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Screening BRCA1 and BRCA2 Mutation Frequencies in Breast Cancer patients of west Iran reveals a novel polymorphism in BRCA1 gene

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Summary. *Introduction:* Breast Carcinoma is the most prevalent cancer in women worldwide. Mutations and polymorphisms of BRCA1 and BRCA2 genes are among the most important predisposing variants BC. The existing variants in these genes are highly heterogeneous, therefore study of these variants in a population could yield different findings from another. As a result, study of such genes seems necessary to determine and screen for the most important mutations in each region to assist in counseling of predisposed individuals. *Material and methods:* This case-control study was conducted on 140 women with breast carcinoma and 140 healthy individuals. After the consent was obtained, 5cc peripheral blood was taken from each individual for molecular tests. Then, the genomic DNA was extracted and Multiplex PCR was run for 185del AG and 5382insC in BRCA1 and 6174 delT in BRCA2. The results of Multiplex PCR were observed on polyacrylamide gel. Then, the samples with shifted bands on the gel were proved with direct DNA sequencing. *Findings:* In the studied samples, none of the above mutations were observed by sequencing, but C>G was detected at position 5265 in the coding region of the BRCA1 gene. In view of the investigations, this variation is a new polymorphism in BRCA1. *Conclusion:* The variant detected in BRCA1 gene causes variation in the third codon of amino acid serine and develops another codon for the same amino acid. Therefore, the detected variant is classified as silent and does not exist in the conserved region. The variant has not yet been reported. As a result, the effect of such variation on increased predisposition to BC or its association with the disease needs study of more samples.

Key words: Breast Carcinoma, BRCA1, BRCA2, polymorphism

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Riassunto. *Introduzione:* Il carcinoma al seno è il tumore più diffuso nella popolazione femminile mondiale. Le mutazioni e i polimorfismi dei geni BRCA1 e BRCA2 sono tra le più importati varianti che predispongono al tumore al seno. Le varianti note di tali geni sono altamente eterogenee, pertanto studi su tali varianti condotti in popolazioni diverse possono portare a più risultati. Di conseguenza, sono necessari studi su tali geni per determinare e selezionare le mutazioni più importanti in ciascuna regione in modo da assistere i pazienti predisposti. *Materiali e metodi:* Questo studio caso controllo è stato condotto su 140 donne con carcinoma al seno e 140 donne sane di controllo. Dopo aver ottenuto il consenso al trattamento dei dati, sono stati prelevati 5 cc di sangue ad ogni individuo per test di biologia molecolare. Successivamente, è stato estratto il

DNA ed è stata eseguita una PCRMultiplex per le mutazioni 185delAG, 5382insC di BRCA1 e 6174delT per BRCA2. I risultati della PCRMultiplex sono stati osservati mediante gel di poliacrilammide. I campioni con le relative bande rivelate dal gel sono stati analizzati mediante sequenziamento del DNA. *Risultati:* Nei campioni presi in esame non sono state riscontrate le mutazioni sopracitate mediante sequenziamento del DNA sebbene, alla posizione 5265 nella regione codificante del gene BRCA1, sia stato rilevata una regione con C>G. Dal punto di vista scientifico, tale variazione è da considerarsi un nuovo polimorfismo del gene BRCA1. *Conclusioni:* La variante riscontrata nel gene BRCA1 comporta variazioni nel terzo codone dell'amminoacido Serina e produce un altro codone per il medesimo amminoacido. Pertanto, la variante osservata è classificata come silente e non è presente nella regione conservata. Tale variante non è mai stata riportata. Ne consegue che l'effetto di tale variazione sull'aumentata predisposizione al cancro al seno o la sua associazione con tale malattia necessita di ulteriori approfondimenti.

Parole chiave: Carcinoma al seno, BRCA1, BRCA1, polimorfismo

Introduction

Breast carcinoma (BC) is the most prevalent malignancy in women approximately in most countries worldwide, with 73% frequency in developed countries and comprising 23% of all cancers. By the reports of the World Health Organization (WHO), this cancer frequency increases by 2% each year (1). In fact, breast carcinoma is responsible for most mortality due to cancer in women worldwide (2). The main type of BC is sporadic and its frequency range has been estimated as 90-95%. The remaining 5-10% comprises familial types (3). By estimates, the patients with BC in Iran are usually younger than those in Europe and the USA, their cancer type is familial and inherited, and this risk factor is more frequent in them (4). The most frequent genes that have been suggested for BC and increase the risk of this disease include PTEN, p53, BRCA2, BRCA1, CDH1, and STK11/LKB1 (2). Of these, the highest risk has been reported for BRCA1 and BRCA2. Increasing the risk of disease by respectively 59-87% and 38-80% and the existing mutations in these two genes seem to be associated with higher-degree breast tumors (5).

The women who inherit a mutant copy of any one of the above genes have 45-65% risk of cancer till the age of 70 years (6). To date, more than 2000 mutations have been reported for BRCA1/2. These mutations include the deletion, insertion, and displacement of nucleotides in the coding and non coding regions (7). Mutation distribution of BRCA1/2 varies in various populations. In the early studies on these genes the mutations 185delAC and 5382insC in BRCA1 and 6174 delT in BRCA2 have been reported in Ashkenazi Jewish populations, and then various mutations were reported for these genes in different populations. Generally, 1.40-1.80 of individuals with one mutation in these genes have higher risk of breast or ovarian cancer (8,9). Despite numerous studies on these genes, screening for mutations in these genes seems highly necessary. BC is a complicated disease which develops due to a variety of environmental and genetic factors and the value of screening for it is focused on timely diagnosis. Screening is aimed to diagnose the disease at a time when it is not still metastatic.

Screening could be implemented by mammography, sonography, or Magnetic Resonance Imaging (MRI) (10). According to the widespread prevalence of this cancer in Iran and its development in younger women, identifying the mutations and their frequency in BRCA1 and BRCA2 genes assists in screening for predisposed individuals because cancer development is, by the estimates, more likely in the individuals with mutation and we can act more wisely prior to cancer incidence.

Materials and method

Sampling and genomic DNA extraction

This descriptive analytical study was conducted per case-control method. Blood samples were collected after the written consent was obtained from the participants and their demographic and clinical data were gathered through questionnaire. One haundred forthy patients with pathologically confirmed diagnosis of disease referring cancer ward of hospital were randomly enrolled as cases and 140 healthy individuals were recruited as control. Five cc peripheral blood was taken from each individual for molecular tests. Then, blood samples were transferred to a tube containing EDTA (0.5 M) as anticoagulant. Genomic DNA was extracted by phenol-chloroform method and the quality and quantity of the extracted DNA were investigated by spectrophotometry (UNICO 2100, USA).

Analysis of mutations

For analysis of the mutations the mutations 185del AC and 5382insC in BRCA1 and 6174 delT in BRCA2, Multiplex PCR was used. To reproduce the mutations, the primers of a previous study (11) were used. Table 1 shows the primers used for each mutation. Polymerase chain reaction (PCR) was carried out using a thermal cycler (ASTEC, PC818 Japan) in a reaction volume of 25 μL containing: 2.5 μL PCR buffer (10X), 3 μL MgCl2 (50 mM), 0.5 μL of dNTP mix, 0.2 μL each of the 14 primers (50 pmol), 100 ng of genomic DNA (1 μL), and 0.5 U of *Taq* DNA polymerase 5U/μL (0.1 μL). Annealing temperature of each region, included in each reaction were between 57-59°C. The initial denaturation phase of 96°C/3 min. Amplification was carried out for 35 cycles, and followed by a final extension of 5-6 minutes at 72°C. PCR products electrophoresis in polyacryamid gel (PAGE) 8% at 45 mA for 1.5 hours and bands were visualized by silver staining.

Result

In this study, the DNA was extracted from the blood samples of women with sporadic and familial breast carcinoma (n=140) confirmed by the physician. Also, blood samples of healthy individuals (n=140) were taken for comparative study. The patients' age ranged 26-77 (mean age: 51.8±11) years and the control individuals' age ranged 16-70 (36.9±10.1) years ($p<0.01$). Investigating the mutations under this study, no variation was observed in BRCA2, but the variant 5382insC of BRCA1 was noted on polyacrylamide gel in allele band of eight samples with nucleotide variation. For confirmation of variation, sequencing was done.

Table 1. Primer sequences using in study and PCR amplicon.

Primer	Sequence of primer	Size
BRCA1 185delAG		
Common forward(P1)	5'-GGTTGGCAGCAATATGTGAA	335
Wild type reverse(P2)	5'-GCTGACTTACCAGATGGGACTCTC	354
Mutant reverse(P3)	5'-CCCAAATTAATACACTCTTGTCGTGACTTACCAGATGGGACAGTA	
BRCA1 5382insC		
Common reverse(P4)	5'-GACGGGAATCCAAATTACACAG	271
Wild type forward(P5)	5'-AAAGCGAGCAAGAGAATCGCA	295
Mutant forward(P6)	5'-AATCGAAGAAACCACCAAEGTCCTTAGCGAGCAAGAGAATCACC	
BRCA2 6174delT		
Common reverse(P7)	5'-AGCTGGTCTGAATGTTCGTTACT	151
Wild type forward(P8)	5'-GTGGGATTTTTAGCACAGCTAGT	171
Mutant forward(P9)	5'-CAGTCTCATCTGCAAATACTTCAGGGATTTTTAGCACAGCATGG	

Sequencing: After the studied samples were observed on the gel, for confirmation and determination of mutation, the samples with definite variation in gel and different bands underwent direct sequencing.

Sequencing indicated no mutation 5382insC, but in the sequence of studied samples was noted a c.5265 C>G variant (figure 1). This variation was found to be a new polymorphism in the gene BRCA1 after the data on conservation in Consurf database region and NCBI databases were investigated. In Figures 1 and 2 is shown the chromatogram of PCR products of the BRCA1 gene and polyacrylamide gel respectively. Figure 3 shows the change results in the disruption protection is desired.

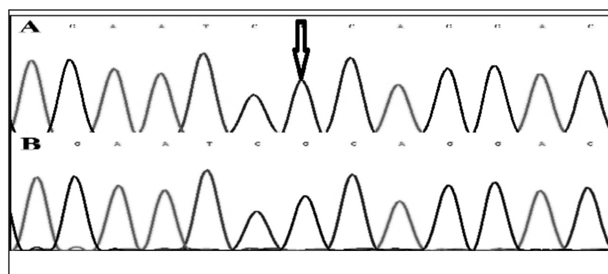

Figure 1. Sequencing results of exon 18 of BRCA1 gene, A: Sample of healthy people and unchanged. B: Sample with the C> G variant.

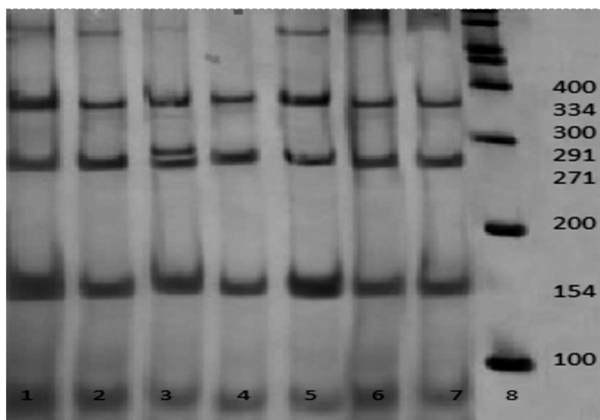

Figure 2. The 8% polyacrylamide gel electrophoresis of multiplex-PCR products using oligonucleotide primers for amplification of *BRCA1* gene. Line 8 is a 100 bp DNA ladder (Fermentas, Germany), line 3 is target variant, and other lines are no change samples.

Discussion

The value of a large number of known mutations and polymorphisms in BRCA1 and BRCA2 has not

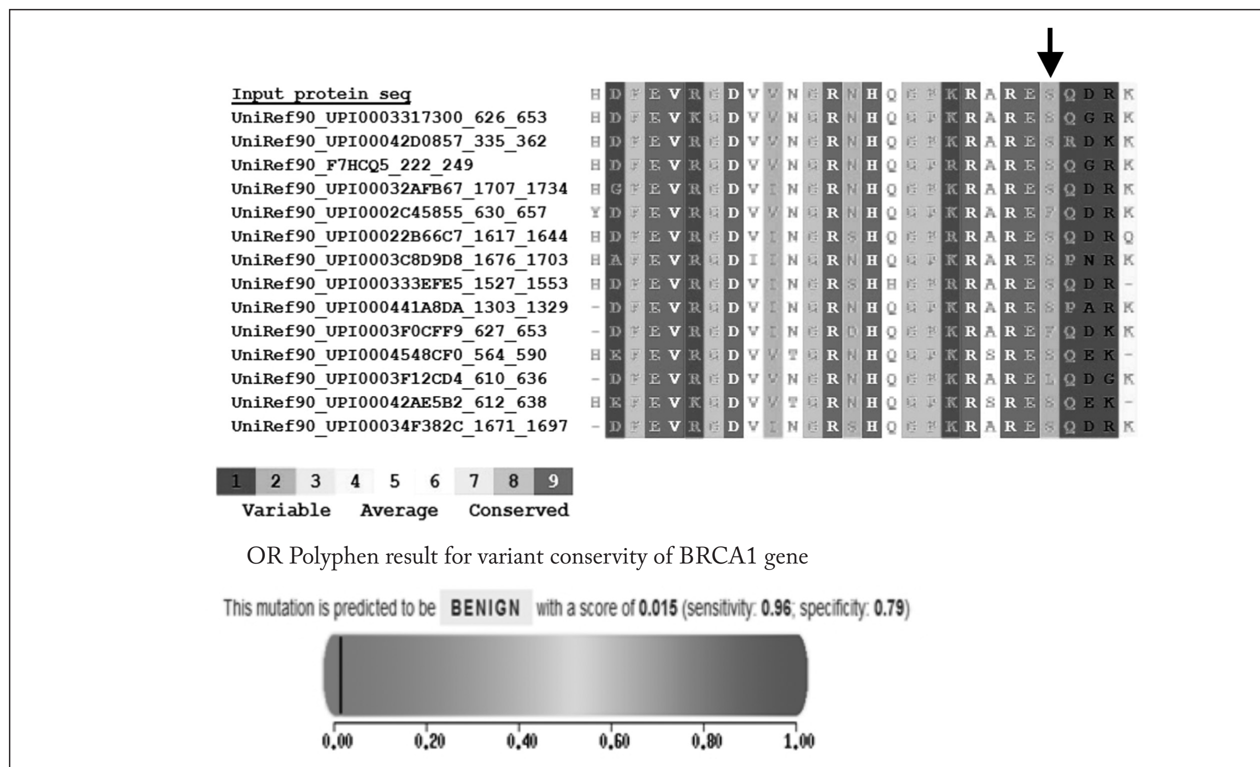

Figure 3. Conservation of disruption in exon 18 of the protein BRCA1- according to the color of the amino acid is modified at the Variable

yet been determined (4). The mutations associated with familial or inherited BC are much more frequent than those associated with sporadic BC. Also, different polymorphisms with progressive or protective role in BC progression have been so far reported. For example, single nucleotide polymorphisms such as S1613G in exon 16 and E1038 in exon 11 have been reported as associated with familial BC in India, Germany, Malaysia, Turkey, and Italy (12-15). However, other polymorphisms like S919S, E879E, and Y1137Y in the gene BRCA1 have not been considered as predisposing factor for the disease (16). In the present study, investigating the 185del AC and 5382insC mutations in BRCA1 and 6174 delT mutation in BRCA2 in the patients with BC, none of the above mutations were found. The 185delAG and 5382insC mutations in BRCA1 and 6174 delT in BRCA2 mutation have been detected as founder mutations in Ashkenazi Jews. These three mutations have been also reported from other populations in Romania (17), Greece (18), Austria (19), Brazil (20), Turkey (21), Russia (22), Lebanon (23), India (24), and Iran (25). In Iran, these mutations were reported in 19% of the patients with familial BC. However, in different studies of Iran's regions, the prevalence of these three mutations have been reported less in various Iranian populations than in other populations (26*).* In this study, none of the above mutations were detected, but, another variant was detected when the samples with variation on polyacrylamide gel were investigated. The detected variant of exon 18 of BRCA1 gene causes variation in the third codon of amino acid serine and develops another codon for the same amino acid. The detected variant is classified as silent. Therefore, as the detected variant does not exist in the conserved region and nucleotide variation has not caused amino acid displacement at this position, it is concluded that this variant is a type of polymorphism. The detected polymorphism has not yet been reported by other studies and its contribution to progression of and/or protection against disease has not been investigated. As a result, the effect of such variation on increased predisposition to breast carcinoma and/or its association with the disease could not be argued by the present study and needs studies of more samples.

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