

RESEARCH ARTICLE

Screening for Del 185 AG and 4627C>A BRCA1 Mutations in Breast Cancer Patients from Lahore, Pakistan

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Abstract

Breast cancer contributes to approximately 23% of the cancer cases identified and 14% of cancer related deaths worldwide. Including a strong association between genetic and environmental factors, breast cancer is a complex and multi factorial disorder. Two high penetration breast cancer susceptibility genes (BRCA1 and BRCA2) have been identified, and germ line mutations in these are thought to account for between 5% and 10% of all breast cancer cases. The human BRCA1 gene, located on 17q, is involved in the regulation of cell proliferation by aiding in DNA repair, transcriptional responses to DNA damage and cell cycle check points. Mutations in this gene enhance cell proliferation and facilitate formation of tumors. Two mutations, the 185 deletion of AG and the 4627 substitution from C to A, are founder mutations in the BRCA1 gene for breast cancer in Asian populations. Allele specific PCR was performed to detect these selected mutations in 120 samples. No mutation of 4627 C to A was detected in the samples and only one of the patients had the 185 del AG mutation in the heterozygous condition. Our collected samples had lower consanguinity and family history indicating the greater involvement of environmental as compared to genetic factors.

Keywords: Allele specific PCR - BRCA1 - breast cancer - founder mutation - Lahore, Pakistan

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Introduction

Breast cancer is a malignant tumor that starts in the cells of the breast; these cancer cells can invade surrounding tissues or metastasize to distant areas of the body. Breast cancer is the second major cause of deaths due to cancer in the women worldwide and contributes to 23% of the cancer cases identified (DeSantis et al., 2014). Typically, breast cancer in South-east Asian region is characterized by aggressive disease affecting younger age women with late stage presentations due to lack of awareness and improper facilities for prognosis and diagnosis (Malik et al., 2002).

Pakistan is one of countries that have high burden of breast cancer with more than half of the patients presenting in advanced stages of disease (stages III and IV) (Hanif et al., 2009). According to internationally accepted guidelines, regular mammography and clinical examination can result in decreased incidence rates of breast cancer among women (Aziz et al., 2003; Ban and Godellas 2014). Pakistan has one of the highest rate of the breast cancer in Asian countries with yearly death rate of 40,000 women (Ayub et al., 2015), and a large proportion of cases is below 40 years. Pakistani population also has highest incidence of genetic breast cancer with 6.7% of BRCA mutations in breast cancer (Asif et al., 2014). The age-standardized rate (ASR) of breast cancer in Karachi,

Pakistan is 51.7 per 100,000 per year and it is considered as the highest rate ever reported for any Asian country, excluding Israel (Liede et al., 2002). The spectrum of the BRCA1 and BRCA2 mutations is heterogenous in diverse population except for few common mutations of the genes (Hansa et al., 2012).

Strong inherited component are identified for 5%–10% of breast cancer cases, while a small fraction of these cases (4%–5%), explained by mutations in high penetrant genes transmitted in an autosomal dominant manner. Studies have shown that BRCA genes are the most commonly mutated genes, while other genes causing hereditary breast cancer are also emerging (Walsh et al., 2010).

Normal expression of BRCA genes is involved in error-free repair of DNA double-strand breaks or in cases where repair cannot be done they help to destroy the damaged cells. If BRCA genes are damaged by a BRCA mutation, cell loses the error-free repair function and this increases the risk for breast cancer (Friedenson 2007). One splice site and five frame shift and truncation mutations in BRCA1 gene (2080insA, 185 del AG, 3889delAG, 4184del4, 4284delGA, and 4627C>A) have been categorized as founder mutations in Pakistan (Rashid et al., 2006).

This study was done to find out the BRCA1 founder mutations 185 del AG and 4627C>A in patients of breast cancer from our local population.

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Materials and Methods

Sample collection

The study included 120 samples (80 patient and 40 control). 2-3 ml of venous blood was collected in EDTA containing vials, from all the patients and controls with informed consent. Patients were collected from the Jinnah Hospital, Lahore and INMOL (Institute of Nuclear Medicine and Oncology) Hospital, Lahore.

DNA isolation and characterization

DNA was isolated with modified choloform phenol extraction method and using kit method (Favorgen Biotech Corp.). Extracted DNA was observed on 1.5% agarose gel.

Allele specific PCR

Allele specific primers for two most common BRCA1 gene mutations were prepared using DNA sequence from GENE BANK of NCBI (National Center for Biotechnology Information) and allele confirmation by Ensembl. Primer complementarity was checked using oligocalc and an additional 3rd last base mismatch was generated to avoid non specific binding (Table 1).

For each sample two reactions of PCR were used. One using normal primer set and other using mutated allele specific set. PCR was done using 1.5mM MgCl₂, 200 μM each dNTP's 0.5 units of Taq polymerase, 0.4μM of each primer, 12 μl of water and 1μg of DNA sample. Thermocycling conditions were 95oC for 4 min, followed by 30 cycles each at 94oC for 30 sec, 55.6oC for 45 sec, 72oC for 1 min, then final extension at 72oC for 10 min and store at 4oC.

Results

Demographic data

A total of 120 samples were collected between December 2013 to May 2014 with mean age of 46.21± 11.6 years (Table 2). Age of disease onset was observed to be 44.3 ± 11.4 years. Among the breast cancer patients 2.5% were the cases of bilateral breast cancer while 97.5% were unilateral 56.3% of patients were presented with grade 3 of disease (Table 3).

Genotyping Results For BRCA1 Mutations

Genotyping was done using allele specific PCR for BRCA1 185delAG and 4627 C to A. One of the samples showed the mutation 185delAG in heterozygous state. Figure 1, represents the bands both from normal and mutated alleles, which demonstrated the presence of the heterozygous condition.

The patient with a positive deletion of AG was a female of 35 year of age she had a family history of breast and ovarian cancers and high degree of consanguinity was observed in her family. She was diagnosed just after few months of initial symptoms (age 34.5 years) and at the time of observance she was at Stage IIIb and Grade 3. Her BMI was 44.4 which placed her in the obese personal group.

In case of substitution of C to A at locus 4627 on exon 15 no mutation was observed in any of the cases assessed in this study.

Table 2. Age Range of Patients at Time of Presentation and Diagnosis

No.	Age range (years)	No of patients	
		At time of presentation n(%)	At time of diagnosis n(%)
1	20 – 30	6 (7.5)	7 (8.75)
2	31 – 40	25 (31.25)	26 (32.5)
3	41 – 50	26 (32.5)	25 (31.25)
4	51 – 60	13 (16.25)	16 (20)
5	61 – 70	9 (11.25)	5 (6.25)
6	71 – 80	0	0
7	81 – 90	1 (1.25)	1 (1.25)

Table 3. Distribution of Patients with Stage and Grade

Sr. No.	Stages	Percentages of patients	Grade	Percent patients
1	I	4.41	1	1.58
2	II	26.47	2	46.03
3	III	36.76	3	52.38
4	IV	32.35	-	

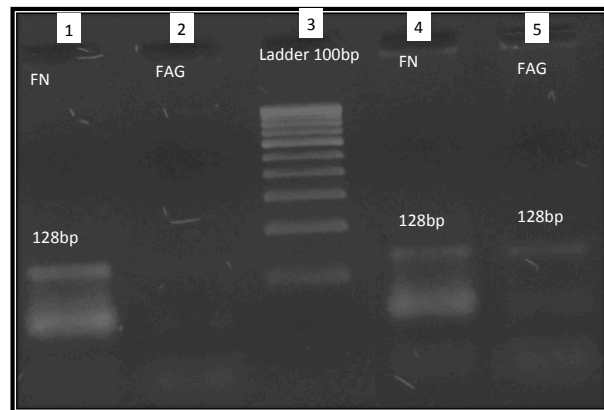


Figure 1. Allele Specific PCR for Mutational Analysis of BRCA1 185delAG (2% agarose gel). (Lane 1: Normal allele without AG mutation in control, Lane 2: Mutated allele without AG mutation in control sample, lane 3: 100 bp DNA ladder, Lane 4: Normal allele without AG mutation in patient, Lane 5: Mutated allele showing AG mutation in patient)

Table 1. Sequences of Primers Used for Allele Specific PCR

No.	Name	Sequence	No. of bases	Product size
1	B1-185-FAG	GCTATGCAGAAAATCTTAGT	20	128 bp
2	B1-185-FN	GCTATGCAGAAAATCTTACAG	21	
3	B1-185-R	GTCTTTTCTTCCCTAGTATGT	21	
4	B1-4627-FN	CCCCTTCTAAATGCCCGTC	19	474 bp
5	B1-4627-FM	CCCCTTCTAAATGCCCGTA	19	
6	B1-4627-R	CACTATGCCTGGCCTGAAA	19	

Discussion

BRCA1 and BRCA2 mutations play critical role in almost 12% of all breast cancer cases. One splice site in addition to four truncation and frame shift mutations in the BRCA1 gene list the founder mutations for breast cancer in local Pakistani population. The most common alterations in the BRCA1 gene, identified so far, include 185delAG and 4627C>A that produce truncated protein (Rashid et al., 2006).

The reported study was conducted on the 120 cases (80 patients and 40 controls) to confirm the presence of these both mutations as founder mutations in Pakistani population. The subject data for both control and patient was collected regarding age, gender, weight, height, body mass index, stage, grade, receptor status and family history. Most of the cases included were between the age range of 31-50 years and the average age of disease onset was 44.33 years that indicated early onset and early diagnosis of the disease. It is reported that the good prognosis is applied to the women of ages 45-49 years (Adami et al., 1986; Assi et al., 2013).

Family history is one of the important factors that contribute to the occurrence of disease. Women with positive family history of the breast / ovarian cancer experience disease differently compared to those without any family history in relation to early onset and metastasis (Petrissek et al., 2000). In the current study 12.5% patients had a positive family history for breast/ ovarian cancer. Specifically the one patient described with mutation (185delAG) had a strong family history and an early disease onset (34.5 years). The rapid spread of cancer ensured that genetic mutations have a strong role in early and aggressive development of cancers.

It can be concluded from the present study that in Pakistani population 185del AG and 4627C>A of BRCA1 may serve as founder mutations only in certain ethnic groups or specified locations. Rashid et al, 2006 described the presence of 185delAG in Pathan and 4627C>A in Punjabi populations. There may be other factors (like obesity) that serve as major contributors in this early age cancer presentation in our local population. So there is a need of proper awareness programs about self examination and early detection of breast cancer. The use of chemotherapy as a treatment therapy for breast cancer should be a top priority. Proper care and management of cancer patients should be ensured, dedicated cancer hospitals should be available and accessible at reasonable costs to every person. If clinicians, genetic counselors and laboratory workers collaborate and share data with each other the incidence of cancer based mortalities can be reduced in Pakistan.

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