Original Article

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Evaluation of Mutations in Exons 7 and 8 of TP53 Gene in Breast Cancer Patients from Azarbaijan

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ABSTRACT

BACKGROUND AND OBJECTIVE: Breast cancer is the most common type of malignancy in women, and TP53 tumor-suppressor gene is one of the most commonly transformed genes in human cancers. Accurate assessment of TP53 gene mutations in cancer patients can play an important role in diagnosis, prognosis, or treatment. This study aimed to identify mutations of this gene in breast cancer patients.

METHODS: In this descriptive study, 102 tumor samples were obtained from female breast cancer patients from Azarbaijan, Iran. All the participants were referred to hospitals of Tabriz during 2007-2009, and their DNA was extracted by Proteinase K. TP53 gene mutations in exons 7 and 8 and intron 7 were investigated using polymerase chain reaction technique and direct sequencing.

FINDINGS: Seven (6.86%) cases of mutation and 14 (13.72%) cases of polymorphisms were identified. Mutations (CGG → CAG) at codon 248 (in two cases) and (CTG → CCG) at codon 257 in exon 7 and G>T mutation in the first nucleotide of intron 7 were observed. In exon 8, GTG>ATG mutation at codon 272, CCT>TCT mutation at codon 278, and three nucleotide deletion at codon 262 were identified.

CONCLUSION: The results of this study showed a different pattern of TP53 gene mutation in female breast cancer patients. Further studies could specify the role of TP53 mutations in the progression of breast cancer.

KEY WORDS: *Breast cancer, Mutation, TP53 gene.*

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Introduction

Breast cancer is the most common malignancy among female population in both developed and developing countries (1). Annually, more than 1.1 million women are diagnosed with breast cancer worldwide, and more than 410000 of them die of this disease. The highest incidence rates are reported in the North America, while the lowest rates are in Africa and Asia (2, 3). On the other hand, lower rates of this malignancy in the East and Central Europe and the Far East are on a growing trend. For instance, the incidence of breast cancer in Japan doubled over the last 40 years (3). While breast cancer in developed countries is on the verge of elimination as a public health threat, and in the Asian countries its incidence rate is soaring and it seems that in the near future, breast cancer would be considered as disease of developing country (1).

In Iran, cancer is the third leading cause of mortality, and annually, 30,000 Iranians die of cancer. Breast cancer is one of the most common malignancies among Iranian women and its prevalence is increasing (4-6). Early stage diagnosis of cancer is of great prominence, since patients who are diagnosed in later stages show less rates of survival (6-8). Thus, reliable prognostic markers are required to help physicians with treatment decision making. Various molecular genetic alterations such as change in oncogenes and tumor-suppressor genes were studied in breast cancer to discover the molecular association between prognosis, clinical characteristics, and phenotypes of breast cancer. *TP53* gene is a tumor-suppressor gene, which is mutated in 15-71% of breast cancer patients (9). P53 is a phosphoprotein transcription factor that regulates the expression of over 2500 target genes, and it is involved in diverse cellular processes including maintenance of genome stability, longevity, metabolism, and most importantly, tumor suppression (9). Its gene is located on the short arm of chromosome 17 (17 p 13.1) and has 11 exons with a length of 20 kb. Natural p53 protein contains 393 amino acids and several structural-functional domains, which consist of two transcriptional activator domains in the N-terminal, a domain rich in proline, DNA-binding central domain, as well as a base and tetramerization domains in the C-terminus (9, 10). This gene is one of most frequently mutated genes in human cancers (10, 11). Accurate evaluation of mutations of this gene in breast cancer patients in the East Azarbaijan province, Iran, can be of great importance for prognosis or treatment. Moreover, identification of the pattern of distribution of somatic mutations or germline of this gene in this region can provide valuable information regarding various environmental mutagens and rare syndrome of Li-Fraumeni in Azerbaijani families. Our previous study showed the frequency and types of mutations in exons 5 and 6 of *TP53* gene (11). This study aimed to identify the mutations of the exons 7 and 8 and intron 7 of *TP53* gene through polymerase chain reaction (PCR) and direct sequencing in breast cancer patients from East Azerbaijan.

Method

Sampling: This descriptive study was performed on 102 breast cancer patients from the Turk population of East Azerbaijan province, Iran, during 2007-2009. The patients were referred to Imam Khomeini, Imam Reza, and Nornejat hospitals in Tabriz for surgery. After obtaining written consent, the samples were selected randomly to have a representative sample of the patient population in the region of East Azerbaijan. According to the standard classification of breast cancer (TNM staging system), the tumoral tissue were classified by a pathologist expert. Tumoral tissue was removed at operation room and was transferred to the laboratory freshly in liquid nitrogen, and was stored at -80°C.
DNA extraction: After pathologic confirmation of the mass, to extract DNA, SE buffer (75mM NaCl; 25mM Na2 EDTA; pH 8.0), sodium dodecyl sulphate (SDS), and Proteinase K were added to a crushed tumor tissue, then it was kept at 40°C for 24 hours, and afterwards, for an hour at 55-60°C. At this point, saturated brine and chloroform were added and it was centrifuged at 3500 rpm. The clear liquid supernatant containing DNA coils appeared by adding cold absolute ethanol. The coil was removed gently by sampler and dissolved into water after elution with 70% ethanol.

Performing PCR: To assess the mutations in p53 gene, the sequencing method was used. For gene amplification from a pair of primers, I6 F5'GCCCTCCCCTGCTTGCC3' and E8R5'TCCACCGCTTCTTGTCCTGC3' were used. The length of the selected fragment for amplification was 682 bp, which included exon 7 (110 bp), intron 7, and exon 8 (137 bp). To amplify this fragment, we added 2.5 µL buffer of PCR10x, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 1u Taq polymerase enzyme, 1 µl of tumor DNA sample, and 0.4 µM of each primer was put into 25 µL microtubes, and then PCR was performed. In this study, the denaturation temperature was 96°C for one minute, primer-binding temperature was 59°C for 45 seconds, and extension temperature was 72°C for one minute at 30-35 thermal cycles.

The PCR product was electrophoresed for 30 minutes with 100 voltage (fig 1). Sequencing: 200 µl of qualitatively approved product of PCR and the primers were sent to Fazabiotech for sequencing. To identify possible changes in the studied samples, the obtained peaks (sequences) were assessed, and then the sequences were blasted with registered sequences at NCBI by Chromas application. The resulting sequences were compared to the normal sequence of p53 gene, using ClustalW2 software. Finally, any variation in the samples was confirmed as genetic alteration after re-comparison with the corresponding peak.

Results

The patients' mean age was 46 years (age range: 23-80 years), which was lower than that of patients with breast cancer in developed countries. The type of disease was invasive ductal carcinoma in 81.41% of the patients. Based on TNM (Tumor Node Metastasis) classification, 62.16% of the cases were at stage III, 28% at stage II, and 9.75% were at stage I at the time of surgical operation. According to the patients' records, 44% of the cases underwent left breast surgery, 54% right breast, and 2% underwent surgery for both breasts. Mass size in 24.6% of the patients was less than 2 cm, in 36.92% 2-5 cm, and in 30.76% of the samples it was larger than 5 cm. In 7.69% of the cases, the disease had developed and involved the patients' skin. Using primers, the 682 bp fragment was obtained including exons 7 and 8 and intron 7 (fig 1).

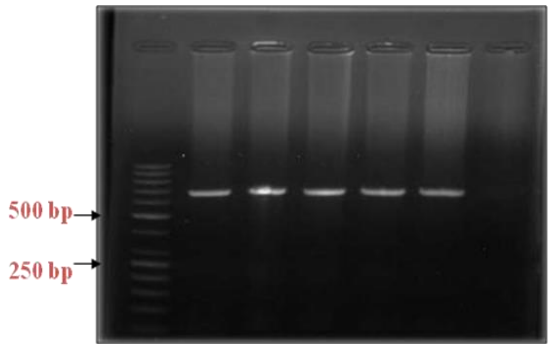

Figure 1. Polymerase chain reaction products of 682 bp fragment containing exons 7 and 8 and intron 7 of *TP53* gene

In this study, 21 (20.58%) genetic alterations were observed, 14 (13.72%) cases of which belonged to polymorphism and seven (6.86%) of them were related to mutants. Mutations (CGG→CAG) at codon 248 (in two cases) and (CTG→CCG) at codon 257 were placed on exon 7. In the first nucleotide of intron 7 in a tumor sample, G>T mutation led to splicing mutations in the intron. In exon 8, GTG>ATG mutation at codon 272 changes the valine to methionine and CCT>TCT mutation at codon 278 replaces proline amino acid with serine. Three nucleotide deletion at codon 262 removes the glycine amino acid of the protein (Table 1). The sequences of the identified mutations are demonstrated in figure 2.

Table 1. Characteristics of the identified mutations in exons 7 and 8 and intron 7 in *TP53* gene

Sample	Codon	Normal codon	Mutant codon	Normal aminoacid	Mutant aminoacid
31	248	CGG	CAG	Arg	Gln
77	248	CGG	CAG	Arg	Gln
101	257	CTG	CCG	Leu	Pro
69	262	GGT	Deletion	Gly	-
95	272	GTG	ATG	Val	Met
58	278	CCT	TCT	Pro	Ser
99	Int7	(14451)	G>T	-	-

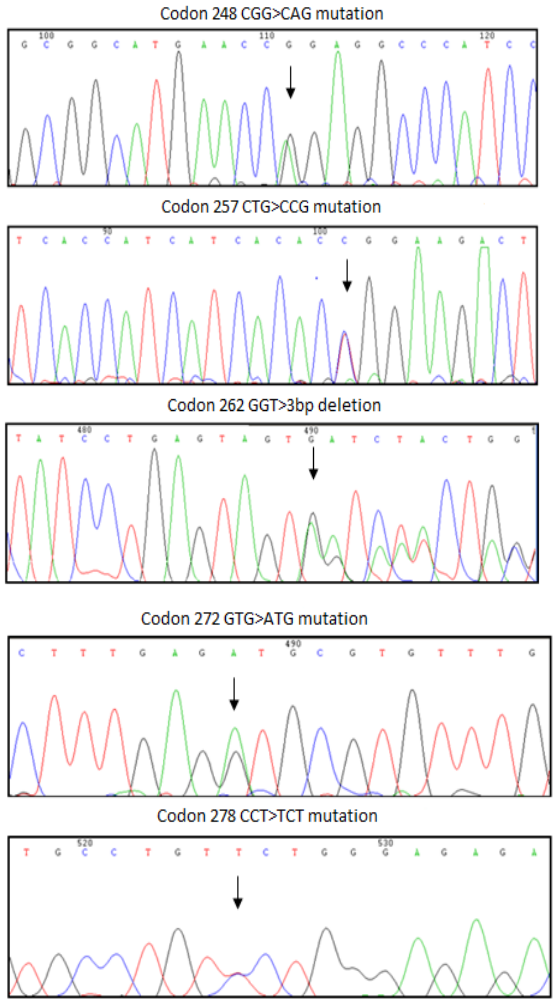

Figure 2. The sequence of samples with mutations in exon 7 or 8 in *TP53* gene; codon number is provided on top of each figure

Discussion

Our results exhibited the presence of seven mutations in exons 7 and 8 and intron 7 of *TP53* gene in the patients. Four mutations were found in exon 7, which occurred at codon 248 in two samples and led to changing arginine amino acid into glutamine. Codon 248 is known as the hot spot of the mutation in patients with breast cancer (9). The current study demonstrated the importance of this point in mutations in Azerbaijani women with cancer. Changing C>T at codon 257 (in patient code 101) turned leucine into proline. In the first nucleotide of intron 7, G>T mutation resulted in splicing mutation in a tumor sample. Changing GTG>ATG at codon 272 of exon 8 converts valine into methionine, and CCT>TCT mutation at codon 278 of this exon replaces proline with serine (9). Three nucleotide deletions in codon 262 removes glycine from protein. These findings show the presence of deletion mutations with missense in exons 5 and 6, which were not observed in the previous studies (11).

The rate of mutations in p53 gene is 15-71% in breast cancer (9). The frequency of *TP53* gene mutation in breast cancer varies depending on ethnic and geographic populations. Investigation of two exons of *TP53* gene in this study, showed 6.86% mutations. In our previous study, the frequency of mutations in exons 5 and 6 of p53 gene was 10.78% (11). In this study, the frequency of *TP53* mutations in exons 5-8 was reported to be 17.64% in Azerbaijani patients with breast cancer. This frequency is comparable with those of France (19%), New Orleans (15%), while it is greater than the frequencies reported from Delhi, India (3%) and less than those reported from the United States (45%), Great Britain (34.5%), and Cashmere (44%) and frequencies reported by IARC (23.56%) (12-14). In a study conducted in Hamadan, frequency of mutations in p53 was determined to be 25% (15). According to the IARC database, R175H and R273H mutations are very common in breast cancer.

However, in the current study, the studied tumor samples did not show the aforementioned mutations. A special pattern of *TP53* mutations was expectable in Azerbaijani women, and similar results were reported in studies conducted in populations with other ethnicities (16-19). The difference in frequency and somatic mutation patterns exhibits the presence of various environmental mutagens. The low frequency of *TP53* mutation may indicate the involvement of other genes in carcinogenic pathway (16, 17). These differences in frequency of p53 mutations in breast cancer might be due to a multitude of factors including evaluation of different ethnic and geographic populations, limited sample sizes, differences in sample selection techniques, diverse sampling and analysis techniques, difference in supply of endogenous and exogenous carcinogens, varied lifestyle, eating habits, and reproductive patterns, as well as cultural and social differences; identification of

the role of each these factors requires further investigation (17, 18). In present study, tumor size, lymph node involvement, and the end-stage of the disease among patients indicate poor screening and lack of early detection programs, and point out the need for modifying screening programs and promoting awareness of women in East Azarbaijan province. During targeted therapy, researchers managed to target and identify the specific molecules or genetic changes that were necessary for tumorigenesis and growth of cancer cell using drugs or small molecules (18, 19). As *TP53* gene mutations are among the most frequent genetic alterations observed in all human cancers, new therapeutic methods were introduced to identify and target p53 changes. Reactivation of mutant p53 protein using small molecules improves the prospect of cancer treatment with lower side effects (20-24). The results of evaluation of *TP53* gene in the East Azerbaijan province demonstrated a special pattern of p53 mutations in Azeri female patients. These features are significantly different from reports of other countries and the findings of IARC database, and represent a different mutation pattern in this region reported for the first time. These results can be employed for effective treatment of patients. Further studies are required to investigate the cause of these differences and to determine the relationship between ethnic-genetic background, lifestyle, adjusted dietary regimen to regional living conditions, and environmental carcinogens.

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