ORIGINAL ARTICLE

CHARACTERIZATION OF MUTATION OF *BRCA1* GENE AT CHROMOSOME 17Q21 IN BANGLADESHI WOMEN WITH FAMILIAL BREAST CANCER

A Siddiqua[1], N Martuza[1], J Tasnim[1], M Rahman[1], IJ Akhi[1]
A Shahnaj[1], MH Rahman[1], N Sultana[1], TJ Shoha[2]

[1]*Dept of Biochemistry, Dhaka Medical College, Dhaka*
[2]*Dept of of Oncology, Anwar Khan Modern Medical College Hospital, Dhaka*

ABSTRACT

Breast cancer is the most common cancer among women in Bangladesh. About 69% cancer death of women is due to breast cancer. In Bangladesh the incidence rate of breast cancer is about 22.5 per 100000 female. Female who carries mutation in the *BRCA1* gene have an 80% chance of developing breast cancer during their lifetime. Due to its high prevalence, genetic testing for *BRCA1* mutation has become an integral part of clinical practice. This study was aimed to characterize the mutation at chromosome 17q21 by targeting *BRCA1* gene among Bangladeshi women with familial breast cancer. This descriptive case series study was conducted in the Department of Biochemistry, Dhaka Medical College, Dhaka, from July 2017 to June 2018. In this study, thirty diagnosed breast cancer patients with family history breast cancer were selected according to the selection criteria from the Department of Surgery and Department of Radiotherapy, Dhaka Medical College, Dhaka. Mutations were detected by Next Generation Sequencing method. Characterization and clinical significance were verified from NCBI genome database. The study parameters were presence of mutation, types of mutation, clinical significance, frequency (%) of mutation. In this study, sixteen distinct mutations were found. Three pathogenic mutations (10%) were found among the patients which were nonsense mutations. Pathogenic mutations were c.5251C>T, c.2158G>T and c.1059G>A. Three mutations of uncertain significance with missense variant, five benign mutations of missense variant and five benign mutations of silent variant were also found among thirty patients. In this study we found that, Bangladeshi women with familial breast cancer had 10% pathogenic mutations. Early screening for *BRCA1* gene mutations might provide better information for health to those who have family history of breast cancer.

Key Words: *BRCA1*, Next generation sequencing, Mutation

Introduction

Breast cancer is a type of malignancy caused by the abnormal growth and uncontrolled cell division within the terminal duct and lobular units of the breast. It has the potential to invade and destroy surrounding normal tissue and spread throughout the body via blood or lymph fluid to new sites[1]. Normally, human cells grow and divide to form new cells when the body needs them. As the cells grow old or become damaged, they die, and new cells take their place. But in cancer, this orderly process breaks down. Cells divide and grow in more abnormal

and excessive fashion. These extra cells divide without stopping and may form growths called tumors or cancers[2]. Breast cancer incidence in developed countries is higher, while relative mortality is greatest in less developed countries. It remains the most common cancer among women in Bangladesh with the incidence rate of 22.5 per 100000 female. It accounts for 69% of women cancer death in Bangladesh[3]. The mortality rate of breast cancer is highly frequent in Bangladeshi women than cervical, esophageal cancer. Five year prevalence rate of breast cancer in Bangladeshi women is observed as 35.6% in 2012 which is higher than any other types of cancer[4]. Several risk factors are responsible for development of breast cancer. Women who are getting Hormonal Replacement Therapy (HRT), Oral Contraceptive Pills (OCP), have history of early menarche and late menopause, are in risk of developing breast cancer due to high estrogen level as it increases breast cell mitosis[5]. Others are non-hormonal risk factors such as, alcohol, smoking, obesity, less physical activity and positive family history[1]. Germline mutations of breast cancer susceptibility genes like *BRCA1, BRCA2, p53, HER2* have been reported to have a high risk for developing hereditary breast cancer[6]. Family history of breast cancer contributes about 20%-25% of breast cancer but only 5%-10% of breast cancer cases demonstrate an autosomal dominant inheritance[7]. Men and women who carry genetic mutation have 50% chance of passing it to each of their children[8]. Female who carries mutation in the *BRCA1* gene have an 80% chance of developing breast cancer during their lifetime and *BRCA2* mutation have 80%-85% chance of developing breast cancer[9]. The prevalence of *BRCA* pathogenic mutation carriers in the general population is estimated at between 1/800 (0.12%) and 1/1000 (0.1%). *BRCA1* and *BRCA2* mutation frequencies in breast and ovarian cancer patients unselected for family history or age at onset are generally low (<1-3% for *BRCA1*)[10]. *BRCA1* gene is a tumor suppressor gene. It produces *BRCA1* protein which repairs DNA double break during cell cycle. So, it has protective function over cancer development as it suppresses tumor formation. If there is any pathogenic mutation present in *BRCA1* gene, *BRCA1* protein loses its DNA repairing property. So, unregulated cell proliferation, clonal expansion, tumor progression, malignant neoplasm, invasion and metastasis take place[11,12]. Location of *BRCA1* gene is in long (q) arm of chromosome 17 at region 2 band 1, from base pair 41,196,312 to base pair 41,277,500 (Build GRCh37/hg19). Gene size is 81kb and *BRCA1* protein has 1863 amino acids. *BRCA1* have 24 exons[13]. Hundreds of mutations have been indentified in *BRCA1* gene but not all mutations are clinically significant. Pathogenic mutations are very likely to cause disease. Benign mutations are unlikely to cause any harmful effect on health. Another type of mutation is 'Variant of uncertain significance'. Its clinical significance or function on health is not known[14]. Several mutation detection procedures are available but next generation sequencing is less time consuming and cost effective[15]. Genetic testing for *BRCA1* gene mutation have become an integral part of clinical practice in United States and other western countries[16]. Detection of *BRCA1* gene mutation carriers is an important step in assessing the risk. The incidence and the type of *BRCA1* mutations differ extensively and may have different geographic and ethnic distribution. However, contribution of mutations in *BRCA1* gene to breast cancer patients in Bangladeshi population remains relatively unexplored. This study is focused on understanding the character and distribution of *BRCA1* gene mutation in patients

with familial breast cancer in Bangladeshi people, which will help to develop awareness among women who have family history of breast cancer.

Materials and Methods

This was a descriptive case series study conducted in the Department of Biochemistry, Dhaka Medical College Dhaka, from July 2017 to June 2018 after receiving approval from Research Review Committee and Ethical Review Committee. Thirty diagnosed breast cancer patients with family history of breast cancer in 1st or 2nd degree relatives were included in this study according to selection criteria. Diagnosis was confirmed by FNAC or histopathology reports. Patients who received complete treatment of breast cancer, diagnosed with any other cancer, any known genetic disease, having systemic disease such as renal failure, hepatic failure, uncontrolled diabetes mellitus were excluded from this study. Written informed consent was taken from the participants. With all aseptic precautions, 5 ml venous blood was collected. All the laboratory procedures were done in NeuroGen genetic laboratory, Panthopath, Dhaka. Laboratory procedure included DNA extraction from blood, polymerase chain reaction, gel electrophoresis, library preparation, sequencing of PCR products and mutation detection. The study parameters were presence of mutations, types of mutations, clinical significance of mutations and frequency of mutations. Clinical significances were verified from NCBI genome database. Statistical analysis was done by using SPSS version 21.

Results

In this case series study, mean±SD age of study group was 47.63±10.35years. Mean±SD BMI of the study population was 20.7±2.1 Kg/m^2.

Table-I: Demographic profile of the study subjects (N=30)

Variable	Mean±SD
Age (years)	47.63±10.35
BMI (Kg/m^2)	20.7±2.1

Table I shows general characteristics of the study group. It shows that, mean±SD age and BMI of study population is 47.63±10.35 years and 20.7±2.1 Kg/m^2 respectively.

Table II: Characteristics of clinicopathological parameters in breast cancer (N=30)

Variables		Number of patients (%)
Triple negative breast cancer:	Yes	17(56.67%)
	No	13(43.33%)
Type of breast cancer:	Ductal	28(93.33%)
	Lobular	2(6.67%)
Staging of breast cancer:	I	8(26.67%)
	II	12(40%)
	III	6(20%)
	IV	4(13.3%)
Grading of breast cancer:	I	7(23.33%)
	II	19(63.33%)
	III	4(13.33%)

Table II shows clinicopathological characteristics of the study population. It shows that (56.67%) patients had TNBC, maximum patients had ductal carcinoma (93.33%), stage II (40%) and grade II (63.33%) breast cancer.

Figures within parenthesis indicates percentage (%). Data were achieved from previous pathological reports of the study population.

Table III: Characterization of *BRCA1* gene pathogenic mutations (N=30)

Variant coordinate	Base change	Amino acid change	Type of mutation	Clinical significance	No of patients having this mutation out of 30 patients	Frequency (%)
1) 41246489 rs80356935	1059G>A	Trp353Ter	Nonsense	Pathogenic	1	3.3%
2) 41245390 rs80356875	2158G>T	Glu720Ter	Nonsense	Pathogenic	1	3.3%
3) 41209095 rs80357123	5251C>T	Arg1751Ter	Nonsense	Pathogenic	1	3.3%

Table III shows mutation position, base change, amino acid change, mutation types, clinical significance, frequency (%) of pathogenic mutation. Total 10% (n=3) pathogenic mutation was present among 30 patients (3 out of 30 patients) of which conversion of Tryptophan, Glutamic acid and Arginine contribute same percentage (3.3%) to become stop codon.

Table IV: Characterization of *BRCA1* gene mutations of uncertain significance (N=30)

Variant coordinate	Base change	Amino acid change	Type of mutation	Clinical significance	No of patients having this mutation out of 30 patients	Frequency (%)
1) 41244936 rs799917	2612C>T	Pro871Leu	Missense	Uncertain Significance	19	63.3%
2) 41244435 rs16941	3113A>C	Glu1038Ala	Missense	Uncertain Significance	18	60%
3) 41243553 rs730881490	3995G>T	Gly1332Val	Missense	Uncertain Significance	1	3.3%

Table IV shows mutation position, base change, amino acid change, mutation types, clinical significance, frequency (%) of mutation of uncertain significance of which conversion of Proline to Leucine is 63.3% (19 patients out of 30 patients), Glutamic acid to Alanine is 60% (18 patients out of 30 patients), and Glycine to Valine is 3.3% (1 patient out of 30 patients).

Table V: Characterization of *BRCA1* gene benign missense mutations (N=30)

Variant coordination	Base change	Amino acid change	Type of mutation	Clinical significance	No of patients having this mutation out of 30 patients	Frequency (%)
1) 41244000 rs16942	3548A>G	Lys1183Arg	Missense	Benign		60%
2) 41223094 rs1799966	4837A>G	Ser1613Gly	Missense	Benign		46.67%
3) 41245471 rs4986850	2077G>A	Asp693Asn	Missense	Benign		6.7%
4) 41199697 rs786201582	2044G>A	Ala682Thr	Missense	Benign		6.7%
5) 41246481 rs1799950	1067A>G	Gln356Arg	Missense	Benign	18 14	3.3%

Table V shows mutation position, base change, amino acid change, mutation types, clinical significance, frequency (%) of benign missense mutation of which conversion of Lysine to Arginine is 60% (18 patients out of 30 patients), Serine to Glycine is 46.67% (14 patients out of 30 patients), Aspartic acid to Asparagine is 6.7% (2 patients out of 30 patients), Alanine to Threonine is 6.7% (2 patients out of 30 patients) and Glutamine to Arginine is 3.3% (1 patient out of 30 patients

Table VI: Characterization of *BRCA1* gene benign silent mutations (N=30)

Variant coordination	Base change	Amino acid change	Type of mutation	Clinical significance	No of patients having this mutation out of 30 patients	Frequency (%)
1) 41245237 rs16940	2311T>C	Leu771=	Silent	Benign	20	66.67%
2) 41245466 rs1799949	2082C>T	Ser694=	Silent	Benign	19	63.3%
3) 41234470 rs1060915	4308T>C	Ser1436=	Silent	Benign	18	60%
4) 41223086 rs144588397	4845T>C	Ala1615=	Silent	Benign	1	3.3%
5) 41243921 rs770579978	3627A>G	Leu1209=	Silent	Benign	1	3.3%

Table VI shows mutation position, base change, amino acid change, mutation types, clinical significance, frequency (%) of benign silent mutation of which codons of serine, leucine and alanine were changed but amino acid were not changed.

Discussion

This study was done to see the mutation and its characterization in *BRCA1* gene of Bangladeshi breast cancer patients. For this purpose a total number of 30 patients with familial breast cancer, (who have 1st or 2nd degree relative with breast cancer) were selected. Mutation in *BRCA1* gene was detected by Next Generation Sequencing (NGS). Gene sequence was compared with human reference genome (GRCh37/hr19). After detection of mutation, characterization, types, frequency, clinical significance of mutations were assessed. In the present study, the mean±SD age of study group was 47.63±10.35 years. Mean±SD BMI of the study population was 20.7±2.1 Kg/m^2. The frequency of triple negative breast cancer in study population was 56.67%, which is similar to the result of Zhang *et al*[17]. They found that women with familial breast cancer have more than a 50% chance of developing triple negative breast cancer (TNBC). This is because of genetic predisposition causes cancer in familial case, not the hormone receptors. Most of the patients had ductal carcinoma (93.33%), stage 2 (40%) and grade 2 (63.33%) breast cancer. After mutation detection, it was found that, 3 pathogenic mutations were present among 30 patients (10%). Three mutations of uncertain significance and 10 benign mutations were found. Similar study was conducted by Troudi *et al.*[18]. They studied 36 breast cancer patients who had history of familial breast cancer. They found four pathogenic mutations among 36 patients, (11.1%), 8 variant of uncertain significance (22.2%), and 12 benign mutations (33.3%) in *BRCA1* gene. That study showed frequency of *BRCA1* pathogenic mutation in women with familial breast cancer in Tunisia is similar to Bangladeshi women with familial breast cancer. Buleje *et al*[19] had found similar type of results which was conducted in Peru to see the contribution of *BRCA1* and *BRCA2* mutations in Peruvian patients. They did case series study with 28 patients. They identified 3 pathogenic mutations present among 28 patients (10.71%), 2 mutations of uncertain significance and 28 benign mutations. Frequency of *BRCA1* gene pathogenic mutations in Peruvian study subjects is similar with study population. Case series study was conducted by Miramar *et al.*[20] similar finding. In their study they included 60 Spanish patients with breast cancer. After mutation analysis, they found 8 pathogenic mutations in *BRCA1* gene among 60 patients (13.3%), and 25% of mutation of uncertain

significance. A case series study was done in Sweden by Loman *et al.*[21] to estimate the genetic influence and to characterize the nature and prevalence of *BRCA1* and *BRCA2* germline mutations in early-onset breast cancer. They included 97 subjects with breast cancer in their study. After gene sequencing they found sixteen (6.7%) mutations present in *BRCA1* gene out of 97 cases. This was slightly lower than our result. This is probably due to Scandinavian groups of people harbor less genetic mutation comparing to other ethnic group. Patmasiriwat *et al.*[22] did a case series study to analyze the breast cancer susceptibility genes *BRCA1* in Thai patients with familial breast cancer. They included 18 patients who have family history of breast cancer. They found 3 pathogenic mutations in 18 patients. They also found 4 nonsense mutations, 1 frameshift mutation, 2 missense mutations, 2 intronic variations and 1 silent mutation. This result shows that frequency is slightly higher than our study result. This is probably they due to the fact that, they had included some intronic regional mutations in their study result. From the result of the study, it is observed that, *BRCA1* mutation characteristics, types, frequency, clinical significance are similar to other studies which were conducted in different ethnic groups worldwide. Frequency of *BRCA1* gene pathogenic mutation ranges from approximately 5%-20% in different ethnic groups and *BRCA1* gene mutation is 10% in Bangladeshi women with familial breast cancer.

According to this study, the frequency of *BRCA1* gene pathogenic mutation is (10%) in Bangladeshi women with familial breast cancer. It provides information about genetic influence and characterizes the nature of *BRCA1* germline mutation in familial breast cancer patients. Early screening of *BRCA1* gene mutations might provide better information for health among the female who have family history of breast cancer.

References

1. Parvin S, Islam MS, Al-Mamun MMA, Islam MS, Ahmed MU, Kabir ER, Hasnat A. Association of *BRCA1, BRCA2,* RAD51, and HER2 gene polymorphisms with the breast cancer risk in the Bangladeshi population. 2016 http://hdl.handle.net/10361/6796, Accessed on 3 February 2018.
2. Rebecca SYW. Apoptosis in cancer: from pathogenesis to treatment. **J of Experi and Clin Cancer Res** 2011; **30**(1): 87.
3. Forazi AR, Chowdhury BRK. Incidence of breast cancer in Bangladesh. **Inter Cancer Nurs News** 2015.http://doi.10.4172/2375-4273.C1.00, Accessed on 5 January 2018.
4. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, *et al.* Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. **Inter J of Cancer** 2015; **136**(5): 359-386.
5. Martin A, Weber BL. Genetic and Hormonal Risk Factors in Breast Cancer. **J of the Nation Cancer Ins** 2000; **92**(14): 1126-1135.
6. Oesterreich S, Fuqua SA. Tumor suppressor genes in breast cancer. **Endo Rela Cancer** 1999; **63**(3): 405-419.
7. Lynch HT, Lynch JF. Breast cancer genetics in an oncology clinic: 328 consecutive patients. **Cancer gene and cyto** 2009; **122**: 369-371
8. Brody LC, Biesecker BB. Breast cancer susceptible genes *BRCA1* and *BRCA2*. **Medicine** 1998; **77**(3): pp. 208-226.
9. Jaure O, Alonso EN, Braico DA, Nieto A, Orozco M, Morelli C *et al.* (2015) *BRCA1* polymorphism in breast cancer patients from Argentina. **Oncology Letters** 2015; **9**(2): 845-850.

10. Balmana J, Diez O, Rubio TI, Cadoso F. BRCA in breast cancer: ESMO clinical recommendation. **Ann of Onco** 2009; **22(6):** 31-34.

11. Knudson AG. Two genetic hits (more or less) to cancer. **Nat Rev Cancer** 2001; **1(2):** 157-162.

12. Yoshida K, Miki Y. Role of *BRAC1* & *BRCA2* as regulation of DNA repair, transcription and cell cycle in response to DNA damage. **Cancer Seicnce** 2004; **95**(11): 866-871.

13. Karami F, Mehdipour P. A Comprehensive Focus on Global Spectrum of *BRCA1* and *BRCA2* Mutations in Breast Cancer. **BioMed Res Inter** 2013; **doi**.org/10.1155/2013/928562, Accessed on 25 January 2018.

14. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. **Gene in Med**: 2015; **17**(5): 405-424.

15. Park HS, Park SJ, Kim JY, Kim S, Ryu J, Shon J. Next-generation sequencing of BRCA1/2 in breast cancer patients: potential effects on clinical decision-making using rapid, high-accuracy genetic results. **Ann of Surg Treat and Res** 2017; **92**(5): 331-339

16. King MC, Marks JH, Mandell JB. Breast & Ovarian cancer risks due to inhehited mutation in *BRCA1* & 2. **Science** 2003; **302**(5645): 643-646.

17. Zhang J, Pie R, Pang Z, Ouyang T, Li J, Wang T, Fan Z *et al.* Prevalence and characterization of *BRCA1* and *BRCA2* germline mutations in Chinese women with familial breast cancer. **Breast Cancer Reas and Treat** 2012; **132**(2): 421-428.

18. Troudi W, Uhrhammer M, Sibille C, Dhan C, Mahfoudh W, Bouchlaka SC *et al.* Contribution of the *BRCA1* and *BRCA2* mutation to breast cancer in Tunisia. **J of Hum Gene** 2007; **52**(11): 915-920.

19. Buleje J, Guevara M, Acosta O, Huaman FDP, Danos P, Murillo A *et al.* (2017) Mutational analysis of *BRCA1* and *BRCA2* genes in Peruvian families with hereditary breast and ovarian cancer. **J of Mol Gene & Genom Med** 2017; **5**(5): 481-494.

20. Miramar MD, Calvo MT, Rodrigues A, Anton A, Lorento F. Genetic Analysis of *BRCA1* and *BRCA2* in breast/ ovarian cancer families from Aragon (spain): two novel truncating mutations and a large genomic deletion in *BRCA1*. **Breast Cancer Res and Treat** 2008; **112**(2): 353-358.

21. Loman N, Johannsson O, Kristoffersson U, Olsson H, Borg A. Family history of breast and ovarian cancers and *BRCA1* & *BRCA2* mutation in population based series of early onset breast cancer. **J of Nation Cancer Ins** 2001; **93**(16): 1215-1223.

22. Patmasiriwat P, Bhothisuwan K, Sinilnikova OM, Chopin S, Methakijvaroon S, Budzioch M *et al.* Analysis of breast cancer susceptibility gene *BRCA1* and *BRCA2* in Thai familial and isolated early onset breast and ovarian cancer. **Hum Mut** 2002; **20**(3): https://doi.org/10.1002/humu.9049, Accessed on 24 January 2018.