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RESEARCH ARTICLE

Expression of BRCA1 mRNA in cancerous and non-cancerous breast tissue of Bangladeshi females attending a tertiary care hospital

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Abstract

Background: Dysfunctions of the BRCA1 (BReast CAncer gene1) gene are reported in cancerous and non-cancerous breast lesions. BRCA1 mRNA expression is variable in breast cancer, while this information is still inadequate in non-cancerous breast disorders. This study aimed to measure BRCA1 mRNA expression in cancerous and non-cancerous breast tissue of Bangladeshi females.

Methods: The cross-sectional study was conducted on 50 breast cancer and 19 non-cancerous females. RNA was extracted from formalin-fixed paraffin-embedded breast tissue. Real-time RT-PCR was done for measuring BRCA1 mRNA. BRCA1 mRNA expressions in cancerous and non-cancerous breast tissue were compared and analysed.

Results: BRCA1 mRNA expression was reduced or absent in most of the cancerous and non-cancerous (consisting of fibroadenoma, fibrocystic disease, ductal hyperplasia and normal breast tissue) breast tissue. Expression of BRCA1 in breast cancer and fibroadenoma was almost similar statistically. All cancers were invasive ductal carcinoma and of grade II, and most of them were sporadic (86%). BRCA1 expression was not associated with reproductive or cancer-related characteristics except consanguinity of marriage. The non-cancerous females were younger than the cancer patients (33.5 versus 43 years, respectively).

Conclusion: The study suggests the necessity of bringing fibroadenoma patients, in addition to breast cancer patients, into the screening programme and analysing the molecular profile because their BRCA1 expression is similar.

Key messages

BRCA1 mRNA expression was reduced or absent in most cancerous and non-cancerous breast tissue. Its expression was similar in both breast cancer and fibroadenoma of the breast. BRCA1 expression may be used for screening of fibroadenoma, fibrocystic disease and ductal hyperplasia of breast to prevent their progression to cancer.

Introduction

Breast cancer is one of the leading causes of cancer-related death worldwide; new cases and breast cancer -induced deaths are more rapidly increasing in the low-and-middle-income countries than in developed countries, and about 6.9% of cancer deaths are reported worldwide due to this cancer [1]. Genetic mutations, environmental factors, obesity and lifestyle changes are associated with the occurrence and prognosis of breast cancer. The incidence of this cancer in Asia is rising substantially due to increased life expectancy along with population growth and acceptance of the Western lifestyle [2, 3].

Mutations in the BRCA1 gene are found to be associated with breast cancer, and the frequency of these genetic mutations varies among ethnic groups and countries [2]. Methylation in BRCA1 promoter region, low expression, and copy number deletions also cause deficiency in BRCA1 and produce similar phenotypic features of tumours due to BRCA1 mutations [4, 5]. Dysfunction of this gene is associated with ovarian, prostatic, gastric and pancreatic cancers in addition to breast cancer [6, 7].

BRCA1 is a DNA repair gene. Its mRNA is expressed at the late G1/early S phase of the cell cycle before DNA synthesis. Expression of the BRCA1 protein closely follows its mRNA. Deficiency in this gene produces low expression of BRCA1 mRNA. Recent studies have shown that BRCA1-related breast cancers have clinico-pathological features that are usually associated with a poor prognosis, such as high -grade oestrogen receptor (ER) and progesterone receptor (PR) negative status, and over expression of the Her-2-Neu [8]. Studies also found that the expression of BRCA1 mRNA influences the effectiveness of chemotherapy and helps in the prediction of survival of the patients [9].

Other than breast cancer, BRCA1 gene mutations are found to be present in some benign non-cancerous breast lesions. Among the breast pathologies, most of the breast lesions are benign. A survey of literature from 1985 to 2019 found around 50% of clinical breast lesions were mastalgia and fibrocystic disease, and 25% were fibroadenomas [10]. Benign breast diseases are evidenced as a risk factor for breast cancer, and the risk is higher with BRCA1 mutations [11].

Although high-income countries have achieved significant progress toward curing women with breast cancer, Bangladesh, like other low-and-middle-income countries, is now starting to recognise the extent and severity of the disease [3]. Breast cancer is the second most common cancer irrespective of gender in Bangladesh, and it is the leading cancer (29.3%) in females, according to a hospital-based cancer registry report [12]. Among the non-cancerous breast lesions, fibroadenomas are also more frequently reported in females of Bangladesh [13]. In this prevailing condition, there is still a paucity of information regarding the molecular characteristics of these breast disorders, including the expression of BRCA1 mRNA in Bangladeshi females.

The aim of this study was to measure the expression of BRCA1 mRNA in cancerous and non-cancerous breast tissue of Bangladeshi females. Thus, this research can provide the opportunity to improve the understanding of the molecular behaviour of breast cancer as well as non-cancerous breast lesions in this population for adopting appropriate therapeutic measures and predicting the prognosis of these diseases.

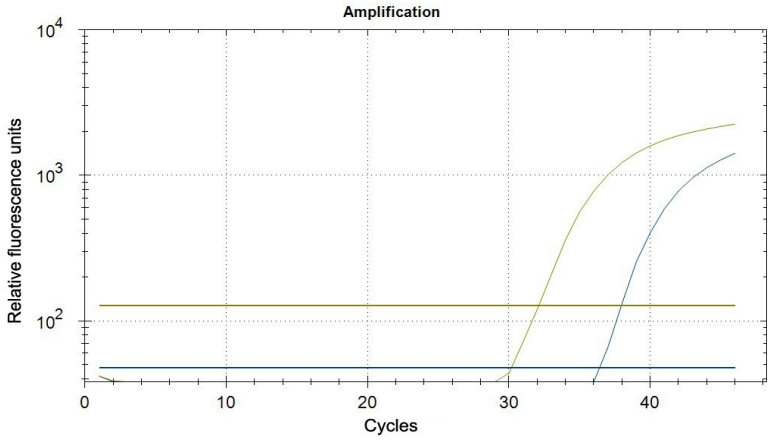

Figure 1 **Amplification curve of BRCA1 (blue) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (green) in log scale of a formalin-fixed paraffin-embedded breast cancer tissue sample**

Methods

This was a cross-sectional study carried out in the Department of Anatomy, Bangabandhu Sheikh Mujib Medical University (BSMMU) from January to December 2022. Molecular tests were done in the Department of Immunology and Molecular Biology laboratory of the National Institute of Cancer Research and Hospital (NICRH), Mohakhali, Dhaka. A memorandum of understanding between the authorities of the concerned departments of BSMMU and NICRH was signed for this research.

Bangladeshi females with breast cancer and benign breast disorders attending NICRH participated in this research. The names and mobile numbers of the histologically diagnosed breast cancer patients and non-cancerous females with benign breast lesions from January 2021 to September 2022 were collected from the register of the Department of Histopathology of NICRH. Inclusion criteria were Bangladeshi female, aged 18 years or above, (i) for breast cancer patient: histologically diagnosed as breast cancer patients; (ii) for females with benign breast disorders: histologically diagnosed as non-cancerous patients with benign breast lesions. Exclusion criteria were having a history of other cancers and chemo or radiotherapy before FFPE block preparation.

Formalin-fixed paraffin-embedded (FFPE) cancerous and non-cancerous breast tissue blocks were collected from the Department of Histopathology of NICRH. A list of 915 breast cancer patients and 126 females with non-cancerous breast lesions was obtained during this period. Among them, 62 breast cancer patients (based on recently prepared tissue blocks) and 27 non-cancerous females (out of 68 available FFPE tissue blocks which had sufficient breast tissue) with benign breast lesions were invited to participate in this study. Fifty (50) breast cancer

patients and 19 non-cancerous females were finally selected from them based on quality and quantity of cancerous and non-cancerous breast tissue in the paraffin blocks (assessed by the histopathologist). Reproductive and cancer-related characteristics were collected from the participants through interviews and from hospital and medical records. BRCA1 mRNA expression in cancerous and non-cancerous breast tissue was measured and compared.

Isolation of RNA

The RNA extraction was done from four (10 µm thick) sequential sections of FFPE breast tissue using a commercial RNA extraction kit (PureLinkTM FFPE total RNA isolation kit, invitrogen by Thermo Fisher Scientific, USA) according to the manufacturer's instruction. The quality and quantity of the extracted RNA was checked by Eppendorf BioPhotometerTM D30 (Eppendorf AG, Germany).

Synthesis of cDNA

The first-strand cDNA synthesis was done by reverse transcription of RNA using Viva cDNA synthesis kit (Vivantis Technologies, Malaysia) according to the manufacturer's protocol. Random hexamer primers were used for cDNA synthesis.

Amplification of cDNA by real-time PCR

Template cDNA was amplified using the CFX96 Bio-Rad Touch Real-Time PCR (Bio-Rad Inc., USA). At first, 18 µl of the reaction mixture was prepared by adding 10 µl of Hot Star Taq Plus Master Mix (Qiagen, USA), 1µl of forward and 1 µl of reverse primers of each of the BRCA1 and GAPDH genes, 1 µl of probes of BRCA1 and 1 µl of probes of GAPDH gene and 2 µl of nuclease -free water. GAPDH was used as a reference gene. Then PCR mixture was prepared by adding 2µl of cDNA and 18 µl of reaction mixture. After that, amplification was performed in a 96-well optical plate at 95°C for 5 minutes, followed by 45 cycles at 94°C for 30 sec, 60°C for 30 sec and 72°C for 1 minute. Real-time PCR was done to measure the expression of these genes. In this study primers and probes for the wild BRCA1 and GAPDH genes were adopted from Egawa *et al.* [14]. The primers and probes were as follows: BRCA1 forward primer 5′-ACAGCTGTGTGGTGCTTCTGTG-3′, reverse primer 5′-CATTGTCCTCTGTCCAGGCATC-3′ and probe FAM-CATCATTCACCCTTGGCACAGGTGT-3′. GAPDH forward primer 5′-TCATTGACCTCAACTACATGGTTT-3′ and reverse primer 5′- GAAGATGGTGATGGGATTTC -3′ and probe JOE-CAAGCTTCCCGTTCTCAGCC-TAMRA.

Table 1 BRCA1 mRNA expression status in cancerous and non-cancerous breast tissue

Types of breast tissue	BRCA1 expression status, number (%)	
	Expressed (n=16)	Not expressed (n=34)
Cancerous tissue (n=50)	16 (32.0)	34 (68.0)
Non-cancerous tissue (n=19)	7 (36.8)	12 (63.2)
Fibroadenoma	3 (16.0)	7 (37.0)
Fibrocystic disease	1 (5.0)	1 (5.0)
Ductal hyperplasia	-	1 (5.0)
Fibrocystic disease with ductal hyperplasia	1 (5.0)	3 (16.0)
Normal breast tissue	2 (10.5)	-

Real-time PCR analysis for the expression of BRCA1mRNA

The expression of the BRCA1 gene was calculated from the quantified cycle threshold (Ct) value. For each sample, the Ct values of BRCA1 and GAPDH were determined (Figure 1). The ∆Ct of BRCA1 was obtained by the formula ∆Ct = Ct of BRCA1 - Ct of GAPDH to calculate the difference. Gene expression level in a given sample was represented as $2^{-\Delta\Delta Ct}$.

Data analysis

Statistical analysis was done using SPSS, version 25, 2017. Expression levels of BRCA1 mRNA in cancerous and non-cancerous breast tissue were compared using the *t* test and, Mann-Whitney U test, as appropriate. The association between BRCA1 mRNA expression status and reproductive and cancer-related characteristics was determined using Chi-square or Fisher's Exact test, as appropriate. All statistical tests were two-sided, and a $P<0.05$ was considered statistically significant.

Ethical concerns

The study was conducted after ethical approval was obtained from the Institutional Review Board of BSMMU and NICRH. Informed written consent was taken from the participant before data collection. Participation in this study was entirely voluntary, and the participants were assured that they would maintain the confidentiality and anonymity of data and that they would have the right to withdraw their participation at any time. The sample was taken from the previously prepared FFPE tissue blocks; thus, there was no chance of injury during sample collection. Permission was obtained from the participants to publish the findings anonymously in seminars, workshops, or journals.

Results

BRCA1 mRNA was expressed in 16 (32%) breast cancer patients in reduced amounts and seven (37%) non-cancerous females. The non-cancerous breast tissue consists of fibroadenoma, fibrocystic disease, ductal hyperplasia and fibrocystic disease with ductal hyperplasia and normal breast tissue. Among the non-cancerous females, BRCA1 expression was observed in three patients (out of 10) with fibroadenoma in reduced amounts, two with normal breast tissue and the others with fibrocystic disease of the breast (Table 1). BRCA1 mRNA expression level ($2^{-\Delta\Delta Ct}$ value) was not significantly different in the cancerous and non-cancerous breast tissue (Figure 2).

Association between the expressions of BRCA1 mRNA in breast cancer tissue and selected reproductive and cancer-related characteristics were analysed (Table 2). BRCA1 mRNA expression was not associated with reproductive and cancer-related characteristics except for consanguinity of marriage ($P=0.03$). Association between the expression of BRCA1 and the histological type and grade of breast cancer was not done, as all breast cancers were in invasive ductal carcinoma and of grade II.

In this study, the majority of the breast cancers were diagnosed at or below the age of 45 years. Most of the cancers were sporadic or non-familial (86%), family history of cancers was reported in seven

patients. Lymph node metastasis was present in almost all patients (96%), whereas distant metastasis was reported in 4 (8%) patients. Hormone receptor negativity was reported in most of the patients (Table 2).

Reproductive characteristics of the breast cancer patients and females with non-cancerous benign breast disorders were not statistically different except the age; the non-cancerous females were relatively younger (mean age 33.5 years) than the cancer patients (mean age 43 years). A comparison of the reproductive characteristics of the participants is shown in Table 3.

Discussion

In this research, the BRCA1 mRNA expression status of breast cancer patients and females with non-cancerous breast lesions was not statistically different. The probable reason might be the presence of 10 fibroadenoma patients out of 19 non-cancerous females. Malignant transformation of fibroadenoma is very rare; only about 100 cases of breast cancer have been reported to arise from this benign lesion worldwide [15]. Burnetti *et al.* found a BRCA1 mutation in a patient with fibroadenoma, which progresses to triple-negative breast cancer [16]. Another case was reported in the USA where carcinoma in situ progressed from multiple bilateral fibroadenomas with BRCA1 mutation [17]. Mutation in the BRCA1 gene is responsible for reduced or absent expression of BRCA1 mRNA [18]. This finding of our research signifies the importance of analysing the mutation profiles of the BRCA1 gene in fibroadenoma patients in addition to breast cancer.

BRCA1 protein is required for the prevention of breast transformation. BRCA1 expression status in non-cancerous benign breast lesions indicates the malignant potential of the breast. Non-expression or reduced expression of this gene in non-cancerous benign lesions requires close monitoring and follow-up, as inactivation of BRCA1 causes blunted ductal development, breast hyperplasia and tumour formation [19]. We have few non-cancerous patients other than fibroadenoma. They were histologically diagnosed as fibrocystic disease, ductal hyperplasia and the concomitant presence of fibrocystic disease with ductal hyperplasia. Non-expression of BRCA1 mRNA was observed more in patients with concomitant presence of fibrocystic disease with ductal hyperplasia in our study. This finding also warns us to keep close monitoring of the patients with non-cancerous benign breast disorders.

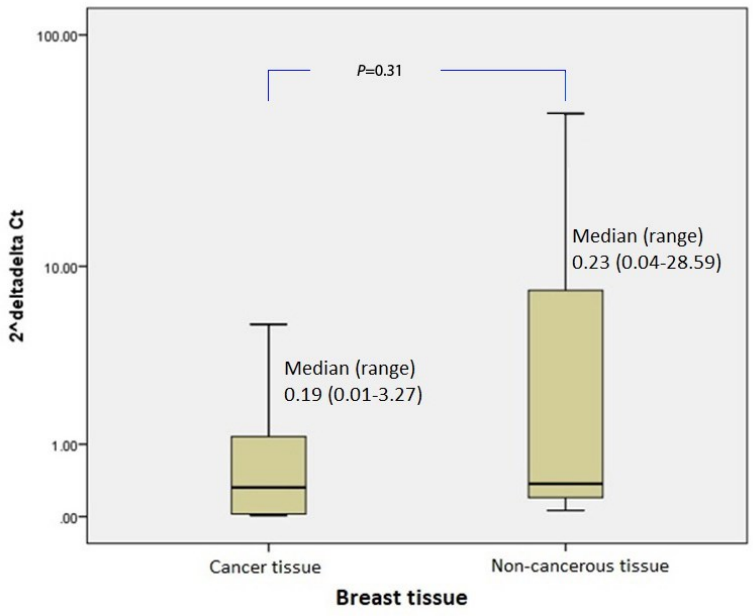

Figure 2 BRCA1 mRNA ($2^{-\Delta\Delta Ct}$ value) expression level in cancerous and non-cancerous breast tissue

Table 2 Association between the expression of BRCA1 mRNA and reproductive and cancer-related characteristics of the breast cancer patients (n=50)

Reproductive and cancer-related characteristic	BRCA1 expression status, number (%)		*P*
	Expressed (n=16)	Not expressed (n=34)	
Age at diagnosis (years)			
28–45	8 (50.0)	26 (76.5)	0.06
46–69	8 (50.0)	8 (23.5)	
Body mass index (kg/m²)			
18.5–24.9	11 (68.8)	19 (55.9)	0.39
≥25	5 (31.2)	15 (44.1)	
Age at menarche (years)			
12–13	15 (93.8)	26 (76.5)	0.24
14–15	1 (6.2)	8 (23.5)	
Age at menopause (years) (n=19)			
37–44	2 (25.0)	5 (45.5)	0.63
45–58	6 (75.0)	6 (54.5)	
History of consanguinity of marriage			
Yes	4 (25.0)	1 (2.9)	0.03
No	12 (75.0)	33 (97.1)	
Number of children			
1–2	11 (68.8)	26 (76.5)	0.73
3–5	5 (31.2)	8 (23.5)	
Duration of breastfeeding (years)			
1–2	4 (25.0)	7 (20.6)	0.73
>2	12 (75.0)	27 (79.4)	
Duration of contraceptive use (years)			
Never used	1 (6.2)	6 (17.6)	0.21
1–5	7 (43.8)	19 (55.9)	
≥6	8 (50.0)	9 (26.5)	
Family history of cancer			
Positive	1 (6.2)	7 (20.6)	0.41
Negative	15 (93.8)	27 (79.4)	
Lymph node metastasis			
Present	15 (93.8)	33 (97.1)	0.54
Absent	1 (6.2)	1 (2.9)	
Other organ metastasis			
Present	1 (6.2)	3 (8.2)	0.99
Absent	15 (93.8)	31 (91.8)	
Her-2/Neu (n=49)			
Positive	5 (33.3)	9 (26.5)	0.62
Negative	10 (66.7)	25 (73.5)	
Oestrogen receptor (n=49)			
Positive	4 (26.7)	5 (14.7)	0.43
Negative	11 (73.3)	29 (85.3)	
Progesterone receptor (n=49)			
Positive	3 (20.0)	5 (14.7)	0.69
Negative	12 (80.0)	29 (85.3)	

Table 3 Reproductive characteristics of the participants

Reproductive characteristic	Breast cancer patient (n=50)	Non-cancerous female (n=19)	*P*
Age (years)[a]	43.0 (9.5)	33.5 (10.0)	0.001
Body mass index (kg/m^2)[a]	24.4 (3.5)	24.2 (3.0)	0.98
Age at menarche (years)[a]	12.8 (0.8)	13.0 (0.7)	0.80
Menstrual status, n (%)			
Menstruating	31.0 (62.0)	16.0 (84.2)	0.13
Postmenopausal	19.0 (38.0)	3.0 (15.8)	
Age of menopause (years)[a]	45.2 (4.6)	43.3 (5.7)	0.63
Consanguinity of marriage, n (%)			
Yes	5.0 (10.0)	1.0 (5.3)	0.99
No	45.0 (90.0)	18.0 (94.7)	
Number of children[b]	2.0 (1.0–5.0)	2.0 (1.0–3.0)	0.56
Total duration of breast feeding (years)[b]	3.0 (1.5–8.0)	3.0 (2.0–6.5)	0.79
Use of contraceptives, n (%)			
No	7.0 (14.0)	4.0 (21.0)	0.48
Yes	43.0 (86.0)	15.0 (79.0)	
Duration of contraceptive use (years)[b]	5.0 (1.0–20.0)	6.0 (1.0–14.0)	0.28

[a]Mean and standard deviation; [b]Median and range

In our study, BRCA1 was expressed in 32% of breast cancer patients. Al Mullah *et al.* observed BRCA1 mRNA expression in 21% of EEPE tissue block. They also observed that the reduced or no expression of BRCA1 mRNA was more in the high histological grade of the tumour [20]. Taylor *et al.* found reduced expression of BRCA1 in non-familiar breast cancer and non-expression in 20% of non-familiar and 20% of familial breast cancer patients. They conducted the research on 142 non-familiar, 25 familial breast cancer patients and 28 non-cancerous females with fibrocystic disease, fibroadenoma and normal breast tissue [21]. In their study, mutation was reported in breast cancer patients with a family history of cancers and loss of heterozygosity (LOH) was reported in non-familial patients. If the normal functional allele of BRCA1gene is lost in LOH, this gene will exhibit non-expression. Silvia *et al.* reported 47% of LOH in the BRCA1 region (17q21) in patients with breast cancer [22]. In addition to LOH, hypermethylation is another cause of non-expression of the BRCA1 gene in breast cancer. The CpG islands of the promoter regions of tumour suppressor genes are usually unmethylated, and hypermethylation or abnormal methylation of these sites causes non-expression of those genes [23]. Hypermethylation at these sites of BRCA1 causes the inactivation of this gene, known as LOH. Esteller *et al.* found hypermethylation in the promoter region of the BRCA1 gene in 13% of primary breast cancer. That study also noticed abnormal methylation was more common in patients with breast cancer at or below the age of 45 years, and it was present in grade II or III invasive ductal carcinoma [24]. Therefore, considering the cancer-related characteristics of our participants, we can assume abnormal methylation or LOH might be a cause of reduced or non-expression of BRCA1 mRNA in our patients.

Taylor *et al.* did not find any correlation of BRCA1 expression with age, menopausal status, hormonal status, histological type and tumor size [21]. Li *et al.* also did not find any association between BRCA1 mRNA expression and clinicopathological characteristics of the breast cancer patients [5]. Our finding is consistent with the findings of these literature.

In our study association was found betweenBRCA1 mRNA expression and breast cancer patients with family history of consanguinity. The progeny of the consanguineous couples has more chance to get homozygous alleles. Thus, the frequency of genetically determined diseases in offspring of consanguineous parents is influenced by the homozygosity of the genes responsible for that conditions [25]. The frequency of breast cancer among the daughters of consanguineous parents varies in different populations. Studies on Pakistani, Croatian and North American population found that the younger women of parents with first-cousin marriages have increased risk of developing breast cancer, whereas, several studies conducted on Arab, North African and non-Jew Israeli population found low incidence of breast cancer in daughters of consanguineous parents [26–28]. Medimegh *et al.* conducted a study on Tunisian breast cancer patients and did not find homozygosity in BRCA1 haplotypes in consanguineous patients with familial breast cancer. They reported more BRCA1 haplotypes homozygotes in healthy consanguineous controls and more heterozygotes in non-consanguineous group. They explained their findings by the facts that there were three possible genotypes in consanguineous families: the first- without mutations gave healthy progeny, the second- with deleterious mutations at heterozygote state and caused breast cancer and the third- the deleterious mutations at homozygote state which was lethal [25]. Mice model studies proved the lethal effects of BRCA1 knockout homozygotes [29], [30]. Moreover, a computer simulated study on the consequences of long term practice of consanguineous marriage on the prevalence of lethal cancer genes found that mutation carrier rate of BRCA1/2 genes decreased six times faster in a highly consanguineous population than non-consanguineous population if spontaneous abortion and gene flow were considered to be absent [31]. From the above-mentioned findings we can assume that the patients from consanguineous parents have factors other than BRCA1 gene responsible for tumourogenesis.

Estimation of BRCA1 expression in breast cancer is important for selection of effective chemo and targeted therapy. Studies found that the cancer cells deficient of BRCA1 or BRCA2 genes are more sensitive to PARP1 inhibitors whereas the cells with normal BRCA expression are unaffected by these drugs [32]. Estimation of BRCA1 expression is required for BRCA replacement therapy.

Limitations

Relatively small number and heterogeneity of non-cancerous females with benign breast disorders may reduce the strength of interpretation of BRCA1 expression status in non-cancerous breast tissue.We found 126 females with non-cancerous benign breast lesions and 915 breast cancer patients in the register of the Department of Histopathology, NICRH during this period. Usually surgery is not done in patient with non-cancerous breast lesions unless it is highly susceptible to malignancy. FNAC (fine needle aspiration cytology) or core biopsy is done for

diagnosis. Redundancy operation of breast is also very low in Bangladesh. Therefore, getting normal breast tissue block is less likely. Inspite of this limitation, we used FFPE tissue blocks, because gene expression status due to somatic and germ line mutations is yielded from the tissue. We included available all (68) FFPE blocks of non-cancerous breast lesions, 27 of which had sufficient amount of breast tissue and ultimately genetic analysis was possible from 19 samples. Among the breast cancer patients, all cancers were invasive ductal carcinoma and of grade II, thus comparison of BRCA1 expression in different types and histological grades was not possible.

Conclusion

This study revealed similar type of BRCA1 expression in breast cancer and fibroadenoma patients. This finding indicates the importance to bring the fibroadenoma patients under screening to prevent progression of cancer by appropriate and timely intervention. This study also suggests more research for analyzing the molecular profile to find out the LOH and methylation pattern of promoter region of BRCA1 gene other than mutations of breast cancer patients as well as fibroadenoma even though it is marked as a benign breast disease.

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Author contributions

Conception or design of the work; or the acquisition, analysis, or interpretation of data for the work: LN, SHZR, FA, ZAY, LN, UHL, SSA, FA. *Drafting the work or reviewing it critically for important intellectual content:* LN, SHZR, FA, ZAY, LN, UHL, SSA, FA. *Final approval of the version to be published:* LN, SHZR, FA, ZAY, LN, UHL, SSA, FA. *Accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved:* LN, SHZR, FA.

Conflict of interest

We do not have any conflict of interest.

Data availability statement

We confirm that the data supporting the findings of the study will be shared upon reasonable request.

Supplementary file

None

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