Original Article

Identification of *BRCA1* Exon15 Mutations in Bangladeshi Ovarian Cancer Patients

Sharmeen S[1], Bashar F[2], Islam R[3], Sultana M[4], Begum KN[5]

ABSTRACT

After cervical and uterine cancer, ovarian cancer is the third most frequent gynecological malignancy worldwide. BRCA1 mutations are the common predisposing factors for ovarian cancer development. Among the South Asian countries, most of studies are focused on Chinese, Indian, Pakistani populations. This study aims to identify BRCA1 exon15 mutations in a Bangladeshi ovarian cancer and benign gynecological disease patient population. This cross-sectional, comparative study was carried out in the Genetic Research Laboratory, Department of Anatomy, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, from March 2020 to July 2021. The mean age at diagnosis of ovarian cancer patients was found 44.9 years and benign gynecological diseases patients was found 35.6 years. Among them, about 40% patients were multiparous and 26.7% had family history of cancer. Upon Sanger sequencing of the targeted region (BRCA1 exon15) of the extracted DNA of thirty (30) ovarian cancer and fifteen (15) benign gynecological diseases patients, a mutation in one ovarian cancer patient was identified. The one mutation in ovarian cancer patient, C.4684C>T (P.Pro1562Ser) where 'C' was substituted by 'T'. This mutation was missense, and it was previously reported in ClinGen Allele Registry. Only 3.33% patient, within the age group of 18-50 years, were found to have mutations in their blood. Among this small samples size, one mutation in blood samples of ovarian cancer patients were identified.

Keywords: *ovarian cancer, sanger sequencing*

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INTRODUCTION

Ovarian cancer is now more in both developed and developing countries. Most of the research have been performed to find out the incidence, prevalence and risk factor of ovarian cancer. A large number of studies related to the mutation are based on American and Caucasian populations. However, some studies have focused on Asian populations. The incidence rate of ovarian cancer in the world is raising significantly. While approximately 90% of ovarian cancers occur sporadically, 10% of women with ovarian cancer have inherited genetic changes that predisposed them to ovarian cancer.[1] *BRCA1* gene encodes a nuclear phosphoprotein that plays a role in maintaining genomic stability, and it also acts as a tumor suppressor. *BRCA1* gene contains 22 exons and locates on 17q21.[1] *BRCA1* and *BRCA2* genes mutation are found in ovarian cancer, and it is estimated that about 44% of women who inherit a harmful *BRCA1* mutation and about 17% of women who inherit a harmful BRCA2 mutation will develop ovarian cancer by the age of 80.[1] An ovarian cancer cluster region (OCCR) in *BRCA1* exon15 has been identified. The prevalence rate of mutation in *BRCA1* exon15 is 9.7% in North Indian population.[2] When the mutation occurs in *BRCA1* gene, the encoded protein leads to

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the loss or reduction of function that makes the blockage in cell differentiation and also causes the defect in mechanism of cell apoptosis.[3]

However, we lack such genetic studies in our population. Therefore, we proposed this study to to identify BRCA1 exon15 mutations in a Bangladeshi ovarian cancer and benign gynecological disease patient population.

METHODS

This cross-sectional, comparative study was conducted in the Genetic Research Laboratory, Department of Anatomy, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. Our sample size in research was 53 in each group. The total sample size was 106 in both group but considering the financial constraint and COVID-19 situation, finally samples were collected from 30 diagnosed ovarian cancer patients for BRCA1 gene and 15 benign gynecological disease patients (ovarian cyst, ovarian teratoma and ovarian mass). With written informed consent, data were collected by the investigators from the patients by filling the data collection questionnaire. At the same time of data collection, each patient was made anonymous with code (study ID number). Safeguards were followed to ensure the confidentiality and security of all information obtained from ovarian cancer patients and benign gynaecological disease patients. Among socio-demographic character related variables patients' age, family history of ovarian cancer or breast cancer among the 1st degree and 2nd degree relatives, their parity and age of menopause (if applicable) were noted down. Mutation related variable included presence of the mutation (insertion, deletion, substitution, frame-shift mutation was considered) in genetic sequencing.

Procedure of DNA processing:

Blood sample collection and preservation: After taking consent, 5 ml of venous blood was collected carefully from each patient with proper safety measures. Three ml of venous blood was kept in EDTA (1mg/ml) containing tube and rest of blood preserved in microbiology lab.

Qualification and quantification of DNA: The quality (purity) and quantity (concentration) of DNA were measured with an integrated device called NanoDrop 2000/2000c spectrophotometer (Thermo Fisher Scientific, USA).[4]

Primer designing: Primers for BRCA1 exon15 was designed using Primer3Plus software. Exon15 (target region) has got 310 bp nucleotides. Both primers (forward primer and reverse primer) were designed such a way that they included both flanking regions (at 5' end and 3' end) of BRCA1 exon15. The General Settings were adjusted as per requirements which are mentioned below: [5]

- Product size was put at Product Size Ranges.
- Primer size was given 18 at Min box, 30 at Max box and optimum size 24 at Opt box.
- Specified primer temperature was 55°C and 65°C at Primer Tm box.
- 5° temperature was selected at Maximum Tm differences box.
- 40-60% GC content was given at Primer GC% box.

Primer validation: The designed primers were validated using OligoAnalyzer Tool of Integrated DNA Technologies (IDT) and Amplicon preparation by polymerase chain reaction (PCR).[5]

Agarose gel electrophoresis of amplicon: ***The product of PCR reaction were visualized using gel electrophoresis.***[5]

Purification of the amplicons: The amplicons were purified before Sanger sequencing. Purification was done using the Promega Wizard® DNA clean-up system, USA.

Qualification and quantification of the purified PCR product: The quantity and quality of the purified PCR products were measured with an integrated device called NanoDrop 2000/2000c spectrophotometer (Thermo Fisher Scientiic, USA) before sequencing. The

Primer	Sequence	Primer position	GC %	Tm (°C)
F	5/- tgtaaattaaacttctcccatt-3/	146716-146737	27.3%	52.6
R	*5/- acctacataaaactctttccag-3/*	*147106-147127*	*36.4%*	*52.2*

Fig.-1. *Primers (Forward and Reverse) with their length, GC content and number of Any and Self-dimer designed by Primer3Plus shows the product size (412 bp).*

ratio of the absorbance at 260/280 was used to see the purity of the DNA and concentration was estimated by measuring the absorbance at 260nm. The 260/280 ratio from 1.7 to 1.9 and the concentration from 10 ng/µl to 20 ng/µl was considered for the DNA sequencing.

Agarose gel electrophoresis of purified PCR product: Before DNA sequencing, the purified products of PCR were run again in agarose gel by using gel electrophoresis. For gel run, one to two microliters purified DNA sample was loaded in the well of the gel and hundred bp step ladder was used as molecular weight marker. After staining with ethidium bromide, the gel was placed in UV transilluminator to visualize the bands of desired targeted region. After validating the purified PCR products by gel run, it was sent for DNA sequencing by Sanger Sequencing.

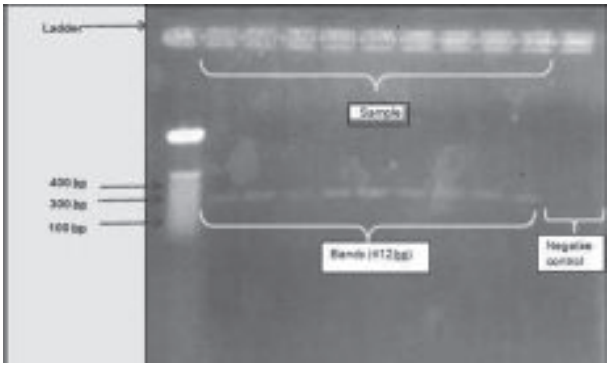

Fig.-2. *Gel electrophoresis of the PCR products on 1% agarose gel. The desired product size (412 bp) of the amplicons is shown in specific bands from lane 2 to 10 comparisons with standard 100 bp DNA ladder in lane 1. The well of the lane 11 was loaded with sample of negative control which shows no band indicating absence of contamination.*

Sanger sequencers generate a four-color chromatogram which represented the results of the sequencing run. For the present research, data obtained from the sequence in ABI files was analyzed using the Chromas® software, version 2.4.3. The interpretation of sequencing was done by following the instruction of 'Interpretation of Sanger sequencing', produced by University of Michigan, 2019.[6] The sequences were compared with the National Centre for Biotechnology Information (NCBI) database by the Basic Local Alignment Search Tool (BLAST) search.[7] The percentage frequency of mutations among the socio-demographic and reproductive characteristics of ovarian cancer and benign gynecological disease patients were analyzed by Statistical Package for the Social Sciences (SPSS) version 25.0. Continuous data were summarized using mean and standard deviation and the difference was determined by the t test. Chi-square tests were applied to compare categorical variables between groups across sociodemographic characteristics. If the P value is <0.05, it was considered as statistically significant.

The general quality control of the Genomic Research Laboratory, Department of Anatomy, Bangabandhu Sheikh Mujib Medical University (BSMMU), is maintained by regular monitoring, promoting a safe laboratory environment, facilitating staff safety. To ensure accuracy, the samples were stored in EDTA containing tube at -20°C for preservation and further use. All laboratory procedures were performed as per standard operating procedures (SOPs) with strict aseptic precaution to avoid contamination.

The study was approved by the Institutional Review Board (IRB) of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh.

RESULTS

A total of thirty adult Bangladeshi ovarian cancer and fifteen benign gynecological disease patients at or above eighteen years of age were selected for this study. The sociodemographic, the reproductive characteristics of ovarian cancer and benign gynecological disease patients were analyzed in this study. The study was aimed to identify mutation in *BRCA1* exon15 by Sanger sequencing in Bangladeshi ovarian cancer patients. Regarding ovarian cancer patients, only one ovarian cancer patient had a history of mutation (3.33%) (Table-I). Probability (P-value) of number of mutations was 0.063, this value was non-significant (P>0.05). Upon Sanger sequencing of the targeted region (*BRCA1* exon15) of the extracted DNA of thirty (30) ovarian cancer patients, one mutation in one patient was identified was caused by substitutions. One mutation identified in one patient, namely C.4684C>T (P.Pro1562Ser) where 'C' was substituted by 'T' which was missense shown in Table-II. The mean age of ovarian cancer was 44.9 years and mean age of benign gynecological diseases was 35.6 years (P=0.114), which was not statistically significant. This result indicates that 38-57 years age group was vulnerable group for ovarian

cancer and benign gynecological disease patients (Table-III). In this study family history of cancer was found in 26.7% of ovarian cancer patients and 53.3% in benign gynecological disease patients (P=0.078). This value was not statistically significant. Among the married women with ovarian cancer were found higher percentage (40%) of multiparous women than ovarian cancer with uniparous and nulliparous women and benign gynecological disease patients were found higher percentage (40.0%) of nulliparous women than benign gynecological disease patients of uniparous and multiparous women. P value was found 0.660; the difference was non-significant, as because multiparous patients in both groups were more affected but multiparity is protective factor for both groups.

Table-I. *Number of mutation between two groups*

Mutation	Ovarian cancer (N=30)		Benign gynaecological disease (N=15)		P value
	Frequency	Percentage	Frequency	Percentage	
Present	1	3.33	0	-	0.063^{NS}
Absent	29	96.7	15	100	
Total	30	100	15	100	

P value reached from Chi-square test; NS=non-significant

Table-II. *Types and groups of mutations with nucleotide and amino acid changes*

Sample ID group	Nucleotide change	Amino acid change	Type
OC-12Nonsynonymous	C.4684C>T	P.Pro1562Ser	Missense

Table-III: *Age distribution of the participants*

Age group	Ovarian cancer (N=30)		Benign gynaecological disease (N=15)		P value
(in years)	Frequency	Percentage	Frequency	Percentage	
18-37	10	33.3	6	40.0	0.114^{NS}
38-57	11	36.7	8	53.3	
58-77	9	30.0	1	6,7	
Total	30	100	15	100	
Mean±SD	44.9±19.1 years		35.6±11.9 years		

P value reached from (Unpaired Student's t-test); NS=non-significant

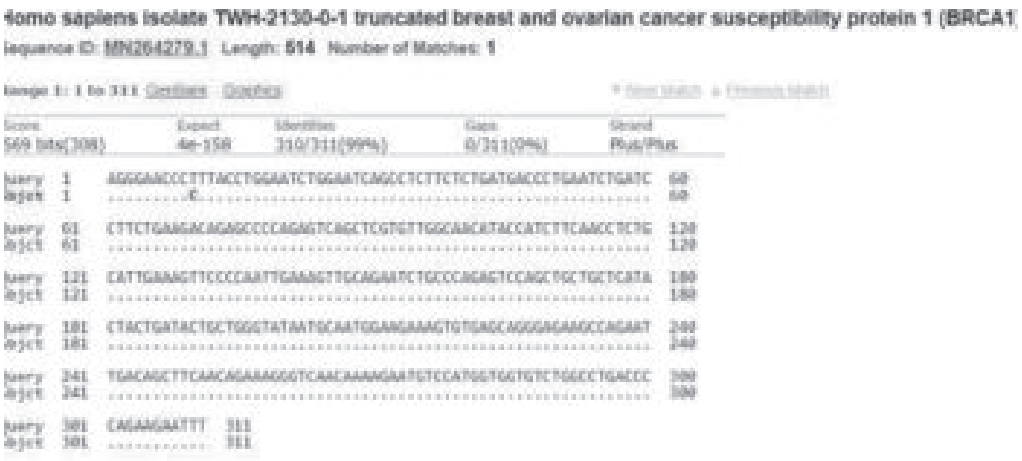

Fig. 3. *Sequencing analysis of sample ID- OC-12 using BLAST search*

Fig.-4: *Sequencing analysis of sample ID- OC-12. Here, at the 'C' has been substituted by 'T' (c.4684 C>T) as shown by the arrow. (using ClinGen Allele Registry).*

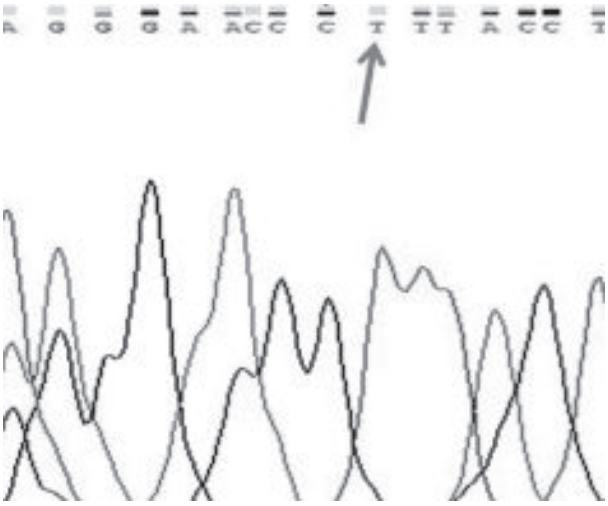

Fig.-5 *Sequencing analysis of sample ID – OC-12. Here, at the 4684th nucleotide position 'C' has been substituted by 'T' (c.4684 C>T) as shown by the arrow (using Chromas software).*

DISCUSSION

In our study, the mutation identified in one ovarian cancer patient, namely C.4684C>T (P.Pro1562Ser) where 'C' was substituted by 'T' which was missence and nonsynonymous group because proline was replaced by serine. In this study, only one ovarian cancer patient had a history of mutation (3.33%). Probability (P value) of number of mutation was 0.063, this value was non-significant because Pd"0.05 was considered as significant. The prevalence rate of ovarian cancer in Bangladesh has 3.3% in the last five years[8], as because the cause of ovarian cancer is not only the mutation of *BRCA1* gene or genetic factor but also the several pathways and risk factors are lead to ovarian cancer. Hansen et al. found that a nucleotide C.4684delcc mutation in two Greenlandic Inuit families with ovarian cancer.[9] In this study, identified mutation in one ovarian cancer patient is C.4684C>T, this mutation is a new variant.

In the present study, the mean age of ovarian cancer patients was 44.9 years, while mean age of benign gynecological diseases was 35.6 years; the incidence of ovarian cancer and benign gynecological disease patients diagnosed at age range between 18 and 77 years. Several studies in Bangladesh related to ovarian cancer found almost similar average age of diagnosis. Hossain et al. reported that the mean age was 53 years (range 25-80 years) in 2017–2018[10] and Saha et al. found that the mean age was 47.44 years (range 20-70 years) in 2013–2015[11] and Deeba et al. also found that the mean age of ovarian cancer patients was 40.6 (range 13-63 years) in 2008–2009.[12] In our study, P value of age at diagnosis was 0.114, this value was not significant, probably because of our small sample size. Another study in Bangladesh done by Chowdhury et al. found 77.77% of ovarian tumors in women aged between 41 and 59 years[13] and our study revealed that the majority of ovarian cancer patients are aged 38 to 57 years, which in congruence with the previous study.

In this study, family history of cancer was found in 26.7% of ovarian cancer patients and 53.3% in benign gynecological disease patients and P value of family history of cancer was 0.078, which was not significant. However, results of another case-control study showed that the risk of ovarian cancer increases in women with a family history of breast, uterine, or ovarian cancer in their mother or sister (P<0.001), which signifies that family history of breast or ovarian cancer is one of the most important risk factors for ovarian cancer.[14]

When compared to women who have three or more children, nulliparous women had a risk of 2.12 (95% CI: 1.81–2.48) of having ovarian cancer.[15] The overall number of nulliparous and uniparous ovarian cancer and benign gynecological disease patients is higher than the multiparous women in this study, and the majority of the multiparous women had two children. Pregnancy may protect against ovarian cancer, according to the findings of various research.[16] According to the findings of a case-control research women who have a live birth (P<0.001) or an induced abortion (P<0.05) have a lower risk of ovarian cancer, and this risk reduces as the number of live birth cases increases (P<0.001).[17]

In the present study, this study was found only one ovarian cancer patient who had a history of early menopause (35 years). Globally, ovarian cancer is most usually detected after menopause, between ages of 60 and 64 years, with the average age of diagnosis being 63 years.[18] Furthermore, ovarian cancer affects 90% of women over the age of 45, and 80% of women over the age of 50.[18] Ovarian cancer affects 60% of women below the age of 50 and 40% of women over 50, according to this study. The mean age of ovarian cancer patients above 45 years was higher than that of patients aged below 45 and the mean age of benign gynecological disease patients below 45 was higher than that of patients aged above 45 years in the present study.

CONCLUSION

Ovarian cancer is caused by a variety of variables, with hereditary factors being one of the most prominent. The mutant patient of this present study was papillary serous cyst adenocarcinoma who had a history of *BRCA1* exon15 mutation and she was a multiparous woman, lactating mother and used oral contraceptive. The identification of *BRCA1* cancer predisposition genes opened the doorway to a new era of genetics-based cancer prevention and risk assessment. *BRCA1* mutation prevalence is high and is nearly identical among women of diverse ethnicities who undergo clinical genetic testing. Although the present study has pointed out some limitation such as sample size and *BRCA1* exon, but the results of the study have shown some hope for another study in the future. Further studies are needed to clarify the mutation analysis of ovarian cancer patients regarding *BRCA1* exon. Clinical genetic testing is an integral component of hereditary ovarian cancer risk assessment and should be considered in all high-risk women.

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