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Original Article

A Study of *BRCA1* Gene Exon 2 Mutation in Bangladeshi Female Breast Cancer Patients

Jayasree Basu[1]*, Zinnat Ara Yesmin[3], Taslima Hossain[2], Rayhan Shahrear[4]

[1]*Assistant Professor(cc), Department of Anatomy, National institude of ENT, Dhaka*

[2]*Associate Professor, Department of Anatomy, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka*

[3]*Assistant Professor, Department of Histopathology, National institude of ENT, Dhaka*

[4]*Assistant Professor, Department of Anatomy, Ibrahim Medical College, Dhaka*

***Corresponding Author:** Dr. Jayasree Basu, E-mail: jayasreetuli15@gmail.com

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ABSTRACT

Background: Breast cancer is the most common malignancy and leading cause of death among women in Bangladesh. Mutations in BRCA genes increase the risk for breast cancer. A large number of distinct mutations and polymorphisms in the *BRCA1* gene have been reported worldwide, a frameshift mutation in *BRCA1* gene exon 2 (185delAG) is one of the commonly reported mutations. Therefore, the study was planned to determine the frequencies of mutation and polymorphisms in *BRCA1* gene exon 2. **Materials and Methods:** A cross-sectional descriptive type of study was done on 100 adult Bengali Bangladeshi female patients with ductal carcinoma of breast of age range between 25 to 70 years by 'selection checklist.' Genomic DNA was isolated from the peripheral blood samples. Amplification of the desired sequence of *BRCA1* exon 2 was checked in gel and then digested with restriction endonuclease enzyme (*Hinf*I). The fragments obtained were analyzed on a gel and photographed under UV light. Sanger sequencing was done on 10 blood samples for confirmation. Sequenced data was analyzed by Geneious Software version 11. **Results:** The onset of cancer was predominantly below 50 years and 44% of patients did not experience menopause as they developed cancer at a younger age. Gel photographs after enzyme digestion showed wild-type bands of 149bp in all samples. The chromatogram reveals wild-type sequence of exon 2 of *BRCA1* gene. **Conclusions:** *BRCA1* mutation status helps us in the cancer risk prediction, selection of therapeutic management and genetic counseling of the patients and families. Therefore, it is necessary to study the whole *BRCA1* gene in our population.

INTRODUCTION

Breast cancer is the second most frequent cancer in overall after lung cancer; 2 million new cases were reported in both sexes worldwide[1]. Incidence and mortality rate were highest in Asia[1], due to noticeable percentage of patients presented as locally advanced or at metastatic stage at the time of diagnosis. In Bangladesh, breast cancer is the most frequently occurring cancer in women approximately 27.4% among female cancers [2] and about 1363 new breast cancer cases were diagnosed in 2014.

Breast cancers may be hereditary or sporadic. Approximately 15–40% of breast cancers are hereditary[3] and occur due to mutation in the *BRCA1*, *BRCA2*, *TP53* and *CHEK2* genes. A germ-line mutation in *BRCA* genes have a lifetime risk of developing breast cancer by 80 to 90% and ovarian cancer by 20 to 50%[4]. Therefore, the identification of breast cancer susceptibility genes is essential to understand the pathogenesis of breast cancer.

BRCA1 gene is located on chromosome 17 (17q12-21), composed of 22 coding exons that code for a 220kd protein[5] of 1863 amino acids[6]. More than 1536 distinct germ-line mutations, polymorphisms and variants have been found in *BRCA1* gene. The c.68-69delAG (BIC: 185delAG) mutation is one of the most common and ancient mutations in the exon 2 occurs in codon 23, resulting in stop codon formation in position 39, which leads to premature termination of polypeptide chain and significant shortening of protein[7]. Study of many countries of Asia also reported 185delAG mutation including in a country close to Bangladesh like India[8]. The increasing incidence of breast cancer demands to find out the genetic basis of breast cancer on Bangladeshi perspective. Several genetic tests are available for the study of *BRCA1* gene exon2. Restriction Fragment Length Polymorphism (RFLP) is a less expensive and less time-consuming procedure that will help patients, families and the government in healthcare management. Several studies

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described the methods of screening of *BRCA1* gene exon 2 by RFLP[5,9].

MATERIALS AND METHODS

The cross-sectional descriptive study was carried out in the Department of Anatomy, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka from March 2017 to February 2018 after getting approval from Institutional Review Board (IRB) of BSMMU. All patients were diagnosed with ductal carcinoma of breast and Informed consent was obtained from each hundred (100) patients. Genetic analysis of *BRCA1* gene exon 2 mutation and polymorphisms were done only in blood samples where sufficient concentration of the DNA was available. Among 100 blood samples 14 are not in working state. Genetic analysis was done as per protocol of genetic research laboratory of BSMMU.

DNA Isolation

Peripheral blood samples (3 ml) were collected and genomic DNA was extracted by using ReliaPrep™ Blood gDNA isolation kit as per manufacturer's instruction. The quantity of the DNA was measured by Nanodrop spectrophotometer.

Amplification

Targeted region was amplified by conventional PCR with the primers (Table 1). Both primers (forward primer and reverse primer) were 171 bp that included both flanking regions (at 5′ end and 3′ end) of exon2 of *BRCA1* gene, where exon 2 is 99 bp in length. Primers were designed by using Primer3Plus software. PCR amplification was performed with 35 cycles in thermal cycler C1000TM-Bio-Rad.

The amplified product was checked by 0.5% agarose gel. After staining by ethidium bromide, the gel was visualized under UV light. The photographs were taken by gel documentation system Bio-Rad (Figure 1).

Restriction Analysis

For PCR-RFLP, purified products were then be digested with restriction endonuclease enzyme *Hinf*I. Restriction digestion was performed in Thermal cycler C1000TM-Bio-Rad at 37°C for 5 hours. The PCR fragments obtained after enzyme digestion were analyzed in 2% agarose gel stained with ethidium bromide. Resolved band was then photographed under UV light and photographed by gel documentation system Bio-Rad.

Sanger Sequencing

The RFLP data analysis was followed by Sanger sequencing of ten family history positive blood samples. After purification with ethanol precipitation, direct sequencing of DNA was done according to the standard protocol provided with the BigDye Terminator Kit® V 3.1 in ABI 3500 Genetic Analyzer. Sequenced data were analyzed by Geneious Software version R 11.

Ethical Implication

The present study was carried out after getting formal approval from the IRB of BSMMU. The study was conducted with the permission of proper authority of the hospitals. All selected breast cancer patients were informed that their DNA samples were used for research purpose only and written consents were obtained from all the patients. All patients were treated equally and with respect at the time of research. They also were informed that they had the right to refuse to participate in or withdraw their names from the study at any time. Each patient was given a special ID number for safeguarding confidentiality and protecting anonymity.

RESULTS

In the present study, The mean age of the patients was 45.55 (±11.22) years and 71% of the patients had the onset of breast cancer before 50 years of age (Figure 2).

Gel electrophoresis revealed that wild-type bands were present in all the samples analyzed (Figure 3). No mutation was found in blood samples. All the samples on restriction digestion with HinfI gave rise to one fragment of size 149 bp and 22 bp bands as per recognized sequence of restriction enzyme. 22 bp bands were small, so it cannot be detected in the 2% gel.

For further query genetic analysis was done by direct DNA sequencing of ten family history positive blood samples. Sequence chromatogram analysis after alignment of samples sequence with reference sequence showed the presence of ten wild-type sequences (normal allele) within 43124017 to 43124115 region of chromosome 17. No pathogenic variation was detected and all the sequence gave wild-type peak in the chromatogram (Figure 4). This indicates the

Table 1. The sequence of oligonucleotide primers and relevant information

Primer	Oligonucleotide sequence	Primer size	Primer Position	GC %	T^m (c)	Product size
FP	GAAGTTGTCATTTTATAAACCTTT	24	43124178	25	55	171 bp
RP	CTGACTTACCAGATGGGACAC	21	43124008	52.38	61.3	

Forward primer, FP. Reverse primer, RP

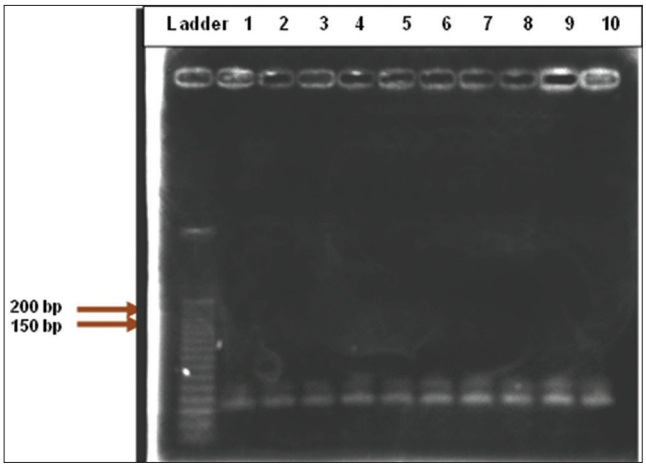

Figure 1. Gel Electrophoresis of the PCR Products on 0.5% Agarose Gel. The desired product size (171 bps) of the amplicons is shown in specific bands from lane 2 to 8 in comparison with standard 50 bps DNA ladder in lane 1

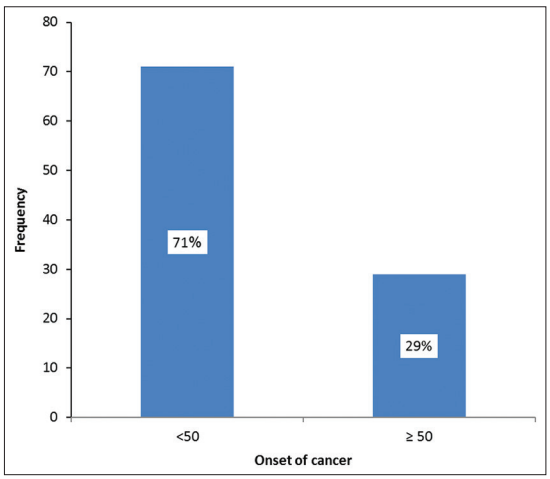

Figure 2. The frequency of onset of cancer in adult Bangladeshi female breast cancer patients (n= 100)

wild-type sequence of BRCA1 exon 2 in adult Bengali Bangladeshi breast cancer patients.

DISCUSSION

There is an established fact that Asian breast cancer patients are, on average, younger than European[10]. In the present study, the mean age was 45.55 (±11.22) and the majority (71%) had the onset of cancer before 50 years, which is similar to another study[11]. Well known reproductive factors (age at menarche, menopause and first pregnancy, breastfeeding, parity) not found to be strongly associated with the breast cancer burden in Bangladesh. We observed that the incidence of postmenopausal breast cancer is comparatively higher (56%), which was similar to study[11].

Various studies had revealed that highly penetrant mutant *BRCA1* constitute a very high risk of breast cancer[12]. In PCR-RFLP procedure, the wild-type allele is normally cut by 'HinfI'. 'HinfI' cuts the 171 bp amplicon giving rise to two fragments size of 149 bp and 22 bp. The mutant allele

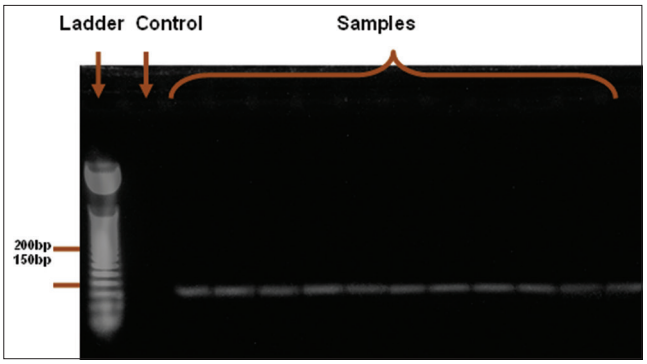

Figure 3. Representative Ethidium bromide-stained agarose gel photograph of enzyme digestion product Here, Lane 1 is a 50bp DNA ladder, lane C- Negative control and other lanes loaded with samples show digested band (wild-type band). Here HinfI cuts the 171bp amplicon of exon 2 giving rise to two fragments of size 149bp and 22bp (not visualized in gel)

was not digested with HinfI enzyme, because cutting site is destroyed. Thereby, the 185delAG allele was intact when treated with the restriction enzyme giving rise to a fragment of size 171bp in homozygous mutation and three bands of 171, 149 and 22 bp in heterozygous mutation, as per sequence of exon 2. In the present study, all the samples on restriction digestion with HinfI gave rise to two fragment size of 149bp and 22bp bands. 22bp bands were small, so it cannot be detected in the 2% gel. So, the results obtained in gel electrophoresis revealed that wild-type bands were present in all the samples analyzed. None of the 86 patients carried the 185delAG mutations.

The present research does not find mutation of *BRCA1* exon 2 in Bengali Bangladeshi female patients of breast cancer, which supports the study from Delhi where Sharma et al. reported that the 185delAG in *BRCA1* was not seen in any of the participants[13]. The 185delAG mutations in *BRCA1* gene were not found in any of the 231 histologically confirmed breast cancer patients by Chakraborty et al[8]. In these two studies, the population is more similar to our study population as they share same ethnicity (Bengali) and many socio-cultural factors and the incidence of *BRCA1* exon 2 mutations appeared to be similar to present study.

This was followed by Sanger sequencing of ten family positive breast cancer patients. Chakraborty et al. also confirmed the absence of mutation by Sanger sequencing[8]. Only family positive cases were selected because inherited mutations in high penetrance *BRCA* genes closely correlated with an increased risk of breast cancer in different ethnic and age groups[14].

Findings of the present study suggested that *BRCA1* 185delAG mutation may not have a strong recurrent effect on breast cancer among the Bengali Bangladeshi population. There might be a mutation in the other exon or non-coding region and other coding regions of *BRCA1* and the existence of other breast cancer susceptibility genes namely *BRCA2*, p53 and PTEN. Also, mutations maybe influenced by the geographical and ethnic origins of the population studied.

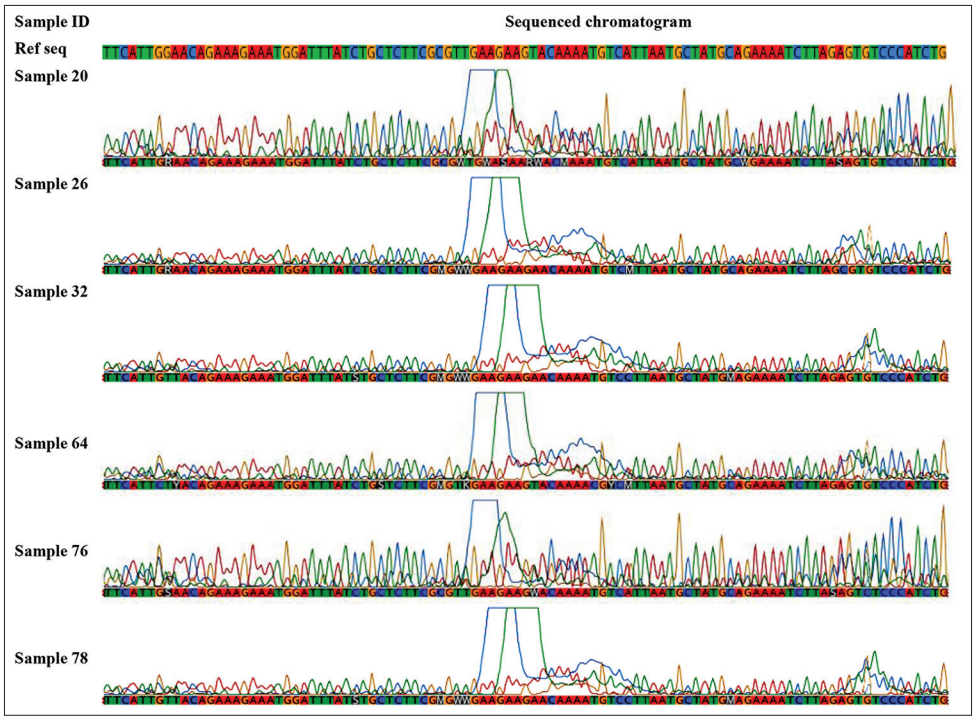

Figure 4. Sequenced chromatogram revealed wild-type sequence (normal allele) in the samples. The upper panel shows the reference sequence of exon2 from NCBI database.

CONCLUSION

In conclusion, the research sequenced was a relatively shorter region of *BRCA1* gene in a limited number of study subjects. Only exonic mutation is targeted in the research, no intronic mutation is studied. That might reduce the probability of finding gene mutation in the study sample. But the findings of present research highlight the importance of screening in a larger cohort of Bangladeshi population in order to explore the variant pattern of these genes and to develop a mutation database for the purpose of accurate interpretation of genetic predisposition to breast cancer.

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CONFLICT OF INTERESTS

There were no potential conflicts of interest with respect to the research, laboratory procedure.

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