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Investigation of Mutations of Exon 11-A of BRCA1 Gene in Women with Breast Cancer in the Northwest of Iran

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ABSTRACT

BACKGROUND AND OBJECTIVE: Breast cancer is the most common cancer in women, which is associated with genetic changes such as mutations in carcinogenic genes and tumor suppressor genes. One of the most important tumor suppressor genes involved in breast cancer is the BRCA1 gene. The mutation in this gene is a common occurrence in human breast cancer. The purpose of this study is to investigate the mutations of exon 11-A of BRCA1 gene in women with breast cancer in the northwest of Iran.

METHODS: In this descriptive study, blood sample were collected form 40 patients with breast cancer whose cancer was diagnosed before the age of 40 years and the exon 11-A of BRCA1 gene was examined using PCR and direct sequencing methods to detect mutations. Sequencing results were analyzed using Chromas software.

FINDING: In the present study, a nonsynonymous mutation was reported as a new mutation of BRCA1 gene for the first time: Ala584Thr mutation was also observed in two samples. The mutations of codon 694 (Ser694Ser) showed a higher incidence (52.5%). Other mutations were observed in codons 693, 356, 486, 550 and 628.

CONCLUSION: Based on the results of this study, mutations and polymorphisms of exon 11 of BRCA1 gene were observed for the first time in the northwestern population of Iran. One new case of mutation was observed in exon 11-A of BRCA1 gene.

KEY WORDS: *Breast cancer, BRCA1, Exon 11-A, mutation*.

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Introduction

Breast cancer is known as the most abundant and most fatal malignancy in women, accounting for about 16% of cancer deaths around the world (1). This disease is the cause of a large number of deaths among women in all developed, developing and underdeveloped countries each year (2).

Breast cancer occurs in both hereditary and non-hereditary forms, and most cases occur in non-hereditary form and without family history of breast cancer (3). Two groups of hereditary predisposing genes with a high incidence of family history of breast cancer have been identified, which include high risk predisposing genes and medium to low risk predisposing genes. BRCA1 is a high risk genotype for breast cancer (4).

BRCA1 gene is a genome-aware gene and plays a role in repairing DNA damage. Therefore, inactivity of this gene results in genomic instability. BRCA1 is located as a tumor suppressor gene on the chromosome 17q21, which has a length of about 100 kb that has 23 exons and encodes 1863 amino acids. Exon 11 encodes the middle portion of the BRCA1 protein that can interact with the Rad51 protein and is involved in repairing DNA with this mechanism. Most people who are prone to high risk breast cancer have mutations in the BRCA1 gene (5).

In a review article by Nematzadeh et al. reviewing 13 studies on BRCA1 mutations, a total of 100 mutations in the BRCA1 gene were reported in these 13 studies, and of these 100 reported mutations, 20 mutations were related to exon 11-A of BRCA1 gene (6). Akbari et al. in 2013 reported the mutations in the BRCA1 gene (7).

Since the incidence of mutation is highly dependent on the geographic location and the ethnicity of the studied population, and the number of mutations detected in BRCA1 is increasing and also because breast cancer is considered as the first identified cancer among Iranian women (5), the aim of this study was to investigate the mutations of exon 11-A of BRCA1 gene in women with breast cancer in the northwest of Iran.

Methods

This descriptive study was conducted in Genetics Laboratory of Tabriz University during the years 2015 to 2016.

Selection of samples: Patients were selected from among the referents to the hospitals of Noor Nejat and Imam Reza in Tabriz based on indicator features, including type of breast tumor, age of the patient diagnosed with breast cancer and gender. A total of 40 women with malignant tumors of the mammary glands with a diagnosis age of 40 years and below were selected for the study. Most patients (96%) had invasive ductal carcinoma and the remaining patients (4%) had invasive lobular carcinoma. Sampling was done after obtaining informed consent from the patients.

DNA extraction and mutation examination: Genomic DNA was extracted from patients' blood samples using a standard salting-out method (8) and was used for mutation examinations.

DNA amplification by polymerase chain reaction (PCR) and direct sequencing: Exon 11 of BRCA1 gene was amplified by PCR using specific primers of this exon (Table 1). Each PCR reaction contained 1.5 µl of genomic DNA, 0.5 µl of deoxynucleotide triphosphates (dNTPs), 0.9 µl of magnesium chloride ($MgCl_2$), 0.7 µl of each of the forward and reverse primers, 0.15 µl of Taq DNA Polymerase, 2.5 µl buffer 10X and 18.95 µl of double – distilled water (the materials were obtained from CinnaGen Co. in Tehran) at a total volume of 25 µl. The PCR program was as follows: Initial denaturation at 94 °C for five minutes. Denaturation at 94 °C for 30 seconds. Annealing temperature (primer binding) was considered at 59 °C for 45 seconds.

The temperature of 72 °C was set for 30 seconds for the expansion stage. 72 °C for five minutes for 35 cycles, final expansion stage. PCR products were observed with 2% agarose gel electrophoresis; then the PCR products were sent to Pishgam Tehran Co. for sequencing and the results of the sequencing were recorded in “Basic Local Alignment Search Tool” (BLAST) and were reviewed using Chromas software.

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Table 1. Primers used for PCR for breast cancer samples

Primer name	Primer sequence (5` > 3`)
Forward Primer[1] 1	ACCTCCAAGGTGTATGAAGTATG
Reverse Primer[2] 1	TGGTAGAAGACTTCCTCCTCAG
Forward Primer 2	ATGAGCTTTAATATGTAAAAGTG
Reverse Primer 2	TTTGTTAACTTCAGCTCTGGG

[1]Forward, [2]Reverse

Results

In this study, most of the mutations identified in the BRCA1 gene were missense substitution (Table 2). A new mutation observed in this study was: Ala584Thr. In two women with invasive ductal carcinoma with a diagnosis age of 34 and 39 years, a new mutation has been identified that has not been reported in any other study. The mutations of codon 694 (Ser694Ser) showed a higher incidence (52.5%) in the studied samples. The missense mutation of Gln356Arg was found in 12.5% of the patients with BRCA1 gene mutation. In two cases with an Arg584Agg mutation, missense mutation of Ser694Ser was also observed. Four mutations with pathogenic effects were identified in three patients, including missense mutations of Phe486Leu, Asn550His, Ser628Arg and Asp693Thy. Chromatograms of the identified mutations are shown in Fig. 1. The number of mutated samples based on codon number is shown in Fig. 2. 47.5% of the identified mutations were of nonsynonymous type and 52.5% were synonymous. The observed single nucleotide polymorphisms include: a > g, g > a, t > c, c > t (Transition), a > c, t > g, g > t (Transversion), and the frequency percentage for each variant was respectively calculated to be 12.5, 2.5, 2.5, 52.5, 2.5, 2.5, 2.5%.

Table 2. Exon 11 mutations of BRCA1 gene in breast cancer patients in the northwest of Iran

Sample number	Codon Number	Natural codon	Mutated codon	Natural amino acid	Mutated amino acid
17, 31, 235, 236, 315	356	CAG	CGG	Gln	Arg
289	486	TTT	CTT	Phe	Leu
289	550	AAT	CAT	Asn	His
243 and 348	584	GCT	ACT	Ala	Thr
238	628	AGT	AGG	Ser	Arg
142	693	GAC	TAC	Asp	Thy
48, 79, 92, 109, 113, 126, 142, 155, 235, 238, 243, 259, 272, 274, 294, 301, 307, 319, 326, 348, 354	694	AGC	AGT	Ser	Ser

Discussion

In this study, a nonsynonymous mutation was reported as the new mutation of the BRCA1 gene for the first time. Because inherited type of breast cancer is often seen in patients with bilateral breast involvement and early diagnosis age (9), these criteria were considered for selecting patients in this study. Because the BRCA1 gene has expanded from genomic DNA with 23 exons at about 80 kb, its mutations will be extensive and wide. For this reason, evaluation of mutations by direct sequencing is limited to the coding and exon regions of this gene (9).

Breast cancer is a multifactorial disease that results from interactions between a number of predisposing factors, including genotype, in one or more loci as well as the environmental factors. Therefore, the disease may be more affected by other predisposing factors compared with BRCA1 mutations (9). Due to these

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predisposing factors, no mutation was seen in 25% of the patients in this study. Exon 11 contains 60% of the sequences encoding the BRCA1 gene (9). In the analysis of exon 11-A of this gene, seven different variants were identified, among which there was a new mutation (Ala584Thr) in this region. More demographic studies are needed to identify the effect of this mutation. In the present study, the mutation of codon 694 has the highest frequency in samples with mutation. This variant was previously reported by Mojgan Dodova et al. (10) as well as a study by Rajasekaran et al. (11). After codon 694, the variant of codon 356 had the highest frequency in the studied samples, which was observed in 12.5% of the samples and was previously investigated in India by Rajasekaran (11) et al. and was reported in another study in UK by Baynes et al. (12) in the BRCA1 gene.

The results of this study are consistent with the previous reports. All of the patients examined in the study of Jalkh et al. suffered from invasive ductal carcinoma and all were under the age of 55 years. In in vitro experiments, it has been shown that mutation in codon 356 results in the loss of BRCA1 protein function (13). The mutation in codon 584, which was observed in two samples (5% of the samples) in the present study and led to the alteration of the amino acid (Ala) to threonine (Thr), has not yet been reported. Changes in each of the codons 486, 550, 628 and 693 were observed in one breast cancer sample, while changes in both codons 486 and 550 were observed in one sample. The mutation in codon 486 (c.1456T > C) has been reported in the analysis of familial breast cancer in a study by Jalkh et al. (14).

The variant of codon 550 reported in this study (c1648A > C) has also been reported previously in the examination of Bulgarian breast cancer patients by Rajasekaran et al. (11). Variants of codon 628 (c.1884T > G) and 693 (c.2077G > T), which have been observed in 2.5% of the samples in the present study, were both reported in the study of Figueiredo et al. (14), in which the patients were below 55 years old, and patients with BRCA1 gene mutations had a mean age of 40 years. Regarding codon 693, a different nucleotide change (c.2077G > A) has already been reported by Figueiredo et al. (14), which has led to the conversion of aspartic acid to asparagine, whereas in the present study, the mutation of codon 693 (c.2077G > T) led to the conversion of aspartic acid to tyrosine. Among the specified variants, one of them is synonymous (Ser694Ser). In this case, a single nucleotide polymorphism does not change the type of amino acid, since most amino acids have multiple anticodon sequences and thus do not alter the structure of the protein. These genetic changes do not affect the relevant protein chain and are considered as neutral mutations (12). No pathogenic effects have been reported regarding these changes so far (9). As a result, though we reported a new mutation in the BRCA1 gene in the northwestern region of Iran, none of the identified defects in this study have been reported in other Iranian provinces according to investigations. However, some of these mutations have already been reported in patients from some other parts of the world. This study is not sufficient to indicate possible mutations in the genes involved in breast cancer and even the BRCA1 gene in the northwestern region of Iran, and therefore, a large cohort study could facilitate the identification of BRCA1-BRCA2-related mutations in this population.

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