+ categories. In the gender analysis, females predominantly represent all BRCA2 score categories, with one male scoring a 3+. Family history analysis indicates that subjects with a positive family history of BRCA2 mutations are primarily associated with higher BRCA2 expression, especially in the 3+ and 5+ categories. Regarding LN status, a notable pattern emerges where the majority of subjects with higher BRCA2 scores (3+,4+ and 5+) have either no lymph node involvement or limited involvement (1 to 5 lymph nodes). This suggests that higher BRCA2 expression might correlate with limited LN involvement. In summary, the data highlights that higher BRCA2 scores are more prevalent in subjects with a family history of BRCA mutations, predominantly in middle-aged subjects, and are associated with limited lymph node involvement, which could suggest a specific pathological profile for BRCA2 expression.

Table 3: Correlation of tumor grading with BRCA1 expression

Subjects (N=68)		**Grade of Tumor**						**p-value#**
		Grade I		**Grade II**		**Grade III**		
		N=12	**%**	**N=36**	**%**	**N=20**	**%**	
BRCA1 Score	0	2	16.7%	1	2.8%	2	10.0%	<0.01*
	1+	0	0.0%	1	2.8%	15	75.0%	
	2+	0	0.0%	17	47.2%	3	15.0%	
	3+	1	8.3%	16	44.4%	0	0.0%	
	4+	6	50.0%	1	2.8%	0	0.0%	
	5+	3	25.0%	0	0.0%	0	0.0%	

Chi-square test *Statistically significant

The correlation between tumor grading and BRCA1 expression was analyzed in a cohort of subjects with varying grades of tumors, revealing significant patterns in BRCA1 score distribution across the different grades. In Grade I tumors, the majority of cases (50%) exhibited a BRCA1 score of 4+, indicating high BRCA1 expression. Additionally, 25% of these Grade I tumors showed a BRCA1 score of 5+, further emphasizing elevated expression levels in lower-grade tumors. Conversely, in Grade II tumors, the distribution was more varied, with a notable 47.2% of cases having a BRCA1 score of 2+ and 44.4% displaying a 3+ score. This pattern suggests a moderate expression of BRCA1 in Grade II tumors. In Grade III tumors, the majority of cases (75%) had a BRCA1 score of 1+, indicating significantly lower expression levels. The statistical analysis revealed a p-value of less than 0.01, highlighting a significant correlation between tumor grade and BRCA1 expression. These findings suggest that higher tumor grades are associated with decreased BRCA1 expression, which may have implications for tumor behavior and prognosis.

Table 4: Correlation of tumor grading with BRCA2 expression

Subjects (N=3)		**Grade of Tumor**						**p-value#**
		Grade I		**Grade II**		**Grade III**		
		N=12	**%**	**N=36**	**%**	**N=20**	**%**	
BRCA2 Score	0	2	16.7%	1	2.8%	3	15.0%	<0.01*
	1+	1	8.3%	2	5.6%	12	60.0%	
	2+	2	16.7%	9	25.0%	4	20.0%	
	3+	3	25.0%	14	38.9%	1	5.0%	
	4+	3	25.0%	9	25.0%	0	0.0%	
	5+	1	8.3%	1	2.8%	0	0.0%	

Chi-square test *Statistically significant

The analysis of tumor grading in relation to BRCA2 expression reveals distinct patterns that underscore varying expression levels across different tumor grades. In Grade I tumors, a significant proportion of cases exhibited elevated BRCA2 expression, with 25% showing a score of 3+ and another 25% demonstrating a score of 4+. This indicates a relatively high expression of BRCA2 in lower-grade tumors. Additionally, 16.7% of Grade I tumors had a BRCA2 score of 2+, suggesting that a majority of these tumors have moderate to high expression levels. In Grade II tumors, BRCA2 expression was more distributed, with 38.9% of cases having a score of 3+, followed by 25% with a 2+ score. This suggests moderate expression levels are predominant in Grade II tumors. In Grade III tumors, the majority of cases (60%) had a BRCA2 score of 1+, indicating low expression levels, while 15% had a score of 0, signifying no expression. The chi-square test revealed a statistically significant correlation ($p < 0.01$) between tumor grade and BRCA2 expression. These findings suggest that higher tumor grades are associated with decreased BRCA2 expression, which could have implications for understanding tumor aggressiveness and potential therapeutic approaches.

DISCUSSION

The study was conducted at the Department of Pathology, Mandya Institute of Medical Sciences, Mandya, from July 2022 to December 2023. It was a cross-sectional study involving biopsy specimens of breast cancer cases referred to the department. The study excluded recurrent and metastatic breast carcinoma specimens. A total of 68 biopsy samples were collected using convenience sampling. Each specimen was fixed in a 10% formalin solution, processed, and embedded in paraffin to preserve tissue integrity. Sections were cut at 4-5 microns for hematoxylin and eosin (H&E) staining to assess tumor and lymph node status, while 3-micron sections were prepared for immunohistochemical staining to evaluate BRCA1 and BRCA2 protein expression. The immunoreactivity was observed and recorded as nuclear. For nuclear staining, the percentage of positive cells was determined by scoring 100-200 cells in the field at x400 magnification from 10 fields in randomly selected areas. Each tumor was assigned one of the following scores according to the ratio of cancer cells;0=score 1,1-25%=score 2,26-50%=score 3,51-75%=score 4 and >75%=score 5. The expression levels of BRCA1 and BRCA2 proteins were correlated with clinical data obtained from patient files.

With a mean age of 51.75 years, the bulk of patients fell into the 41–50 and 51–60 age categories. While Hedau et al. found a comparatively younger mean age among Indian patients, Honrado et al. and Saha et al. found that breast cancer is more frequent in middle-aged women.[1,6,7] While BRCA2 expression did not substantially correlate with age in the current research ([Table 2]; p=0.091), BRCA1 expression did [Table 1]; (p=0.016).

98.5% of the study population was female, which is in line with other research showing that women are disproportionately affected by breast cancer.[1-4] The sole male patient in our investigation also showed BRCA protein expression, which is consistent with the findings of Honrado et al. This suggests that, while being rare, male breast cancer may have similar molecular features. However, neither BRCA1 nor BRCA2 expression was substantially correlated with gender [Table 1 and 2].[6]

A positive family history of breast cancer was seen in just 14.7% of patients. Hereditary BRCA mutations are more prevalent in patients with a positive family history, especially at younger ages, according to earlier research by Singh et al.[8] However, neither BRCA1 nor BRCA2 protein expression was substantially correlated with family history in the current investigation (Tables 1 and 2), indicating that altered protein expression may also occur in sporadic breast tumours.

Grade II tumours were the most prevalent, followed by Grade III and Grade I tumours, according to histopathological grading. Tumour grade and BRCA protein expression were shown to be significantly inversely correlated. As tumour grade increased, BRCA1 expression declined considerably ([Table 3]; $p<0.01$), with Grade I tumours mostly exhibiting robust expression (4+ and 5+) and Grade III tumours primarily displaying low expression (1+). In a similar vein, higher-grade tumours had considerably reduced BRCA2 expression ([Table 4]; $p<0.01$), with Grade III tumours primarily showing weak or nonexistent staining. These results are consistent with those of Saha et al. and Hussein et al., who found that aggressive, poorly differentiated breast carcinomas had lower BRCA expression.[7,9]

Relatively early-stage illness was indicated by the majority of patients' lack of lymph node involvement. The relationship between lymph node status and BRCA expression was not statistically significant, despite the fact that tumours with little or no lymph node metastases tended to have greater BRCA1 and BRCA2 expression (Tables 1 and 2). Similar findings have been documented by Saha et al., indicating that nodal metastasis may not be accurately predicted by BRCA protein expression alone.[7]

Overall, the current investigation showed that intermediate levels of expression (1+ to 3+) were the most common for BRCA1 and BRCA2 proteins. While age only shown relevance for BRCA1 expression (Table 1), the most notable discovery was the substantial correlation between decreased BRCA1 and BRCA2 expression and rising tumour grade [Table 3 and 4]. These results corroborate earlier findings that BRCA protein expression reduction is linked to tumour growth and might be a helpful predictive indicator for breast cancer.[1,6,7]

CONCLUSION

Breast cancer is the most commonly diagnosed cancer and the leading cause of cancer death among females worldwide. BRCA genes produce proteins essential for repairing damaged DNA. When mutated, these tumor suppressor genes lose their ability to repair DNA, significantly increasing the risk of breast cancer development. Statistical analysis confirms significant correlations between age, tumor grade, and BRCA expression, emphasizing the importance of these factors in understanding breast cancer progression and treatment outcomes.

Limitation of the study: The present study has certain limitations that should be considered while interpreting the findings. First, the study was conducted at a single tertiary care center with a relatively small sample size of 68 cases, which may limit the generalizability of the results to the wider population. Second, evaluating long-term clinical outcomes, disease-free survival, and overall survival in connection to BRCA1 and BRCA2 protein expression is not possible due to the cross-sectional research design. Third, molecular genetic testing for BRCA1/BRCA2 mutations was not done to verify the underlying genetic changes; instead, only immunohistochemistry assessment of BRCA1 and BRCA2 protein expression was carried out. Fourth, there was no correlation found between BRCA expression and other significant prognostic and predictive biomarkers, including oestrogen receptor (ER), progesterone receptor (PR), HER2/neu status, and Ki-67 proliferation index. Larger multicentric prospective trials with molecular analysis and long-term follow-up are necessary since the lack of follow-up data made it impossible to assess treatment responsiveness and the prognostic importance of BRCA1 and BRCA2 expression.

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