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**Original Research Article** **Anatomy**

# Analysis of BRCA1 Exon 2 Mutations in FFPE Tissues of Bangladeshi Bangali Females with Breast Cancer

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## Abstract

***Background*:** Among all breast cancers, 5 to 10% are hereditary and related to mutation of BRCA1 and BRCA2 gene. To date, extensive researches have been carried out for detection of mutations in the exon2 of BRCA1 gene. Genetic study on these two genes from formalin-fixed paraffin-embedded tissue in Bangladeshi population has not been reported so far. ***Objective*:** To identify mutation in exon2 of *BRCA1* gene from formalin-fixed paraffin-embedded tissue of adult Bangladeshi Bangali female patients with breast cancer. ***Methods*:** The research was cross-sectional descriptive type of study. The study was conducted on Adult Bangladeshi Bangali female patients with breast cancer aged more than 18 years. A questionnaire was developed to record information on socio-demographic and reproductive characteristics. The data was analyzed by SPSS (Statistical package for the social sciences) version 23.0. The whole sequence of exon2 of chromosome 17 was amplified by short range PCR using the Gotaq master mix and primer sequence. The amplicons were confirmed by 2% agarose gel electrophoresis and sequencing was done by Sanger sequencer. Data analysis was performed using Chromas® software version 2.33 and Mega 7 software. The quary sequences were compared with the NCBI database. ***Results*:** Average age of the breast cancer patients was 45.46 (± 11.52) and the mean age at menarche was 12.46 (± 0.646). In this study, new mutations along with known mutations were found in exon2 of *BRCA1* gene of eight adult female breast cancer patients. The mutations are insertions (C>A, C>T, T>A, G>T, G>C, T>C, A>T, C>G), deletion (C_) and single nucleotide substitution (T-C, G-A, T-G, C-G, T-A, G-C, C-A) in types. ***Conclusions*:** The aim of the research was to identify mutation in exon2 of *BRCA1* gene. Eight patients have mutation in their DNA sequence revealed from formalin-fixed paraffin-embedded tissue. Further studies are required to evaluate whether these mutation contribute to breast cancer or not. Identification of known mutation along with new mutation with this small sample size emphasizes the importance of exploration the genetic makeup of Bangladeshi population to develop a database for proper screening and genetic counseling of the disease.

**Keywords:** BRCA1, Exon 2 Mutations, Breast Cancer.



## INTRODUCTION

Breast cancer is not only the most common malignancy in women worldwide but also one of the major causes of death throughout the world [1]. It constitutes 23% of all cancers among women and is the second most common cancer for both sexes [2].

*BRCA1* (breast cancer type 1 susceptible protein) (OMIM 113705) is a tumor suppressor gene located in 17q21 with a length of 100kb. Germ line mutations in *BRCA1* and *BRCA2* genes account for genetic predisposition and increased risk of breast and ovarian cancers [3]. Among all breast cancers, five to ten percent are related to the mutation

of *BRCA1* and *BRCA2* gene [4]. The germ line mutations are usually point mutations and are commonly frame shift small insertions or deletions, nonsense mutations or mutation affecting the splicing sites [5]. Women with mutant *BRCA* genes have a lifetime risk of developing breast cancer by 80-90% [3]. Loss of *BRCA1* alleles or low expression of the *BRCA1* gene in a large proportion of sporadic breast cancer cases supports the role of *BRCA1* in the development of sporadic breast cancer [5]. Twenty-two out of twenty-four exons is code for a 220kd protein of 1863 amino acid which is characterized by a zinc-binding RING finger domain at the amino terminus and *BRCA1* carboxyl-terminal (BRCT) domain at the carboxyl terminus. *BRCA1* serves as an important central component in multiple biological pathways that regulate cell cycle progression, centrosome duplication, DNA damage repair, cell growth, and apoptosis. Disease-associated mutations are distributed over the entire coding regions of the *BRCA1* gene and these demonstrate considerable ethno-geographic variation [6, 7]. The prevalence of *BRCA* mutations in Asian breast cancer patients is almost similar to that of other ethnic population [8]. But the frequency of these genetic mutations varies among ethnic groups and countries. Breast cancer predisposition has been demonstrated due to founder mutations originated from a single ancestor. Exon2 is one of the most common mutation sites in the *BRCA1* gene [9]. A frame shift mutation occurs in codon 23, exon2 and results in the creation of the STOP codon. This alteration leads to premature termination of translation of the protein [10].

Identification of mutations in exon2 of the *BRCA1* gene makes a great value in cancer prognosis, treatment and should permit early risk prediction. These would reduce those risks reliably and safely before the occurrence of the disease and administer more individualized advanced therapies for better clinical responses. Research using *BRCA1* exon2 gene mutation of breast cancer would help us in making assumptions on how this disease is transmitted from generation to generation in a study population. Several types of research have been done on the mutation of exon2 of the *BRCA1* gene from formalin-fixed paraffin-embedded tissue worldwide.

## OBJECTIVE

To identify mutation in exon2 of BRCA1 gene from formalin-fixed paraffin-embedded tissue of adult Bangladeshi Bangali female patients with breast cancer.

## METHODOLOGY

**Study Design:** Cross sectional descriptive study.

**Place and Period of the Study:** The research was carried out in the Genetic research laboratory, Department of Anatomy, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. The period of research was span from March 01, 2018 to February 28, 2019.

**Study Participants:** A total of 50 breast cancer patients with invasive ductal cell carcinoma type were selected from OPD and in-patients Departments of National Institute of Cancer Research & Hospital, Mohakhali, Dhaka. All patients were diagnosed by oncologist and histopathologist. A selection checklist was designed by the researcher to check the inclusion criteria in selecting patients. Informed consent was obtained from each patient.

Detailed history of the patients and families were undergone using a semi-structured data collection sheets.

**Inclusion Criteria**

Selection of patients was mainly based on the following criteria-

- Bangladeshi Bangali.
- Sex: Female.
- Age >18yrs.
- Diagnosed breast cancer patients of ductal cell carcinoma variety.

**Exclusion Criteria**

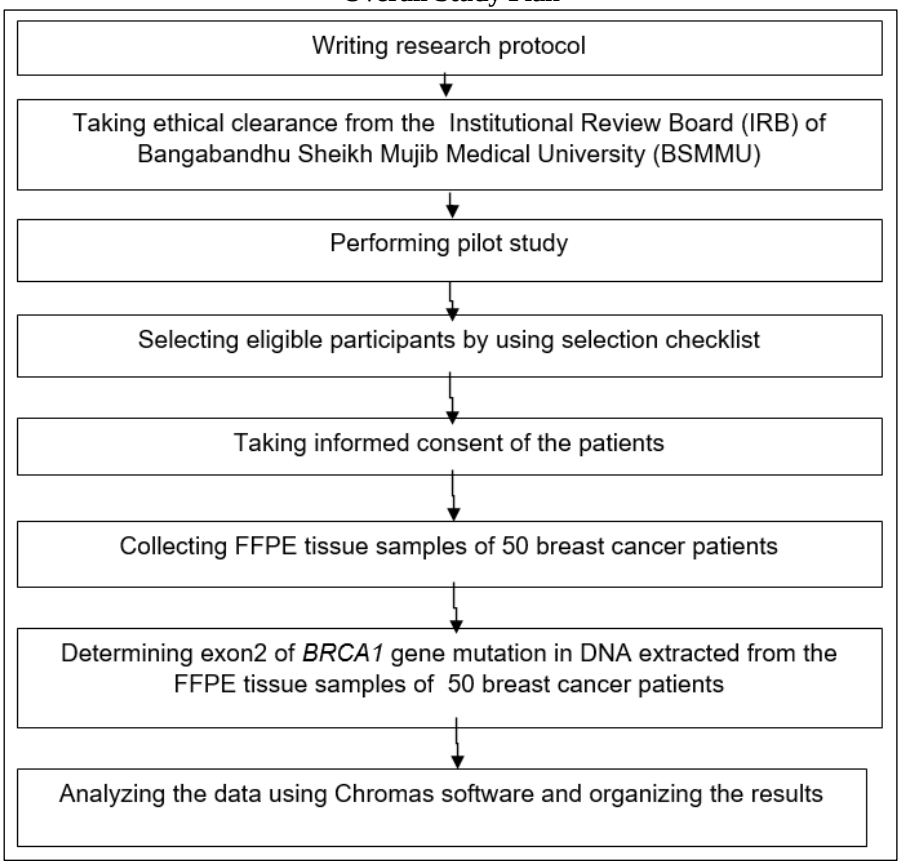
- History of ovarian cancer, pancreatic cancer.
- History of chemotherapy and radiotherapy.

**Sample Size**

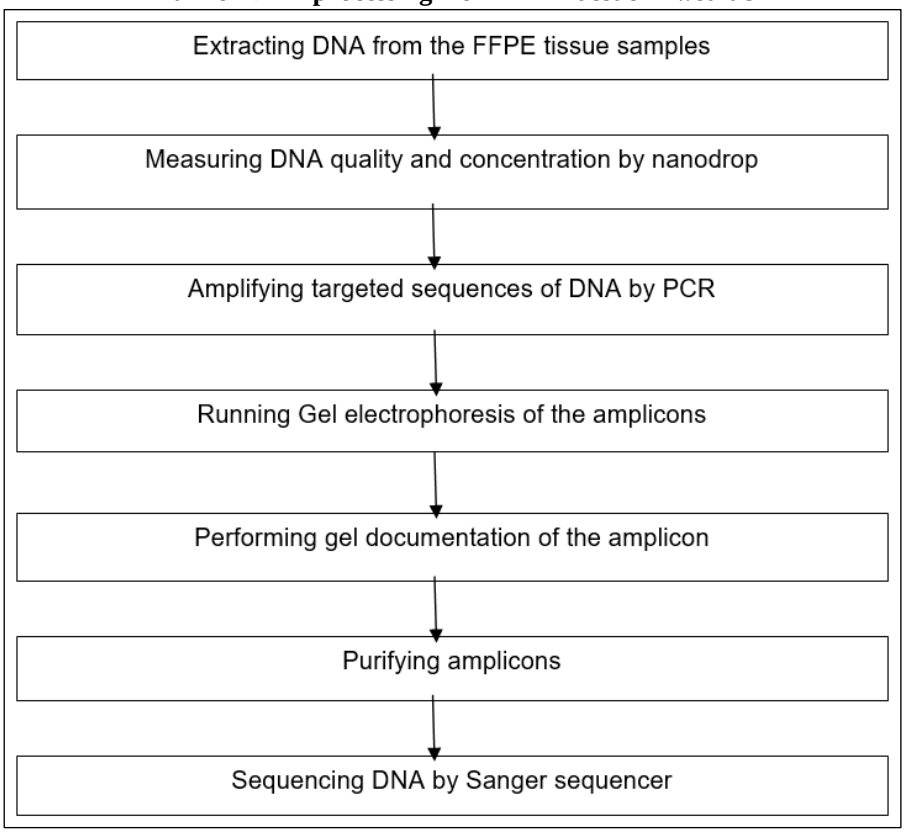
Samples of formalin-fixed paraffin-embedded breast cancer tissue of 50 adult Bangladeshi Bangali female breast cancer patients of invasive ductal cell carcinoma variety were collected from the Department of Histopathology of the National Institute of Cancer Research and Hospital, Mohakhali, Dhaka.

**Sampling Technique:** Patients were selected using convenient sampling technique.

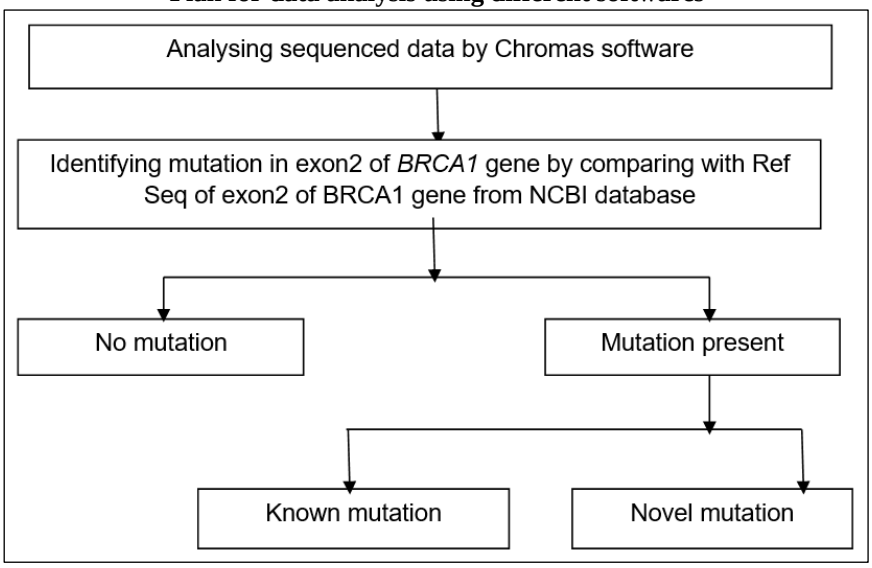
**Overall Study Plan**




**Plan for DNA processing from FFPE tissue in wet lab**



**Plan for data analysis using different softwares**




**Procedure of History Taking and Data Collection**

After taking IRB, patients were selected using 'Selection Checklist'. A semi-structured data collection sheet was constructed with the selected variables. After getting consent from the patients, data were collected by the present researcher herself and filled up the data collection sheet.

Detailed history was taken regarding maternal and paternal family for three to five generations who have had the same diagnosis as the patients or have other phenotype related with breast cancer (including ovarian, colon, prostate and lung cancer that could represent the expression of the closely resemble genetic abnormality) and recorded in data sheets. If any information was required, the telephone calls were made to obtained missing values from the patients. Histories of multiple generations were helped to demonstrate the distribution of cancers in the family.

Hospital records and investigation files were carefully examined for investigation findings. The notes were taken for filling of semi-structured data collection sheets from mammography, FNAC and biopsy reports whether available.

## RESULTS

Among the 50 adult Bangladeshi Bangali female breast cancer patients, all of them were more than 18 years aged. The age limit is divided into four groups. 4 out of 50 patients belong to 18-30 age groups, 32 patients belong to 31-50 age group which comprises highest frequency (64%) in the study population. 14 patients belong to 51-70 age group (28%). The frequency of age group 71-90 is 0%.

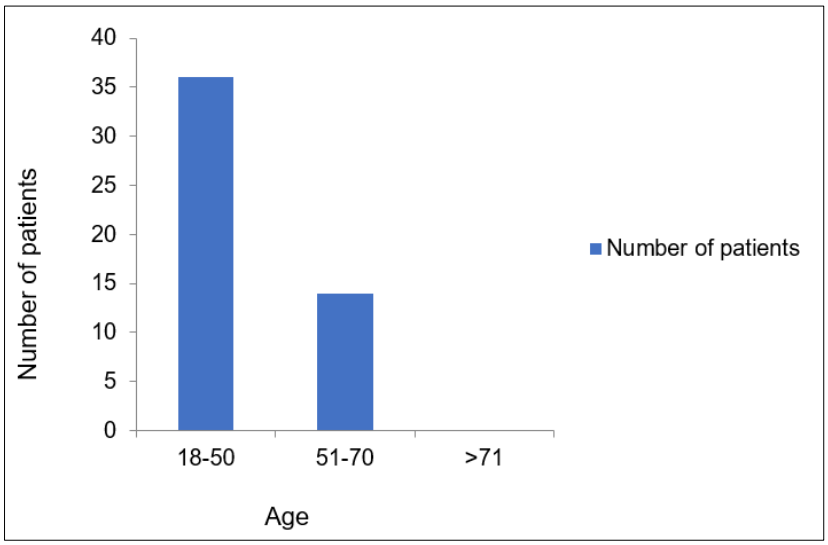



**Fig-1: Number of patients in different age limit**

Among 50 adult breast cancer patients 31 patients were triple negative (ER/PR/HER2) representing 62% of the total study population. 19 patients were positive to these hormone receptors comprising 38% which is shown in a pie chart in Figure.

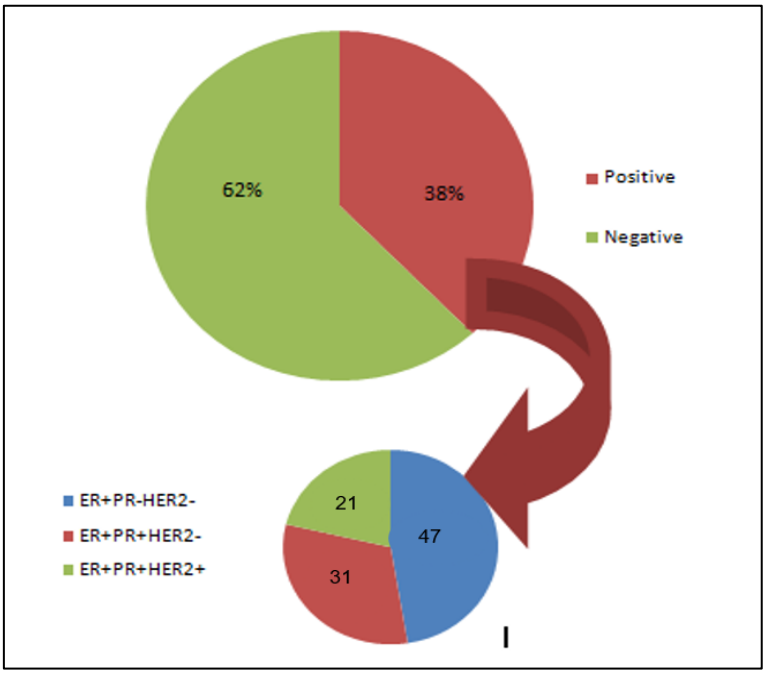



**Fig-2: The frequency of hormone receptor responsiveness of 50 adult breast cancer patients**

The chromatogram report revealed by Sanger sequencing is the raw unprocessed data of the research. The chromatogram shows good noise free peaks which indicated that the extracted DNA shows good quality.

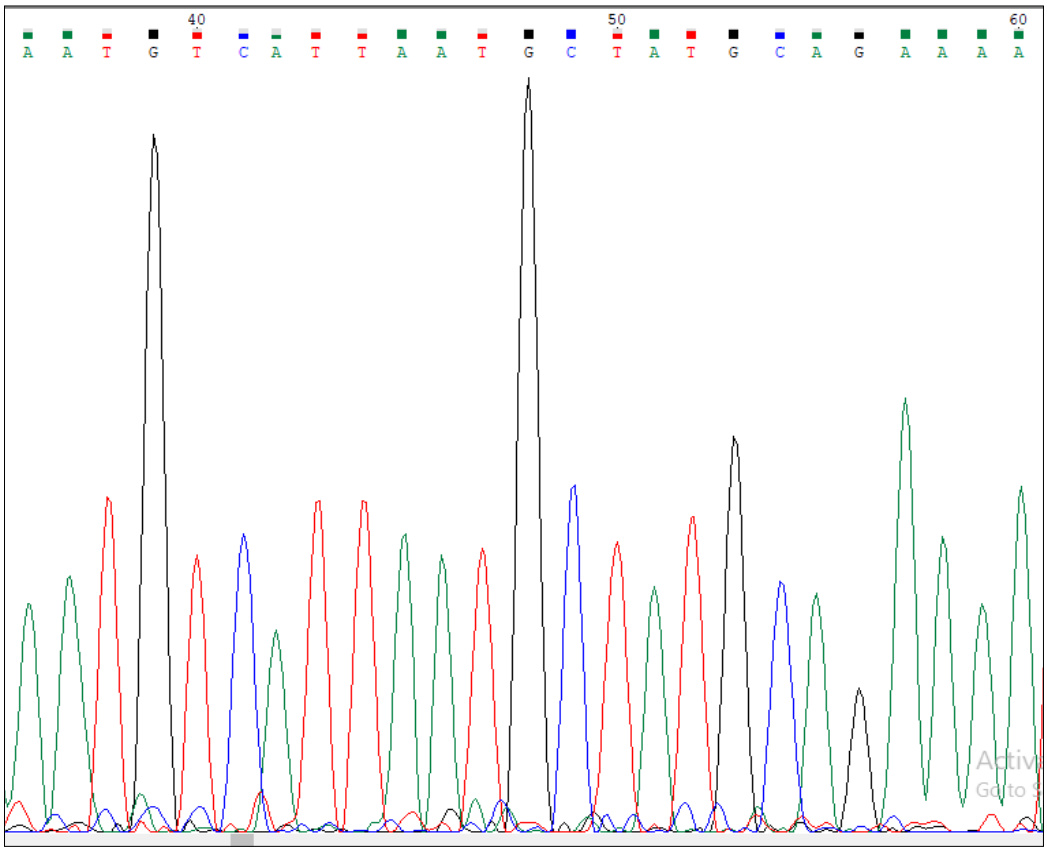



**Fig-3: Chromatogram showing the peaks of the base pairs**

**Table-1: Similarity of identification in percentage of exon2 of *BRCA1* gene with NCBI database**

| Sample ID | Description of Reference | Percentage Similarity |
|---|---|---|
| 1F | Homo sapiens clone 103_P-13_EXON2-FP_D03 breast cancer1 (BRCA1) gene and partial cds | 97% |
| 1R | Homo sapiens BRCA1 DNA repair associated (BRCA1), Ref Seq Gene (LRG_292) on chromosome | 100% |
| 2F | Homo sapiens clone 103_P-13_EXON2-FP_D03 breast cancer1 (BRCA1) gene and partial cds | 91% |
| 2R | Homo sapiens BRCA1 DNA repair associated (BRCA1), Ref Seq Gene (LRG_292) on chromosome 17 | 99% |
| 3F | Homo sapiens clone 103_P-12_EXON2-F_E04 Breast cancer1 (BRCA1) gene, partial cds | 100% |
| 3R | Homo sapiens clone 103_P-10_EXON2-F_D04 Breast cancer1 (BRCA1) gene, partial cds | 100% |
| 4F | Homo sapiens clone I103_P-9_E10 Breast cancer 1 (BRCA1) gene, partial cds | 100% |
| 4R | Homo sapiens clone I103_P-3_P-2_E10 Breast cancer 1 (BRCA1) gene, partial cds | 100% |
| 5F | Homo sapiens clone I103_P-10_EXON2-F_D04 Breast cancer 1 (BRCA1) gene, partial cds | 100% |
| 5R | Homo sapiens BRCA1 DNA repair associated (BRCA1), Ref Seq Gene (LRG_292) on chromosome 17 | 100% |
| 6F | Homo sapiens isolate TWH50701 truncated breast and ovarian cancer susceptibility protein 1 (BRCA1) gene, exon 2 and partial cds | 99% |
| 28F | Homo sapiens clone I103_P-13_EXON2-FP_D03 breast cancer 1 (BRCA1) gene, partial cds | 90% |
| 28R | Homo sapiens BRCA1 DNA repair associated (BRCA1), Ref Seq Gene (LRG_292) on chromosome 17 | 95% |
| 8F | Homo sapiens BRCA1 DNA repair associated (BRCA1), Ref Seq Gene (LRG_292) on chromosome 17 | 99% |
| 9F | Homo sapiens clone I103_P-12_EXON2-F_E08 breast cancer 1 (BRCA1) gene, partial cds | 100% |
| 9R | Homo sapiens clone I103_P-9_EXON2-F_D07 breast cancer 1 (BRCA1) gene, partial cds | 100% |
| 10F | Homo sapiens clone I103_P-13_EXON2-FP_D03 breast cancer 1 (BRCA1) gene, partial cds | 100% |
| 10R | Homo sapiens BRCA1 DNA repair associated (BRCA1), Ref Seq Gene (LRG_292) on chromosome 17 | 100% |
| 11F | Homo sapiens clone I103_P-8_EXON2-F_A07 breast cancer 1 (BRCA1) gene, partial cds | 100% |
| 11R | Homo sapiens breast cancer 1, early onset (BRCA1) gene, complete cds | 100% |
| 13F | Homo sapiens clone I103_P-12_EXON2-F_E08 breast cancer 1 (BRCA1) gene, partial cds | 100% |
| 13R | Homo sapiens clone I103_B-133_P-2_C10 breast cancer 1 (BRCA1) gene, partial cds | 99% |
| 15F | Homo sapiens isolate TWH-1308-0-1 truncated breast and ovarian cancer susceptibility protein 1 (BRCA1) gene, complete cds | 100% |
| 15R | Homo sapiens BRCA1 DNA repair associated (BRCA1), Ref Seq Gene (LRG_292) on chromosome 17 | 100% |
| 16F | Homo sapiens BRCA1 (BRCA1) gene, partial cds | 99% |
| 17F | Homo sapiens clone I103_P-3_P-2_E10 breast cancer 1 (BRCA1) gene, partial cds | 100% |
| 17R | Homo sapiens BRCA1 DNA repair associated (BRCA1), Ref Seq Gene (LRG_292) on chromosome 17 | 100% |
| 18F | Homo sapiens clone I103_P-13_EXON2-FP_D03 breast cancer 1 (BRCA1) gene, partial cds | 89% |
| 18R | Homo sapiens clone I103_P-12_EXON2-F_E08 breast cancer 1 (BRCA1) gene, partial cds | 89% |
| 19F | Homo sapiens BRCA1 DNA repair associated (BRCA1), Ref Seq Gene (LRG_292) on chromosome 17 | 100% |
| 19R | Homo sapiens clone I103_P-13_EXON2-FP_D03 breast cancer 1 (BRCA1) gene, partial cds | 100% |
| 20F | Homo sapiens clone I103_P-13_EXON2-FP_D03 breast cancer 1 (BRCA1) gene, partial cds | 100% |
| 20R | Homo sapiens isolate TWH-1308-0-1 truncated breast and ovarian cancer susceptibility protein 1 (BRCA1) gene, complete cds | 100% |
| 21F | Homo sapiens BRCA1 DNA repair associated (BRCA1), Ref Seq Gene (LRG_292) on chromosome 17 | 100% |
| 21R | Homo sapiens breast cancer 1, early onset (BRCA1) gene, complete cds | 100% |
| 22R | Homo sapiens breast cancer 1, early onset (BRCA1) gene, complete cds | 99% |
| 24F | Homo sapiens clone I103_P-13_EXON2-FP_D03 breast cancer 1 (BRCA1) gene, partial cds | 92% |
| 44F | Homo sapiens clone I103_P-13_EXON2-FP_D03 breast cancer 1 (BRCA1) gene, partial cds | 92% |
| 44R | Homo sapiens isolate TWH-1308-0-1 truncated breast and ovarian cancer susceptibility protein 1 (BRCA1) gene, complete cds | 94% |
| 29F | Homo sapiens clone I103_P-13_EXON2-FP_D03 breast cancer 1 (BRCA1) gene, partial cds | 98.89% |
| 29R | Homo sapiens BRCA1 DNA repair associated (BRCA1), Ref Seq Gene (LRG_292) on chromosome 17 | 100% |

| Sample ID | Description of Reference | Percentage Similarity |
|---|---|---|
| 46F | Homo sapiens clone I103_P-13_EXON2-FP_D03 breast cancer 1 (BRCA1) gene, partial cds | 94.53% |
| 48F | Homo sapiens clone I103_P-12_EXON2-F_E08 breast cancer 1 (BRCA1) gene, partial cds | 100% |
| 34F | Homo sapiens BRCA1 DNA repair associated (BRCA1), Ref Seq Gene (LRG_292) on chromosome 17 | 100% |
| 38R | Homo sapiens clone I103_P-13_EXON2-FP_D03 breast cancer 1 (BRCA1) gene, partial cds | 100% |
| 39F | Homo sapiens isolate TWH-1308-0-1 truncated breast and ovarian cancer susceptibility protein 1 (BRCA1) gene, complete cds | 100% |
| 42F | Homo sapiens clone I103_P-12_EXON2-F_E08 breast cancer 1 (BRCA1) gene, partial cds | 98.89% |
| 42R | Homo sapiens BRCA1 DNA repair associated (BRCA1), Ref Seq Gene (LRG_292) on chromosome 17 | 100% |
| 45F | Homo sapiens isolate TWH-1308-0-1 truncated breast and ovarian cancer susceptibility protein 1 (BRCA1) gene, complete cds | 95.87% |
| 49F | Homo sapiens clone I103_P-10_EXON2-F_D04 breast cancer 1 (BRCA1) gene, partial cds | 100% |
| 50F | Homo sapiens BRCA1 DNA repair associated (BRCA1), Ref Seq Gene (LRG_292) on chromosome 17 | 100% |

**Table-2: Findings of the present study**

| Sample number | Sequence | Base number | Nucleotide change | Type of mutation |
|---|---|---|---|---|
| 01 | R | 8 | T-A | Substitution |
| | | 189 | C-A | Substitution |
| 02 | R | 175 | G-A | Substitution |
| 09 | R | 3 | T-A | Substitution |
| | | 25 | C>G | Insertion |
| | | 27 | T>A | Insertion |
| | | 181 | C-A | Substitution |
| 10 | F | 01 | G>C | Insertion |
| 17 | F | 14 | G>C | Insertion |
| 33 | F | 07 | A>C | Insertion |
| | | 08 | T>C | Insertion |
| | | 12 | T>A | Insertion |
| 35 | F | 07 | T>A | Insertion |
| | | 59 | C>T | Insertion |
| | | 66 | CT>A | Insertion |
| | | 81 | C>A | Insertion |
| | | 105 | C_ | Deletion |
| | | 108 | C>T | Insertion |
| | | 160 | T-C | Substitution |
| 44 | F | 22 | G>T | Insertion |
| | | 29 | C>A | Insertion |
| | | 32 | C>A | Insertion |
| | | 54 | G-A | Substitution |
| | | 73 | C>T | Insertion |
| | | 74 | C>T | Insertion |
| | | 78 | T>C | Insertion |
| | | 82 | T-G | Substitution |
| | | 86 | G>C | Insertion |
| | | 91 | C-G | Substitution |
| 44 | R | 26 | C>TG | Insertion |
| | | 34 | T>A | Insertion |
| | | 62 | T-A | Substitution |
| | | 67 | A>T | Insertion |
| | | 72 | C>T | Insertion |
| | | 91 | T-C | Substitution |
| | | 95 | CA>T | Insertion |
| | | 100 | G>C | Insertion |
| | | 103 | G>C | Insertion |
| | | 108 | T>A | Insertion |

| Sample number | Sequence | Base number | Nucleotide change | Type of mutation |
|---|---|---|---|---|
| | | 153 | C>TA | Insertion |
| | | 158 | G-C | Substitution |
| 47 | F | 69 | C>T | Insertion |
| | | 73 | C>A | Insertion |
| | | 100 | C>T | Insertion |
| | | 110 | C>T | Insertion |
| | | 130 | C>G | Insertion |

***F-Forward**
***R-Reverse**

The sequences edited by Chromas software were aligned by Mega7 software with themselves along with the reference sequence of NCBI database. The sequences showed some similarities with the reference sequence of NCBI. The sequences were also found similar in some base pairs among themselves.

The base pairs which were not similar with the reference sequence of NCBI obtained changes of nucleotide which might be mutation.

**Table-3: Types of mutation present in the study**

| Types of mutation | Sample No. |
|---|---|
| Single nucleotide substitution | 1R, 2R, 9R, 35F, 44F, 44R |
| Insertion | 9R, 10F, 17F, 33F, 35F, 44F, 44R, 47F |
| Deletion | 35F |

Among the types of mutations, insertions are found at highest frequency of 71.8%, substitutions are found 26% and deletions are at only 2.2%.

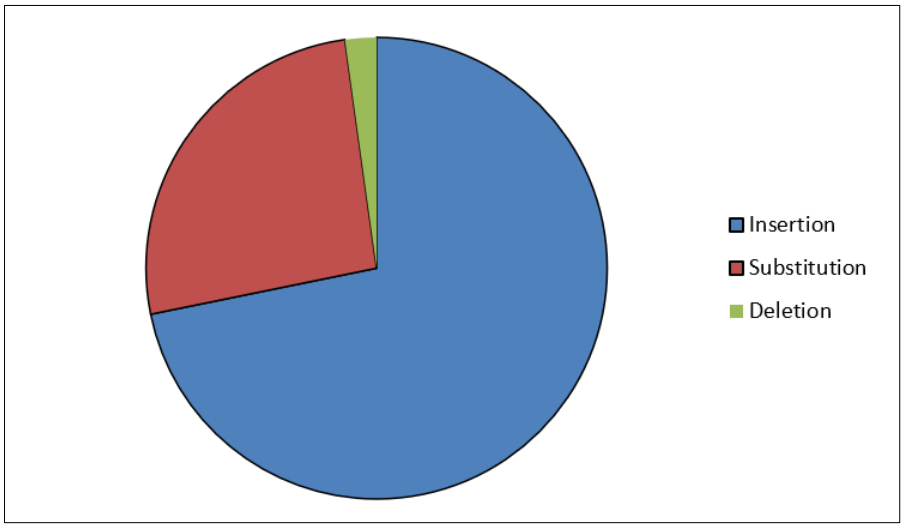



**Fig-4: Pie chart showing the frequencies of mutations found in this study**

## Discussion

In the present study it was found that, 62% of the study population was triple negative (ER negative/ PR negative/ HER2 negative) where as in Nepal, Shrestha *et al.*, 2016 found 55.64% of study population who were triple negative. Rest of the population found hormone receptor positive (38%). Among the total study population, 18% were ER positive, 12% were PR positive and only 8% were HER2 positive. Yip *et al.*, 2009 have found 6.7% of study population who were ER positive, none of them were PR positive and 15.4% were HER2 positive.

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Comparison with the different population are shown in Fig. 5, 6, 7, 8.

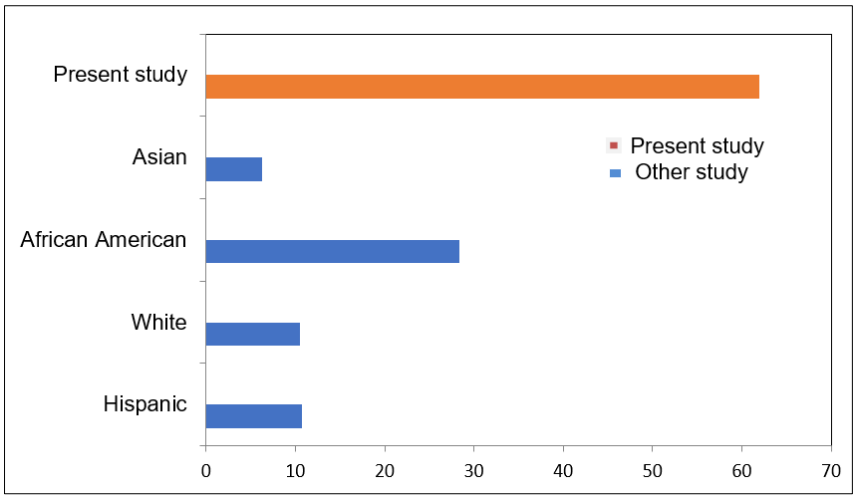


**Fig-5: Comparison of percentage of triple negative hormone receptor status**

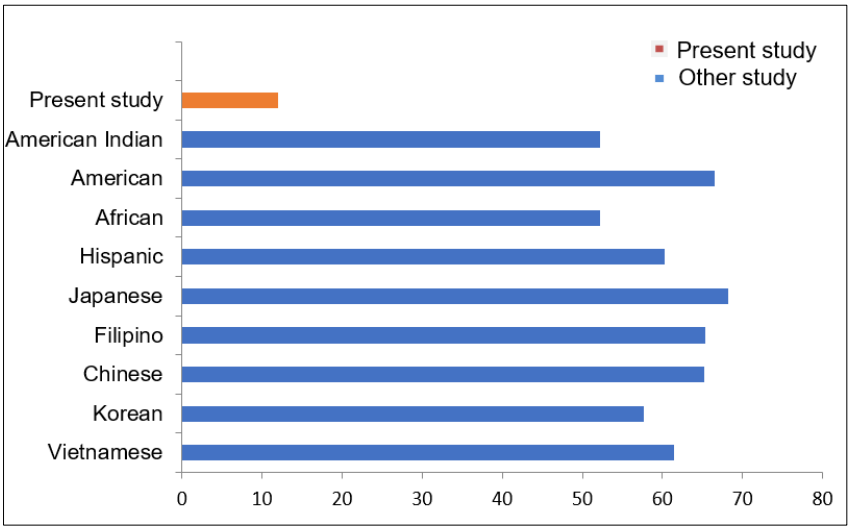


**Fig-6: Comparison of percentage of estrogen receptor positive status**

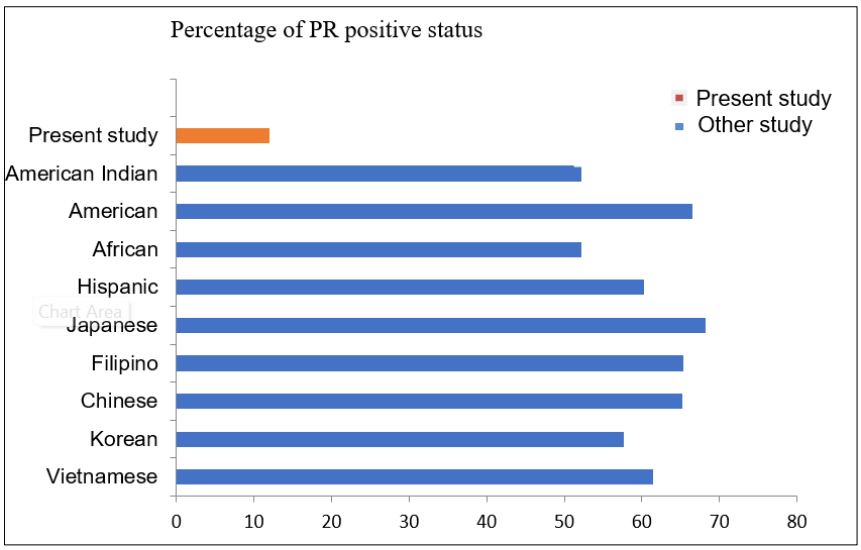


**Fig-7: Comparison of percentage of progesterone receptor positive status**

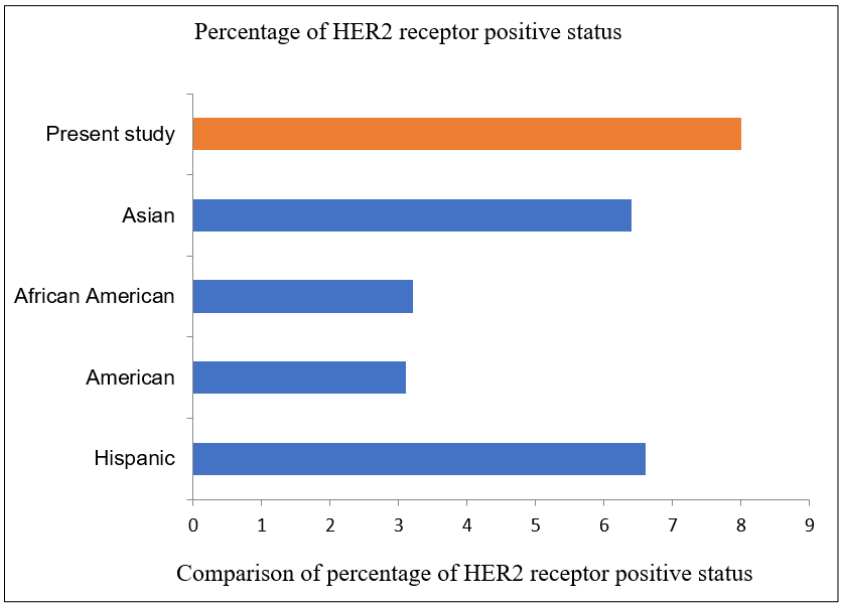


**Fig-8: Discussion on genetic analysis of the breast cancer Patients**

## DISCUSSION

The finding of this study is consistent with the findings of Guo *et al.*, 2016, p. 2255. They worked on FFPE tissues of breast cancer patients and the chromatogram reports revealed novel mutation (C>T, G>C and A>T) in exon2 of *BRCA1* gene. A study by Bernstein *et al.*, 2002 have stated that, they sequenced 12 DNA samples derived from FFPE tissue and 45-55% rate of correct identification of frameshift (deletion and insertion) mutations obtained. This study group has reported that this rate was dependent on the age of the blocks being used. According to Mangold *et al.*, 2010, none of the founder mutations was detected in *BRCA1* gene from formalin-fixed paraffin-embedded tissue of US population [12].

DNA isolated from formalin-fixed paraffin-embedded tissues has been used for analysis of DNA alterations in disease states. Samples were taken from archival FFPE tissue for analysis of *BRCA1* mutations. Multiple mutations found in histologically normal tissue of two cases were not present in matched tumor tissue. On the other hand, DNA from two separate blocks of normal tissue contained different mutations. These observations were inconsistent and suggested that mutations detected in fixed tissues may be artifacts of tissue preservation and not present in the original unfixed tissues. For further confirmation, blood was obtained from two cases for whom mutations were found in fixed normal tissue. DNA from their unfixed lymphocytes did not contain the mutations found in fixed normal tissue. Thus, mutations found in formalin-fixed paraffin-embedded tissues can be artifacts of tissue preservation.

Several studies have done on peripheral blood of breast cancer patients to detect mutation in exon2 of *BRCA1* gene. A study by Mehrgou & Akouchekian, 2016 have revealed two mutations (deletion) in exon2 of *BRCA1* gene on Iranian breast cancer patients [12]. Among the Algerian population, a study revealed three novel mutations in exon2 of *BRCA1* gene (G>A, C>G, T>C) [13]. They have also found two known mutation of exon2 (C>T, A>C).

Findings of the present study suggested that *BRCA1* founder mutation (185delAG) may not have a strong recurrence on breast cancer among the Bangladeshi Bangali population. This finding is analogous with the findings of Chakraborty *et al.*, 2015, p. 7930 where no founder mutations were detected in any of the subjects [10]. Akilzhanova *et al.*, 2013 also stated that, no recurrent or founder mutations were observed in *BRCA1* gene among the breast cancer patients of Kazakhstan [14]. A study by Sharma *et al.*, 2014 reported that, the 185delAG mutation in *BRCA1* gene was not found in any of the participants from Delhi, India [15].

## CONCLUSION

The study was aimed to identify mutation in exon2 of *BRCA1* gene. Among 50 adult breast cancer patients 9 patients have several nucleotide changes in their DNA sequences revealed from FFPE tissue. These are insertions, deletions and single nucleotide substitution types of mutations. These findings require further studies along with clinical correlation to established the contribution of these mutations to breast cancer. Identification of known variant along with new mutation with this small sample size emphasizes the importance of exploring the genetic makeup of Bangladeshi population to develop a database for proper screening and genetic counseling of the disease.

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