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Potential role of BRCA1 protein expression as a prognostic tissue biomarker in breast carcinoma: an immunohistochemical and clinicopathologic study from South India

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ABSTRACT

Introduction. BRCA1 dysfunction is a hallmark of both hereditary and sporadic breast cancer. BRCA1 protein expression can be lost by germline mutation, somatic mutation or promoter hypermethylation. This study aimed to explore BRCA1 dysfunction in breast cancer patients by immunohistochemistry and to study its association with prognostic factors.

Material and methods. BRCA1 protein expression was assessed by immunohistochemistry on formalin fixed paraffin embedded tissue blocks of 110 invasive breast carcinoma patients. Furthermore, the clinical findings and tumor features associated with BRCA1 dysfunction were characterized.

Results. Reduced BRCA1 immunoreactivity was observed in 19% of breast cancer cases. Although these patients presented with aggressive tumor characteristics, statistical significance was observed only with presence of lymphovascular emboli ($p < 0.05$). These results suggest that loss of BRCA1 protein expression is associated with an aggressive phenotype of breast carcinoma.

Conclusions. Immunohistochemistry for BRCA1 protein expression in tumor tissues may provide a less expensive screening tool to identify BRCA1 dysfunction due to genetic or epigenetic alterations.

Key words: breast cancer, BRCA1, biomarker, immunohistochemistry, PARP inhibitor

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Introduction

Breast cancer is a highly heterogeneous disease with different biological behaviors, therapeutic responses, and clinical outcomes among the various subtypes. The highly penetrant breast cancer susceptibility gene *BRCA* was discovered in the early nineties which accounts for almost 60% of hereditary breast cancers [1]. Our knowledge on breast carcinogenesis comprising of morphological, immunohistochemical and molecular characterization has improved ever since the discovery of these genes.

BRCA1 gene is located on chromosome 17q21 which consists of 23 coding exons and encodes a nuclear protein with 1863 amino acids [2]. They are tumor suppressor genes encoding proteins that are essential

in the maintenance of genome stability through repair of double stranded DNA breaks by error free homologous recombination pathway. Therefore, cells that lack BRCA proteins are unable to repair these defects. This deficiency results in the repair of these DNA lesions by potentially mutagenic mechanisms such as non-homologous end joining and single- strand annealing. Several other genes like *EMSY, RAD51C, ATM* and Fanconi anemia genes are also involved in homologous recombination mediated DNA repair. Pathogenic mutation in these genes is also associated with breast and ovarian cancer predisposition [3].

It is important to note that, in addition to germline *BRCA1* gene mutations, BRCA protein deficiency can be seen in sporadic breast cancers due to somatic mutations or epigenetic *BRCA* gene silencing as a consequence of promoter hypermethylation [4]. This concept is referred to as *BRCA*ness where histopathological and molecular features like triple negative phenotype will be similar to *BRCA1/2* germline mutation-related breast cancers. Genetic aberrations in other homologous recombination-related genes could also lead to *BRCA*ness [5].

Individuals are selected for genetic testing based on clinical characteristics where there is a high chance of missing potential germline mutation carriers due to small families, inheritance through unaffected men and development of tumors at an older age. Also genetic testing offered nowadays is time consuming and expensive. Moreover, this does not identify other mechanisms of BRCA protein deficiency. So the need arises to develop and validate new tissue biomarkers for the detection of BRCA dysfunction. Immunohistochemistry is a cost-effective method which can be used as a screening test for the detection of BRCA dysfunction. Only very few studies have been done all over the world depicting loss of BRCA protein expression by immunohistochemistry. Studies using immunohistochemistry for genetic screening has not been conducted in Southern part of India so far.

The purpose of this study was to identify breast cancer patients with BRCA1 dysfunction by immunohistochemistry and to investigate its association with various clinicopathologic factors. This information obtained will help us in elucidating if clinical, morphological and immunohistochemical features could predict BRCA1 dysfunction in breast cancer.

Material and methods

The present study was conducted over a period of one year between March 2019 and March 2020 in a Tertiary Care Center in Kerala, South India after obtaining approval from the Institutional Ethics Committee (PIMSRC/E1/388A/33/2014). This study was composed of 110 women with a diagnosis of breast carcinoma selected from the Department of Pathology of the Institute. Patients with a histopathological diagnosis of invasive breast carcinoma were included in the study. Mesenchymal tumors, lymphomas, prior treatment elsewhere, those with recurrence and patients not consenting for genetic analysis were excluded from the study.

Informed consent was obtained from all the participants involved in the study. Epidemiological data such as age at diagnosis, personal history of cancer, family history of cancer were obtained from these patients using a prestructured questionnaire.

Histopathologic parameters such as tumor subtype and grade were evaluated using H&E stained slides. Tumor grade was assessed using the Nottingham histological score.

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue sections using anti-BRCA1 antibodies (Biogenex, Rabbit polyclonal antibody). Three micrometer-thick sections were obtained on charged slides and incubated at 60–70C for 30 minutes. This is followed by deparaffinization and hydration through descending grades of alcohol. Antigen Retrieval was done with TRIS EDTA buffer for 15–20 minutes. The slides were then rinsed in distilled water and Tris buffered saline 2 minutes each. An endogenous peroxidase blocking agent (3% H_2O_2) was added for 10 minutes on the section. The slides were incubated with primary antibody and were conjugated with streptavidin Horse Radish Peroxidase (HRP). Diaminobenzene tetrahydrochloride (DAB) was used as the chromogen. The slides were counterstained with hematoxylin and examined under the microscopy. The reaction was considered positive if more than 10% of the cells showed distinctive nuclear staining [6]. The stromal cells served as internal positive control as they retain a normal copy of *BRCA1*.Slides without the primary antibody were used as negative control.

Hormone receptor (estrogen and progesterone) expression, HER2/neu overexpression and Ki-67 proliferation were also studied by immunohistochemical staining on formalin fixed paraffin embedded tissues. Estrogen and progesterone receptors were considered positive when $\geq 1\%$ of cell nuclei were positively stained. For Her2/neu testing, only complete circumferential membranous staining in >10% of tumor cells (score of 3) were considered positive. Ki67 was considered high if more than 20% of cells showed positive nuclear staining.

Clinical parameters studied included patient age at initial diagnosis, size of the tumor, status of regional lymph nodes and the number of lesions at the time of diagnosis.

Association between BRCA1 immunohistochemical status and clinicopathological factors were evaluated using Fisher's Exact Test and $\chi2$ test.

Results

Patient characteristics

Women studied were in the age group ranging from 31 to 96 years. Mean age at the time of visit was 55.9 years (SD = 11.3). The highest number of breast cancer cases were in the age group 51–60 years which was around 35% followed by 29% each in the age group 41–50years and above 60 years. Approximately 7% were diagnosed under 40 years of age.

Family history of cancer was seen in six patients. One patient presented with two primary cancers. Ten patients presented with multifocal tumor in the same breast. Axillary lymph node metastasis was noted in 42% of cases. Tumor size more than 2 cm was observed in 75% of cases.

Histopathological characteristics

The predominant histological type was invasive carcinoma of no special type (NST) which was seen in 89% of cases. Histological grading of the tumors was done which revealed 30% of grade 1 tumors, 51% grade 2 tumors and 15% of grade 3 tumors. Metaplastic carcinoma of breast was not graded. Hormone receptor positivity was seen in 68% of tumors. HER2/neu overexpression was observed in 16% of tumors. Seven percent of tumors were triple positive (ER+, PR+, HER2+ve) and 23% of tumors were triple negative (ER-, PR-, HER2-ve). Our study showed a high Ki67 expression in 54% of cases (Tab. 1).

BRCA1 immunohistochemistry findings

Of the 110 breast cancer cases, 19% (21/110) showed loss of BRCA1 expression and 81% (89/110) showed intact BRCA1 nuclear staining (Tab. 2). All the cases showed cytoplasmic positivity. Cases with intact BRCA1 staining showed moderate to strong staining in >10% of tumor nuclei (Fig. 1). Majority of the cases showed strong staining in more than 50% of tumor cells. Cases with loss of BRCA1 expression showed either complete absence of staining or weak staining in < 10% of tumor nuclei (Fig. 2).

Clinical and histopathological characteristics of women with altered BRCA1 expression

Of the 21 breast cancer cases with loss of BRCA1 expression, nine were below 50 years of age. 11 patients with altered BRCA1 expression had axillary lymph

Table 1. Clinical and pathological characteristics of the study participants

Parameters	Number of cases (n = 110)	Percentage
Age at initial diagnosis		
< 40 yrs	9	7%
41–50 yrs	31	29%
51–60 yrs	39	35%
> 61 yrs	31	29%
Family history	6	5%
Lymph node metastasis	46	42%
Histological grade		
Grade 1	33	30%
Grade 2	56	51%
Grade 3	16	15%
Histological type		
Invasive breast carcinoma of no special type (NOS)	89	80%
Invasive carcinoma with medullary features	4	3.6%
Invasive lobular carcinoma	3	2.7%
Metaplastic carcinoma	4	3.6%
Mucinous carcinoma	3	2.7%
Invasive papillary carcinoma	2	1.8%
Apocrine carcinoma	1	0.9%
Others (mixed tumors)	3	2.7%
Tumor size		
< 2 cm	82	75%
> 2 cm	28	25%
Lymphovascular emboli	27	25%
Estrogen receptor (ER)		
Positive	75	68%
Negative	35	32%
Progesterone receptor (PR)		
Positive	71	65%
Negative	39	35%
HER2 overexpression		
Positive	9	22.5%
Negative	31	77.5%
Ki67 expression		
High	59	54%
Low	51	46%

node metastasis. Fifteen patients presented with tumor size more than 2 cm. None of the cases had family history of cancer. The histological type seen in 20 cases with altered BRCA1 expression was invasive carcinoma of no special type. Among the cases with

Table 2. Proportion of breast cancer patients with reduced BRCA1 expression

BRCA1 protein expression	Number of cases (n = 110)	Percentage
> 10% (Retained)	89	81%
< 10% (Loss)	21	19%

NORMAL BRCA1 IMMUNOREACTIVITY
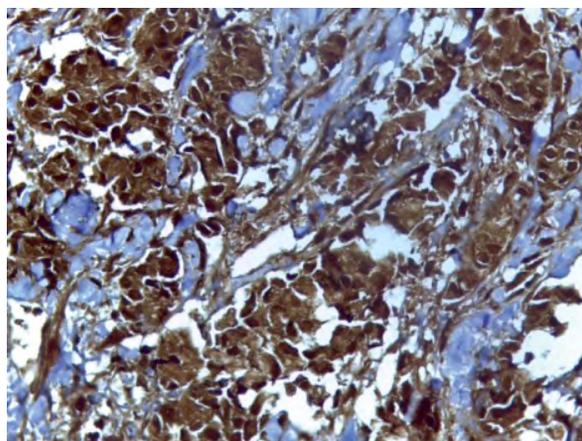
Figure 1. Retained BRCA1 protein expression in tumor nuclei, 40× (normal)

ALTERED BRCA1 IMMUNOREACTIVITY
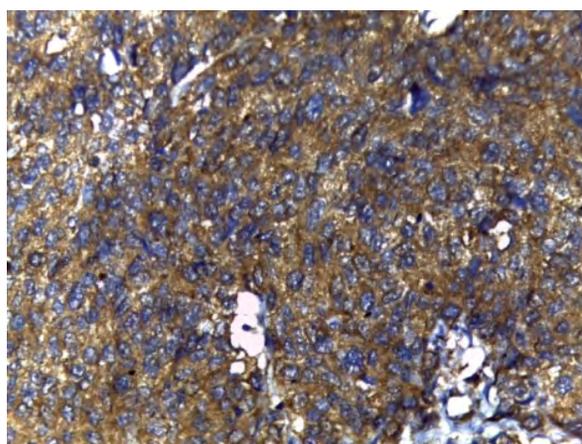
Figure 2. Loss of BRCA1 protein expression in tumor nuclei, 40× (abnormal)

loss of BRCA1 expression, 14 were grade 2 tumors and 5 grade 3 tumors. Triple negative breast cancer was observed in six cases and Her2/neu overexpression in two cases. Lymphovascular emboli was observed in 9 cases. Ki 67 expression was high in sixteen cases with altered BRCA1 expression.

Statistical significance

It was observed that presence of lymphovascular emboli showed association with loss of BRCA1 expression statistically ($p \leq 0.05$). All other clinicopathologic variables (family history, number of lesions, histological grade, stage, hormonal receptor and HER2/neu expression, Ki67) were not found to be statistically significant.

Discussion

Breast cancer is the most commonly diagnosed cancer in females with an incidence of 2.3 million cases, representing 11.7% of all cancer cases and 6.9% of all cancer related deaths in 2020 [7]. The state of Kerala in South India has been seeing a rise in the number of breast cancer cases over the last few years [8]. Moreover, a good number of cases in India are seen in younger age groups when compared to women in western countries [9]. This stress the importance in identifying the cause for the current trend in the state. Genetic predisposition is one of the reasons for early onset breast cancer which is usually aggressive in nature. *BRCA1* is the most commonly mutated gene in hereditary breast cancer. Genetic testing for germline mutation is not routinely done in most Indian centers due to the high cost involved. This has urged the need to identify and validate new tissue biomarkers for prognostic and therapeutic purposes. The role of BRCA1 protein in tumor tissues can be investigated in these patients by immunohistochemistry where genetic testing cannot be performed. Immunohistochemistry is a cost effective, easy to perform laboratory method to assess the expression of various proteins in tumor tissues for diagnosis, localization and detection of dysfunctional proteins. Several studies have also reported other mechanisms such as somatic mutation and promoter hypermethylation for reduced BRCA protein expression in tumor tissues [10].

The benefit of identification of BRCA gene mutations has been well established over the years. Novel targeted therapies such as Poly-(ADP) ribose polymerase inhibitors (PARPi) and platinum based chemotherapeutic agents have been developed for BRCA associated cancers [11]. Olaparib and talazoparib are the PARP inhibitors currently approved for treatment in patients with advanced breast cancer associated with germline BRCA mutation [12]. Although the use of PARP inhibitors is currently restricted to germline BRCA mutated breast cancers, trials are underway evaluating its role in the management of breast cancers exhibiting BRCAness phenotype and homologous recombination deficiency [13]. Recent studies have shown promising results regarding the use of PARP inhibitors in sporadic breast cancers with BRCA dysfunction.

Large number of studies have been done on BRCA gene mutation in breast cancer worldwide. However, BRCA1 protein expression in tumor tissues of breast cancer patients is less known. This study was therefore undertaken to assess the expression of BRCA1 protein in female breast cancer patients from Kerala and to investigate its association with clinical and pathological factors. The aim of this study was to detect BRCA1 dysfunction and identify tumor characteristics relating to dysfunction in formalin-fixed paraffin-embedded tissues.

In the current study, we identified 19% of women with altered BRCA1 protein expression in tumor tissues. Majority of women showed intact BRCA1 staining in the tumor tissues. We observed both nuclear and cytoplasmic staining in all the cases. Our results also demonstrated that loss of BRCA1 expression was associated with aggressive tumor characteristics. The breast carcinomas with reduced BRCA1 expression were high grade tumors. Seventyone percent of cases with altered expression had large tumor size. Triple negative phenotype was observed in 29% of tumors with altered expression. Another interesting observation was that 43% of women with reduced BRCA1 expression were below 50 years of age.

According to a recent study by Israa A Hussein et al. [14], BRCA1 protein expression was reduced in 79.5% which is quite high when compared to our study. They demonstrated a significant relationship of BRCA status with advanced stage, higher grade of the tumor and hormone receptor negativity. Priyadarshini et al. reported absence of BRCA1 staining mostly in tumors of large sizes and with higher histologic grades [15]. These findings are in line with our study.

Deepti Verma et al. observed a significant association of reduced BRCA1 expression with HER2/neu positivity. We observed HER2/neu overexpression in 10% cases with reduced expression. In addition, they also showed association of altered expression with large tumor size and high-grade tumors which was statistically significant [16]. These findings are similar to the observations seen in our study.

Another study done in Portugal where BRCA1 immunohistochemistry was done using monoclonal antibodies and was correlated with BRCA1/2 genetic screening results. This study showed loss of BRCA1 expression in 80% of cases with germline BRCA1 mutation indicating high specificity for the prediction of BRCA1 carriers with immunohistochemistry using monoclonal antibodies [17]. Different types of antibodies for BRCA1 proteins are commercially available at present. Controversies regarding the subcellular localization of BRCA1 have been existing for the last few years. Formalin fixed paraffin embedded sections of breast cancer showed a variety of staining patterns ranging from predominantly nuclear, both nuclear and cytoplasmic and mainly cytoplasmic. This variability in the subcellular localization of BRCA1 protein could be due to the specificity of the antibodies used in various studies to detect the protein. In our study we used a polyclonal antibody which showed both nuclear and cytoplasmic positivity.

Kazuaki Miyamoto et al. [4] observed reduced BRCA1 immnoreactivity in 62% of sporadic breast cancers where none of the cases harbored BRCA1 mutations thereby showing other mechanisms like promoter hypermethylation as the cause for the reduced expression. Another study by Hedau et al also observed a decline in the protein expression of BRCA1 in 50% of sporadic breast cancer cases [18].

Wen-Ying Lee [19] reported a higher incidence of loss of BRCA1 nuclear expression in younger women with breast cancer which was seen to be associated with large tumor size and high proliferation rate. This observation is consistent with our study findings where 43% of the cases with reduced BRCA1 expression were below 50 years.

Rakha et al. [20] showed complete loss of BRCA1 nuclear expression in 15% of breast cancer cases which was correlated with high-grade, advanced lymph node stage, larger size, vascular invasion, negative estrogen and progesterone receptor.

Similar findings were also observed in a Japanese study by Yoshikawa et al. [21] where 28% of sporadic breast cancer cases also showed reduced BRCA1 expression in addition to 79% of *BRCA1* associated breast cancers.

Several studies have been done on BRCA1 protein expression in ovarian cancers also in the past. According to a study by J. L. Meisel et al. [22], BRCA1 immunohistochemistry was found to be abnormal in 36% of ovarian cancers of which 52% was due to germline mutation and the remaining due to somatic mutation and promoter hypermethylation. Two other similar studies done by Karuna Garg et al. and Tarinee Manchana et al. on ovarian cancer patients also showed loss of BRCA1 expression by immunohistochemistry in 47% and 20% of cases [23, 24].

Therefore, our study demonstrated BRCA1 dysfunction in tumor tissues of a subset of breast cancer cases which was seen to have tumor characteristics like higher grade, high proliferative index, large tumor size and presence of lymphovascular emboli. Reduction of BRCA1 protein expression may be considered as an additional prognostic factor. Our study indeed has limitations as we were unable to obtain the mutation status in these patients. It is imperative to conduct large scale studies to assess the clinical usefulness of immunohistochemistry as an alternative to the more expensive molecular testing especially in low resource settings and also to select patients likely to benefit from targeted therapies.

Conclusions

In summary, immunohistochemistry is a promising tool in detecting loss of BRCA1 protein expression which could be due to genetic or epigenetic alterations. Reduced or loss of BRCA1 protein expression plays a significant role in the development of breast cancer. Majority of the cases with loss of protein expression presented with aggressive tumor characteristics. These findings indicate that there are tumor characteristics which suggest the presence of BRCA1 dysfunction in breast cancer patients. Thus, knowledge of BRCA1 expression in tissues could provide additional clinically relevant information in breast cancer patients.

Ethical approval and consent to participate

The study was done after obtaining approval from the Institutional Ethics Committee (No.PIM-SRC/E1/388A/33/2014) of Pushpagiri Institute of Medical Sciences, Tiruvalla, Kerala, India.

Informed consent to participate in the study has been taken from all the study subjects.

Consent for publication

Consent has been taken from the participants for publishing their clinical details and other relevant data in journals.

Availability of data and materials

All data generated or analyzed during this study are included in this article for publication.

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Authors' contributions

Dr Reeba Mary Issac: data collection, manuscript writing — original draft preparation, investigation, software. Dr Prema Saldanha: conceptualization and design of the work, methodology, critical revision of the article. Dr Jessy M.M: conceptualization and design of the work, supervision, review and editing, validation. Dr Rebecca Mathews: methodology. Dr Bindu Kumari: methodology. Dr Tiju Chacko: methodology, writing — review and editing.

Conflict of interest

The authors have declared no conflicts of interest.

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References

1. Lima ZS, Ghadamzadeh M, Arashloo FT, et al. Recent advances of therapeutic targets based on the molecular signature in breast cancer: genetic mutations and implications for current treatment paradigms. J Hematol Oncol. 2019; 12(1): 38, doi: 10.1186/s13045-019-0725-6, indexed in Pubmed: 30975222.
2. Takaoka M, Miki Y. BRCA1 gene: function and deficiency. Int J Clin Oncol. 2018; 23(1): 36–44, doi: 10.1007/s10147-017-1182-2, indexed in Pubmed: 28884397.
3. Stoppa-Lyonnet D. The biological effects and clinical implications of BRCA mutations: where do we go from here? Eur J Hum Genet. 2016; 24 Suppl 1: S3–S9, doi: 10.1038/ejhg.2016.93, indexed in Pubmed: 27514841.
4. Miyamoto K, Fukutomi T, Asada K, et al. Promoter hypermethylation and post-transcriptional mechanisms for reduced BRCA1 immunoreactivity in sporadic human breast cancers. Jpn J Clin Oncol. 2002; 32(3): 79–84, doi: 10.1093/jjco/hyf020, indexed in Pubmed: 11956301.
5. Domagala P, Hybiak J, Cybulski C, et al. BRCA1/2-negative hereditary triple-negative breast cancers exhibit BRCAness. Int J Cancer. 2017; 140(7): 1545–1550, doi: 10.1002/ijc.30570, indexed in Pubmed: 27943282.
6. Kim D, Jung W, Koo JaS. The expression of ERCC1, RRM1, and BRCA1 in breast cancer according to the immunohistochemical phenotypes. J Korean Med Sci. 2011; 26(3): 352–359, doi: 10.3346/jkms.2011.26.3.352, indexed in Pubmed: 21394302.
7. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021; 71(3): 209–249, doi: 10.3322/caac.21660, indexed in Pubmed: 33538338.
8. Breast and cervical cancers on the rise in Kerala, says Health Ministry- The New Indian Express. https://www.newindianexpress.com/states/kerala/2019/dec/21/breast-and-cervical-cancers-on-the-rise-in-kerala-says-health-ministry-2078944.html.
9. Malvia S, Bagadi SA, Dubey US, et al. Epidemiology of breast cancer in Indian women. Asia Pac J Clin Oncol. 2017; 13(4): 289–295, doi: 10.1111/ajco.12661, indexed in Pubmed: 28181405.
10. Secord AA, Berchuck A, Cerami E. Cohort of Ovarian Carcinomas. 2014; 37(1): 138–146.
11. Urbina-Jara LK, Rojas-Martinez A, Martinez-Ledesma E, et al. Landscape of Germline Mutations in DNA Repair Genes for Breast Cancer in Latin America: Opportunities for PARP-Like Inhibitors and Immunotherapy. Genes (Basel). 2019; 10(10), doi: 10.3390/genes10100786, indexed in Pubmed: 31658756.
12. Dutil J, Teer JK, Golubeva V, et al. Germline variants in cancer genes in high-risk non-BRCA patients from Puerto Rico. Sci Rep. 2019; 9(1): 17769, doi: 10.1038/s41598-019-54170-6, indexed in Pubmed: 31780696.

13. Keung MY, Wu Y, Vadgama JV. PARP Inhibitors as a Therapeutic Agent for Homologous Recombination Deficiency in Breast Cancers. J Clin Med. 2019; 8(4), doi: 10.3390/jcm8040435, indexed in Pubmed: 30934991.

14. Hussein IA, Ahmed STh, Hameedi AD, et al. Immunohistochemical Expression of BRCA1 Protein, ER, PR and Her2/neu in Breast Cancer: A Clinicopathological Study. Asian Pac J Cancer Prev. 2020; 21(4): 1025–1029, doi: 10.31557/APJCP.2020.21.4.1025, indexed in Pubmed: 32334465.

15. Dehuri P, Kanungo S. Utility of evaluation of P53 and BRCA1 in invasive breast cancers: An immunohistochemical study. Indian J Pathol Oncol. 2019; 6(1): 123–127, doi: 10.18231/2394-6792.2019.0022.

16. Verma D, Agarwal K, Tudu SK. Expression of breast cancer type 1 and its relation with expression of estrogen receptors, progesterone receptors, and human epidermal growth factor receptor 2/neu in breast carcinoma on trucut biopsy specimens. Indian J Pathol Microbiol. 2018; 61(1): 31–38, doi: 10.4103/IJPM.IJPM_393_16, indexed in Pubmed: 29567881.

17. Vaz FH, Machado PM, Brandão RD, et al. Familial breast/ovarian cancer and BRCA1/2 genetic screening: the role of immunohistochemistry as an additional method in the selection of patients. J Histochem Cytochem. 2007; 55(11): 1105–1113, doi: 10.1369/jhc.7A7209.2007, indexed in Pubmed: 17625228.

18. Hedau S, Batra M, Singh UR, et al. Expression of BRCA1 and BRCA2 proteins and their correlation with clinical staging in breast cancer. J Cancer Res Ther. 2015; 11(1): 158–163, doi: 10.4103/0973-1482.140985, indexed in Pubmed: 25879355.

19. Lee WY. Frequent loss of BRCA1 nuclear expression in young women with breast cancer: an immunohistochemical study from an area of low incidence but early onset. Appl Immunohistochem Mol Morphol. 2002; 10(4): 310–315, doi: 10.1097/00129039-200212000-00004, indexed in Pubmed: 12607598.

20. Rakha EA, El-Sheikh SE, Kandil MA, et al. Expression of BRCA1 protein in breast cancer and its prognostic significance. Hum Pathol. 2008; 39(6): 857–865, doi: 10.1016/j.humpath.2007.10.011, indexed in Pubmed: 18400253.

21. Yoshikawa K, Honda K, Inamoto T, et al. Reduction of BRCA1 protein expression in Japanese sporadic breast carcinomas and its frequent loss in BRCA1-associated cases. Clin Cancer Res. 1999; 5(6): 1249–1261, indexed in Pubmed: 10389907.

22. Meisel JL, Hyman DM, Garg K, et al. The performance of BRCA1 immunohistochemistry for detecting germline, somatic, and epigenetic BRCA1 loss in high-grade serous ovarian cancer. Ann Oncol. 2014; 25(12): 2372–2378, doi: 10.1093/annonc/mdu461, indexed in Pubmed: 25281711.

23. Garg K, Levine DA, Olvera N, et al. BRCA1 immunohistochemistry in a molecularly characterized cohort of ovarian high-grade serous carcinomas. Am J Surg Pathol. 2013; 37(1): 138–146, doi: 10.1097/PAS.0b013e31826cabbd, indexed in Pubmed: 23232854.

24. Manchana T, Tantbirojn P, Pohthipornthawat N. immunohistochemistry for screening of mutation in epithelial ovarian cancer patients. Gynecol Oncol Rep. 2020; 33: 100582, doi: 10.1016/j.gore.2020.100582, indexed in Pubmed: 32529018.