RESEARCH ARTICLE

Molecular profiling of BRCA1/BRCA2 gene variants in Pakistani breast cancer patients

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Abstract

Objective: To determine the frequency of genetic variants in breast cancer types 1 and 2 gene in breast cancer Pakistani patients.

Method: The case-control study was conducted at the Islamic International Medical College and Pakistan Railway Hospital, Rawalpindi, Pakistan, from October 2022 to August 2023, and comprised females with breast cancer in group A, with an age range of 23–83 years (mean 51.9 ± 10.8 years) and as many healthy, age-matched female controls in group B, with age range of 26–82 years(mean 57.8 ±10.1 years). Peripheral blood samples were taken from all the participants. Allele-specific tetra amplification refractory mutation system-polymerase chain reaction was used to determine the frequency of c.4485G>A, c.4508C>A, c.5278G>C and c.5503C>T genetic variants in breast cancer type 1 gene, and c.92G>A and c.3109C>T genetic variants in breast cancer type 2 gene. Data was analysed using SPSS 27.

Results: Of the 336 female participants 168(50%) were in each of the 2 groups. None of the group B controls had genetic variants in breast cancer type 1 and 2 genes. In group A patients, 4(2.3%) had the variant c.4485G>A in breast cancer type 1 gene, while other breast cancer type 1 variants were negative. Also, 9(5.3%) group A patients had variants c.92G>A and c.3109C>T in breast cancer type 2 gene.

Conclusion: Cost-effective, polymerase chain reaction-based genetic testing could effectively identify mutant variants of breast cancer types 1 and 2 genes in the Pakistani population.

Keywords: Breast cancer, BRCA1, BRCA2 genes, Allele-specific ARMS-PCR, Genetic testing. (JPMA 75: 37; 2025)

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Introduction

Breast cancer is a global health concern, being the most commonly diagnosed cancer and a leading cause of cancer-related deaths among women worldwide.[1] In Pakistan, breast cancer is the most prevalent form of cancer, ranking first among the top 10 malignancies in 2022.[2] The risk of developing breast cancer has risen, with approximately one in nine Pakistani females facing a lifetime risk of being diagnosed with the disease.[3] According to the World Health Organisation (WHO) 2020 cancer report for Pakistan, the prevalence of breast cancer was 12%, and the incidence was 14.5%.[4] Unfortunately, due to delayed referrals to appropriate healthcare facilities and late diagnoses, the mortality rate for breast cancer patients in Pakistan remains high.[5]

Approximately 5-10% of all breast cancer cases are caused by genetic factors.[6] The main genetic cause is germ-line mutations in the breast cancer type 1 (BRCA1) and breast cancer type 2 (BRCA2) genes, which are associated with breast and ovarian cancers.[7] These genes are considered the most significant susceptibility genes for breast cancer. Inherited mutations in BRCA1 and BRCA2 genes account for about 3% of breast cancers.[8] While BRCA1 and BRCA2 genes normally protect against certain tumours, mutations in these genes can impair their function. Recent research has revealed that these genes play important roles in two essential cellular processes: deoxyribonucleic acid (DNA) damage repair and regulation of transcription.[9]

A recent study has identified several variants in BRCA1 and BRCA2 genes by Next Generation Sequencing (NGS).[10] According to results from a prior study, BRCA1 and BRCA2 mutations are responsible for 1 in 8 cases of early-onset breast cancer in Pakistan, and 1 in 4 cases of hereditary breast/ovarian cancer.[11] The literature indicates that germ-line BRCA mutations exhibit high penetrance in breast and ovarian cancers, mostly following an autosomal dominant inheritance pattern.[12] In women at increased risk for breast cancer, the identification of a mutation in breast cancer genes BRCA1 and BRCA2 has important implications for screening and prevention counselling. Mutations in BRCA1 or BRCA2 can result in defects in DNA repair, and can be targetted for developing new therapies specifically designed for individuals with these mutations. In families where a

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mutation has been identified, it is possible to definitively determine whether unaffected female relatives carry the mutation, which forms the basis for predictive genetic counselling.[13]

In Pakistan, genetic testing of BRCA1 and BRCA2 mutations has not been established yet, while Sanger sequencing and NGS have been employed in Pakistan for identifying BRCA mutations. These methods entail substantial costs and demand specialised technical expertise, rendering them unsuitable for widespread public screening.

The current study was planned to determine the frequency of genetic variants in BRCA1 BRCA2 genes in breast cancer Pakistani patients.

Subjects and Methods

The case-control study was conducted at the Islamic International Medical College (IIMC) and Pakistan Railway Hospital (PRH), Rawalpindi, Pakistan, from October 2022 to August 2023. After approval from the IIMC ethics review committee, the sample size was calculated using an online calculator[14] while assuming 10% prevalence of BRCA genes[6] while keeping 95% confidence level, 5% margin of error, and a 1:1 patient-to-control ratio. To enhance the strength of the study, the sample size was inflated by >85%. The sample was raised using non-probability convenience sampling technique.

Those included were females who were diagnosed cases of breast cancer, in any stage of breast cancer, of all ethnicities, undergoing breast cancer treatment, and willing to participate. Those excluded were females not willing to participate, and males. Those enrolled were placed in group A, and as many healthy, age-matched females with age ranging +/- 3 years were enrolled in control group B having no personal or family history of breast cancer or any other malignancy. All the participants furnished informed consent.

Venous blood samples were collected via phlebotomy, and were transported to the laboratory in vacutainers containing sodium ethylenediaminetetraacetic acid (EDTA). They were preserved at 4-8 degrees Celsius until further analysis. Genomic DNA was isolated from the blood samples, according to the standard Chelex method[15], by using a 7% Chelex solution, and the extracted DNA was stored at -20°C before amplification. The quantification and purity of the extracted DNA samples were done by measuring the absorbance at 260nm and 280nm (A260/A280) on Evolution ™ One/One Plus UV-Vis Spectrophotometer (Thermo Fisher Scientific, Cat#840-341400, Waltham, MA, USA). Allele-specific primers with different lengths were designed by using an online tool[16,17] and checked by the National Centre for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST).[18,19] The Variants of BRCA1 and BRCA2 genes were genotyped using tetra-primer amplification refractory mutation system-polymerase chain reaction (T-ARMS PCR) in a thermal cycler (Major Sciences, United States). This was done to determine the frequency of c.4485G>A, c.4508C>A, c.5278G>C and c.5503C>T genetic variants in BRCA1 gene, and c.92G>A and c.3109C>T genetic variants in BRCA2 gene. The PCR reaction mixture, with a total volume of 25 µl, was prepared in a 0.2 ml PCR tube. It contained 2 µl of DNA template (~100 ng/µl), 12.5 µl of master mix (including 4 mM magnesium chloride, Deam Taq Green PCR Master Mix Cat#K1081, Thermo-Scientific), 1 µl of primer mix (each primer at 5 pmol/µl), 1.25 µl of 5% dimethyl sulfoxide (Pierce ™DMSO, Sequencing Grade Cat#20688 M/s Thermo Fischer Scientific), and up to 8.25 µl of PCR-grade water (Ultrapure™ DNase/RNase-Free Water Invitrogen, Life Technologies Limited, Cat# 10977-023, Paisley, PA49RF, UK) to reach the final volume. PCR reaction was run according to the following conditions; holding time at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 sec, annealing at 58°C for 30 sec, extension at 72°C for 30 sec and final extension at 72°C for 5 minutes. The amplified products of each reaction were separated on 2% agarose gel premixed with 1% ethidium bromide solution in 1x Tris/Borate/EDTA (TBE) buffer and compared to 100bp and 50bp Gene Ruler ladders [(The Gene Ruler 100 bp and 50 bp Plus DNA Ladder, ready-to-use (Thermo Fisher Scientific, Cat. No. SM0323, Vilnius, Lithuania)] The gel was placed on an ultraviolet (UV) trans-illuminator [GeneBox SN.DR4V2/2987, model No. GBox F3 (Gene Box, UK)] Amplified DNA fragments appeared as white bands against a dark background. A permanent record of the gel was kept by capturing a picture in secure Saccharomyces Genome Database (SGD) format with a UV camera, and the images were stored in a gel documentation system.

Data was analysed using SPSS 27. Frequencies and percentages were determined for descriptive statistics.

Results

The age range of females in Group A (cases) was 23 to 83 years, with a mean age of 51.9±10.8 years. In Group B (controls), the age range of females was 26 to 82 years, with a mean age of 57.8±10.1 years. Of the 336 female participants 168(50%) were in each of the 2 groups. None of the group B controls had genetic variants in BRCA1 and BRCA2 genes. In group A patients, 4(2.3%) had the variant c.4485G>A in BRCA1 gene with mutant heterozygous 4485 GA genotype, while other BRCA1 variants were found negative with homozygous 4508CC, 5278GG and 5503CC wild-type genotype. Also, 9(5.3%) group A patients had variants c.92G>A and c.3109C>T with heterozygous 92GA and 3109CT genotypes in BRCA2 gene (Table 1).

Table-1: Distribution of genetic variants among breast cancer patients (n=168).

Gene	Genetic Variants	Homozygous wild (W) n(%)	Homozygous mutant (M) n(%)	Heterozygous n(%)
BRCA 1	c.5278G>C	168(100)	0	0
BRCA 1	c.4508C>A	168(100)	0	0
BRCA 1	c.5503C>T	168(100)	0	0
BRCA 1	c.4485G>A	164 (97.6)	0	4(2.38)
BRCA 2	c.92G>A	163(97.02)	0	5(2.97)
BRCA 2	c.3109 C>T	164 (97.6)	0	4(2.38)

BRCA: Breast cancer.

Table-2: Distribution of breast cancer (BRCA) types 1 and 2 gene variants with clinical and personal features of the study population.

Parameter	BRCA gene 1		BRCA gene 2	
	Positive	Negative	Positive	Negative
Age at time of diagnosis				
<35years	3 (1.7)	9 (5.3)	5(2.97)	7 (4.1)
>35 years	1 (0.5)	155 (92.2)	4 (2.3)	152 (90.4)
History of Menopause				
Pre-menopause	2 (1.1)	68 (40.4)	6(3.5)	27 (16.0)
Post-menopause	2 (1.1)	96 (57.14)	3 (1.7)	132(78.5)
Family history				
Present	3 (1.7)	23 (13.6)	5(2.97)	20 (11.9)
Absent	1 (0.5)	141 (83.9)	4 (2.3)	139(82.75)

Overall, 13(7.7%) patients were positive for BRCA1 and BRCA2 genes. Of them, 8(61.5%) patients exhibited a positive family history of breast cancer, while 5(38.4%) did not have any family history of the disease. At the time of diagnosis,

8 (61.5 %) patients were aged <35 years and 5(38.5%) were aged >35 years (Table 2).

Discussion

The current study aimed at establishing a cost-effective PCR-based genetic test focussing on BRCA1 and BRCA2 genetic variants in breast cancer cases.

A total of 336 study participants including patients (n=168) and healthy controls (n=168), no mutation was detected for 3 of the BRCA1 gene variants; c.4508C>A, c.5278G>C and c.5503C>T. These findings differ from a study which documented a notable number of mutations in these specific variants of the BRCA1 gene[11]. However, some mutations were detected in BRCA gene variant; c.4485G>A and BRCA2 gene variants c.92G>A and c.3109C>T. But, owing to limitations in both time and resources, the current study could not undertake the necessary sequencing protocols essential to authenticate and verify the mutations identified. These sequencing procedures are crucial to establish the precision and reliability of the detected mutations.

In the present study, mutations were detected in 7.7% of the patients, with 4 mutations found in BRCA1 and 9 mutations in BRCA2, corresponding to 2.3% and 5.3% respectively. This contrasts with the findings from a study which indicated that BRCA1 mutations were 5 times more prevalent than BRCA2 mutations[11]. Discrepant outcomes were documented in 2 studies conducted in South India[20] and Saudi Arabia[21]. Consistent results were reported in other Asian studies from China[22], Malaysia[23], Korea[24] and Indonesia[25], where BRCA2 mutations were observed at an equal or a higher frequency than BRCA1 mutations.

In the current study, around 7.7% of the sample had genetic changes in either the BRCA1 or BRCA2 genes, indicating that while BRCA gene variations play a role in increasing the risk of breast cancer, they are not the sole contributors. The risk of developing breast cancer can result from a complex interplay of multiple factors, extending beyond BRCA genes. These factors encompass other genetic factors, lifestyle factors, and environmental influences.

In the current study, 8(61.5%) patients with mutations in BRCA1 and BRCA2 genes were diagnosed with breast cancer at a younger age, specifically, <35 years. This was further substantiated by the elevated proportions of BRCA-positive patients within the group that had a family history of breast cancer. This suggests that these genetic variations emerged with a distinct family or hereditary trend, implying a direct connection with breast cancer family history. This finding is quite noteworthy and suggests a potential link between these genetic mutations and the onset of breast cancer at an earlier stage in life.

It underscores the importance of genetic screening and counselling, especially for those with a family history of breast cancer, as it can help identify those at higher risk and potentially lead to more proactive medical management and surveillance. These findings are in concordance with earlier studies.[26,27]

In the current study, most patients identified with mutations were within the pre- menopausal age group, which was consistent with previous studies.[20,28]

The current study has some limitations as whole gene sequencing to identify novel variants could not be conducted due to time and financial constraints.

Conclusion

The prevalence of the genetic variations of interest was notably higher in individuals aged <35 years at the time of diagnosis, suggesting a potential association with an increased risk of early-onset breast cancer. Genetic testing and screening strategies for this age group may be helpful. Additionally, individuals with a family history of breast cancer showed a higher frequency of detected mutations, emphasising the importance of genetic counselling for

families carrying BRCA-mutated genes.

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Author Contribution:

BG: Concept, design, drafting, data analysis and revision.
MA: Concept, design, final approval and agreement to offer specialized knowledge.
AR: Revision and final approval.
ZB: Data acquisition and interpretation.
NZ: Drafting.
AA: Data acquisition, interpretation and agreement to offer specialized knowledge.