Asian Pacific Journal of Cancer Nursing
Asian Pacific Journal of Cancer Nursing, 20250806
Research Articles/ Original Work

Mutation in Exon 7 of BRCA1 Gene in Bangladeshi Women with Triple Negative Breast Cancer

Prianka Saha	Department of Biochemistry, TMSS Medical College, Bogura, Bangladesh.
Tonmoy Burmon	Department of Transfusion Medicine, Shaheed Ziaur Rahman Medical College, Bogura, Bangladesh.
Maskura Benzir	Department of Anatomy, TMSS Medical College, Bogura, Bangladesh.
Monzarin Mahal	Department of Biochemistry, Dhaka Medical College, Dhaka, Bangladesh.
Afia Shahnaj	Department of Biochemistry, Dhaka Medical College, Dhaka, Bangladesh.
M. Hafizur Rahman	Department of Biochemistry, Dhaka Medical College, Dhaka, Bangladesh.
Md. Kaoser Bin Siddique	TMSS Health Research Foundation, TMSS Grand Health Sector, TMSS, Bogura, Bangladesh. TMSS Nursing College, Bogura, Bangladesh.

Background: Triple negative breast cancer (TNBC) is an invasive subtype of breast cancer associated with various risk factors. It has poor prognosis due to lack of targeted therapies. About 60-80% of patients carrying BRCA1 gene mutations have TNBC phenotype and the frequency of BRCA1 mutation is 10-15% in TNBC. In Bangladesh, very few studies are conducted regarding this occurrence and it needs further evaluation. Our research aim is to identify the mutation in exon7 of BRCA1 gene in Bangladeshi women with TNBC.

Method: This cross-sectional descriptive study was carried out in the Department of Biochemistry, Dhaka Medical College, Dhaka in collaboration with Institution for Population and Precision Health, The University of Chicago, USA from January 2022 to December 2022. Total thirty-four (34) Bangladeshi female TNBC patients were selected. Two ml venous blood was collected from arm vein, kept in Ethylenediaminetetraacetic acid (EDTA) containing tubes, preserved at -20 degree C and sample was sent to the University of Chicago on Dry ice. DNA library was prepared by using Twist Library Preparation EF Kit 2.0. For mutation detection, Next-generation sequencing method was used.

Result: The mean ± SD age of patients was 43.71±9.84 years and BMI was 23.95±3.38. About 52.9% of female had history of taking contraceptive methods and 70.6% patients were multiparous, remaining 14.7% were nulliparous and 14.7% were primiparous. 5.9% patients had positive family history and No mutation was found in exon 7 of BRCA1 gene. The frequency of mutation was 0.0%, as this study population was relatively small.

Conclusions: This result suggests that Bangladeshi women suffering from TNBC have no mutation in exon 7 of BRCA1 gene. A large-scale investigation is needed to establish these findings.

Introduction

Breast cancer is identified as a complex disease, including a heterogeneous group of tumours [1]. It

is the second most prevalent cancer of women in the world. The incidence of breast cancer constitutes 23% of all cancer patients and also account for 14% of cancer related death [2]. The prevalence of breast cancer is also high in Bangladesh and it accounts of 32.8% for last five years [3]. Breast cancer is not only a single disease but also a multifaceted disease comprised of various biological subtypes and diverse natural history. It presents a spectrum of clinical, pathological and molecular features with different prognostic and therapeutic values. Breast cancer is classified into four subtypes based on immunohistochemistry (IHC) profile. The IHC classification correlates well with intrinsic gene expression microarray categorization; Luminal A, Luminal B, Her-2 positive and Triple negative breast cancer [4].

Triple negative breast cancers (TNBC) share a typical gene expression pattern; it shows the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 expression [5]. It accounts for about 10 to 20% of all cases of breast cancer [6]. It is more aggressive subtypes of breast cancer with higher risk of spreading [7]. It is significantly associated with large tumor size, younger age at onset, high grade and a worse prognosis than other subtypes of breast cancer [1]. The exact etiology of breast cancer is not clearly understood. Different interrelated factors, such as genetics, hormones, environment, sociobiology etc can influence breast cancer development [8]. Genetic factors play a vital role in the initiation, promotion, and progression of mammary neoplasia [9]. The prevalence of BRCA1 mutations has been 10 to 15% in TNBC patients. The BRCA1 gene is located in the long arm of chromosome 17 and has 24 exons which encode 1863 amino acids [10]. BRCA1 genes have more than 600 mutations, including deletion, insertion, frame-shift, duplication, disruption of splice site, and many single nucleotide substitutions in the coding and non-coding sequence [11].

TNBC risk factors are an early age at the onset of the disease, multiparity, higher premenopausal BMI and positive family history of breast and/or ovarian cancer. Age at menarche, menopause are not associated with TNBC risk [12, 13].

The incidence and prevalence of breast cancer are higher in developed countries, but the mortality is more in developing nations. This is due to lack of basic knowledge on cancer, poor health facilities, unavalible access to treatment, late screening and detection [8].

Early detection of breast cancer and timely starting treatment has an important role in reducing the mortality and improving the prognosis of disease [14]. Latest breakthroughs in molecular biology and immunotherapy, very specific targeted therapies have contributed to more efficient and specific treatment protocol in breast cancer patients [15]. But triple negative breast cancer is a challenge in clinical practice because of lack of therapeutic target in comparison with other hormone receptor positive breast cancer [16] .

The exact relationship between the BRCA1 gene and Triple Negative Breast Cancer requires to be further evaluation [7].There is very little research on the Bangladeshi population that uses BRCA mutations. It is now necessary to determine the genetic basis of Triple negative breast cancer from a Bangladeshi perspective because the incidence of TNBC is also increasing day by day.

This study is designed to identify any mutation in exon 7 of the BRCA1 gene in Bangladeshi women with Triple Negative Breast Cancer (TNBC). The identification of mutation in exon 7 of BRCA1 gene in Bangladeshi women with TNBC is important for several reasons. First, it highlights the need for genetic testing and counselling of women with TNBC, especially in high-risk populations. Few Indian studies showed novel mutation in exon 7 of BRCA1 gene in breast cancer patient. Second, understanding the specific genetic alterations that contribute to TNBC in Bangladeshi women can lead to the development of targeted therapies that can improve the outcome of this aggressive form of breast cancer.

Detection of frequency of mutation in exon 7 of BRCA1 gene in Bangladeshi women with triple negative breast cancer may help to understand the mechanisms behind this association and to

develop effective therapies for this population.

Materials and Methods

A cross-sectional descriptive study included 34 diagnosed TNBC patients (age 25-65 years) selected from the department of surgical Oncology, Bangabandhu Sheikh Mujib Medical University (BSMMU) and department of Radiotherapy, Dhaka Medical College (DMC) in January- December, 2022 by purposive sampling technique. 34 patients were diagnosed breast cancer by histological examination. In all cases, the diagnosis of triple negative breast cancer was confirmed by immunohistochemistry. For each patient, a detailed questionnaire was completed.

After taking informed written consent, two millilitres of venous blood was collected from the arm vein of each patient with all aseptic precautions. Blood was collected in EDTA containing tube before starting chemotherapy or radiotherapy for each patient. Then test tubes were labelled for identification and blood was preserved at -20 degree C. Whole Blood was sent to the University of Chicago on Dry ice for the subsequent DNA extraction and sequencing.

DNA was extracted using Quick-DNATM MiniprepKit [17]. Quality control and concentration was measured by Nanodrop 1000. For the targeted DNA sequencing, custom designed Twist Next Generation Sequencing Kit [18] was used.

Library preparation: Enzymatic fragmentation was done to get a fragment size of 180-220 base pair. Then Twist Universal Adapters were ligated on both ends. Then the ligated DNA fragment was PCR amplified using Unique Dual-Indexed (UDI) Primer mix. The library size and concentration was measured by using Bioanalyzer.

Target enrichment: We generated the library with a unique pair of index for 34 samples. We combined all 34 library to make a dual indexed library pool. Then we hybridized the pool with the custom designed probes. After hybridization, streptavidin beads (magnetic) were added to the hybridization pool. Streptavidin would only attach to the double stranded amplified enriched library. These enriched library were then captured and purified by magnetic beads and the other DNA fragments were washed away. Then only enriched library was PCR amplified again. This is the final product for sequencing which is again checked on Bioanalyzer mainly to check the base pair sizing. For concentration, we used Quntus. Based on the concentration and the fragment size, we calculated the nano-molar concentration for the pooled amplified library.

Final enriched library

The DNA sequencing was done on illumina platform using Miseq instrument. A V3 chemistry and appropriate flow cell was used. Miseq Reporter software was used to process the base cell data and generate BAM file after aligning the sequencing reads against human reference genome hg19. We obtained high quality reads -90.7% of the reads were of >=Q30 score.

Mutation analysis was done by microarray technique.

Results

The study findings are systematically presented through descriptive tables and illustrative figures. Demographic characteristics demonstrated that participants had a mean age of 43.71 ± 9.84 years and mean BMI of 23.95 ± 3.38 kg/m², with ages ranging from 25 to 65 years (Table 1, Figure 1).

Variable	Mean ± SD
Ages (years)	43.71 ± 9.84
BMI (kg/m2)	23.95 ± 3.38

Table 1. Demographic Profile of the Study Subjects (N=34).

Figure 1. Histogram Showing the Frequency of Patients in Different Age Group (years) among the Study Subjects.

Contraceptive history analysis revealed that 52.9% (n=18) of Bangladeshi TNBC patients had utilized contraceptive methods, with oral contraceptives being the most prevalent (47.1%), followed by implant and injectable methods (2.9% each), while 47.1% reported no contraceptive use (Table 2).

Contraceptive used	Frequency(n)	Percentage (%)
Used	18	52.9
Implant	1	2.9
Injectable	1	2.9
Oral	16	47.1
Not used	16	47.1

Table 2. Contraceptive Used by the Study Subjects (N=34).

Reproductive history indicated that the majority of participants were multiparous (70.6%, n=24), while nulliparous and primiparous women each comprised 14.7% of the cohort (Table 3).

Parity	Frequency (n)	Percentage (%)
Nullipara	5	14.7
Primipara	5	14.7
Multipara	24	70.6

Table 3. Obstetrics History of the Study Subjects (N=34).

Family history assessment revealed that only 5.9% (n=2) of participants had a positive family history of breast cancer, with 94.1% reporting no familial predisposition (Table 4).

Family history of breast cancer	Frequency(n)	Percentage (%)
Yes	2	5.9
No	32	94.1

Table 4. Family History of Breast Cancer of the Study Subjects (N=34).

Genetic analysis of the BRCA1 gene showed no detectable mutations or variants among the study population (Table 5).

Total Patients	Number of variants	Percentage (%)
34	No variants	0.00%

Table 5. Number of Variants of Study Subjects.

The BRCA1 gene structure, including exons and introns, was visualized (Figure 2), and nucleotide

base composition was color-coded with adenine (red), cytosine (blue), guanine (yellow), and thymine (green) for comprehensive genomic analysis (Figure 3).

Figure 2. Exons and Introns of BRCA1 Gene (411196.3-41277.4KBps).

Figure 3. Nucleotide bases of BRCA1 gene (41244807-41245003).

Discussion

The present study was undertaken to detect mutation in exon 7 of BRCA1 gene in Bangladeshi women with Triple Negative Breast Cancer by using next-generation DNA sequencing method. For this purpose, thirty four female triple negative breast cancer patients attended in Radiotherapy department of Dhaka Medical College Hospital and General Surgery (with Surgical Oncology) department of BSMMU Hospital, Dhaka were selected for sample being confirmed with history of disease and laboratory investigation reports. In the present study, the mean ± SD age of patients was 43.71±9.84 years. Lee et al., 2011 showed that mean ± SD at diagnosis of triple negative breast cancer patients was 41±0.049 which favours this study. Another study also showed mean age of triple negative breast cancer patients was 48 years [13] which is slightly higher than this current study.

In case of all patients, the height and weight were measured to obtain BMI before collection of samples. In this current study, the mean ± SD of BMI was 23.95±3.38 kg/m^2 in all subjects. TNBC patients had higher premenopausal BMI than other subtypes of breast cancers and showed the mean ± SD of BMI was 28.4±2.4 kg/m^2 which has much similarity with current study [12]. Multiparity was a risk factor for TNBC [19]. In this current study, (24/34) 70.5% of study subjects were multipara. So, multiparity has strong association with TNBC. Another study was conducted where almost 75.38% patients with TNBC were multiparous [20]. So it is almost favourable with this present study.

Over use of oral contraceptives led to increased risk of TNBC [21]. There are no association between oral contraceptives and TNBC [12]. So, studies of contraceptives use had mixed results. In this current study, 52.9 % patients used different types of contraceptives and also 47.1% patients had no contraceptives history. So, this current study had slightly higher percentages of contraceptives used than not used of any method which supports Baranska et al., 2022 [21].

Family history was an important factor for breast cancer. A positive family history of breast/ovarian cancer had germline BRCA1 mutaion in 21% cases [22]. But, in this present study, due to a very small study subjects there were only (2/34) 5.9% subjects had positive family history. About 4.3% of breast cancer patients have positive family history in Bangladeshi population [23]. But, in contrast, frequency of positive family history was higher (47.5%) in Taiwanese population [24].

In this present study, sequencing of exon 7 of BRCA1 gene was carried out by next generation sequencing of all blood samples. Out of thirty four (N=34) cases, no mutation was detected which showed percentage of mutation was (0.0%).

In this study, there was no mutation in exon 7 of BRCA1 gene. But other exons like exon 10,11,12 showed so many varients respectively. This result was similar with another study which was conducted by Duzkale and Kandemir. 2021 [25]. They also found variation in exon 10,11,12 but did not in exon 7 of BRCA1 gene. The germline BRCA1 and BRCA2 deleterious mutations and variants of unknown clinical significance associated with breast/ovarian cancer: a report from North India selected total 206 cases and found 45 BRCA1 positive patients. Among them one novel mutation was identified in exon 7. Their mutation was detected in one case (3.2%) [26].

From the result of the study, it is observed that, frequency of mutation in exon 7 of BRCA1 gene is quite different to other studies which were conducted in different ethnic groups of the world in different time. Mutation characteristics, types, clinical significance may vary in different ethnic groups. So, it is required to further investigate the mutation in exon 7 of BRCA1 gene in Bangladeshi women with Triple Negative Breast cancers in a large scale.

Acknowledgements

The authors would like to thank Dr. Muhammad Kibriya, Research Associate & Professor, Dr. Farzana Jasmine, Research Associate & Assistant Professor and Dr. Habibul Ahsan, Dean, Institution for population and Precision Health, The University of Chicago, Chicago, USA. This study also acknowledge support received from all the post graduate students, group members, faculties, clinicians of Dhaka Medical College and all the staff of Biochemistry Department of Dhaka Medical College for their support in conducting this study. The authors would also like to acknowledge Rtn. Dr. Md. Matiur Rahman, Chief of TMSS Grand Health Sector and the colleague of department of Biochemistry, TMSS Medical College, Bogura for their cordial support towards research and publication. The authors are grateful to all the participants who provided valuable data for this study.

Declarations

Author Contributions Statement

PS – Concept, Data Collection and preparation of data, primary processing of the material and their verification. PS, MM, AS, MHR – Overall supervision, Statistical processing and analysis of the material, writing the text of the article (material and methods). PS, TB, MB – Writing the text of the article (introduction, result, discussion). PS, MM, AS, MHR – Concept, design and control of the research, approval of the final version of the article, PS, MKBS – revised whole manuscript and finalized. All authors approved the final version of the manuscript.

Funding

This research was supported by minimal institutional funding, which covered only transportation costs and questionnaire printing expenses.

Ethics approval and consent to participate

This study strictly followed the ethical guidelines by Bangladesh medical research council 2014 and the Helsinki Declaration 2013 (revised) involving human participants in research. The design of the study, data collection procedure, presentation of the data and citation comply with the standard Committee on Publication Ethics (COPE) guideline.

Institutional review board statement

Ethical clearance and permission has been obtained from Dhaka Medical College, University of Chicago Institute for Population and Precision Health and Bangabandhu Sheikh Mujib Medical University (BSMMU).

Informed consent statement

Written consent was obtained from the participants.

Consent to publication

Not applicable

Availability of data and materials

All data will be made available upon reasonable request from the corresponding author.

Competing interests

The authors declare no competing interests.

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