Short Communication

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The Association between ERBB4 Gene Polymorphism (SNP: Rs1972820g>A) and the Risk of Breast Cancer

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ABSTRACT

BACKGROUND AND OBJECTIVE: Breast cancer is the most common cancer in women and the leading cause of cancer death in women worldwide. The ERBB4 gene, a member of the tyrosine kinase family, has been identified as both a tumor suppressor and an oncogene in breast cancer. In this study, the association between rs1972820G>A polymorphism of ERBB4 gene and the risk of breast cancer in northwestern Iran was investigated.

METHODS: This case-control study was performed on 89 women with breast cancer and 98 healthy women with no family history of cancer. Clinical characteristics of patients included age (less than, greater than or equal to 49 years and unknown), involved side (right, left, both and unknown), type of tumor (ILC, IDC, other types and unknown), tumor grade (I, II or III), tumor size (T_1, T_2, T_3 and unknown) and lymph node involvement (N_0, N_1, N_2, N_3 and unknown). Single nucleotide polymorphisms were examined by TETRA-ARMS-PCR technique.

FINDINGS: The genotypic distribution of sick and healthy individuals was 66% and 56% for AA genotype, 9% and 6% for GG genotype and 25% and 38% for AG genotype, respectively. The frequency of Allele A was 147 and 140 and the frequency of G Allele was 49 and 38 in patients and controls, respectively ($p>0.05$).

CONCLUSION: The findings of this study showed that there is no significant relationship between genotypic and allelic distribution of rs1972820 polymorphism of ERBB4 gene with increased risk of breast cancer and clinical features of patients in northwestern Iran.

KEY WORDS: *Breast Cancer, Single Nucleotide Polymorphism, Association Evaluation, ERBB4 Gene*.

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Introduction

Breast cancer is the leading cause of cancer mortality among women worldwide (1). According to GLOBOCAN estimates in 2020, 2.26 million women are diagnosed with breast cancer worldwide, which is 24.5% of the total number of women with cancer in this year. Breast cancer has been the most common cancer among women in this year (2). In Iran, breast cancer accounts for about 22% of cancers among women. Breast cancer is the most common cancer among Iranian women and the age of onset of this disease in Iran is 10 years less than the age of onset in Western countries (1).

The ERBB4 gene is located on chromosomal position 2q34 (3) and its size is 1.16 megabase (Mb) and has 28 exons (4). This gene is a member of the epidermal growth factor receptor family and a subfamily of tyrosine kinases (3). The mRNA of this gene is subjected to periodic splicing and four variants of JM-a, JM-b, CT-a (CYT-1) and CYT-2 (CT-b) are obtained. The variants differ in extracellular domains or in C-terminal cytoplasmic sequences (5). The ERBB4 gene encodes a 180 kDa protein in breast cancer cells, normal skeletal muscle tissue, heart, and brain (6), and the resulting protein, ERBB4/HER-4, is involved in cell division, migration, differentiation, and apoptosis (7).

ERBB4 protein has 4 isoforms, including JM-a CYT-1, JM-a CYT-2, JM-b CYT-1 and JM-b CYT-2. These isoforms have an extracellular domain for juxtamembrane region (JM), transmembrane domain (TM), and cytoplasmic domain (CYT). The intracellular region has a tyrosine kinase domain (8). No difference in the degree of ligand binding was detected among JM variants (9). Single nucleotide polymorphism (SNP) is the result of a single nucleotide mutation at the genome level. The occurrence of polymorphisms in the regulatory and coding regions can lead to gene mutations, changes in protein structure, disruption of the cell cycle, and ultimately the occurrence of various cancers (10).

Various studies in different populations and types of cancers have examined the association between changes in different SNPs of the ERBB4 gene and the function of this gene in breast cancer, colorectal cancer, prostate cancer, hepatocellular carcinoma, lung cancer, and polycystic ovary syndrome, and in some cases, have been associated with these cancers and syndromes (7, 11-14). The investigation of the association between ERBB4 gene polymorphisms and various diseases has shown the association between this gene and polycystic ovary syndrome (11), hepatocellular carcinoma (13) and breast and colorectal cancer (7). ERBB4 has been identified as both a tumor suppressor gene and an oncogene in breast cancer (15). The role of ERBB4 in cancer is complex and affects other members of the ERBB family and the tissue in which ERBB4 is expressed (16). 82% of breast cancer samples are positive for the ERBB4 gene. Patients with higher ERBB4 expression showed better survival than patients with higher ERBB1-3 expression (15). Association between mononucleotide polymorphisms rs13423759, rs1836724, rs11895168 and rs62626347 of ERBB4 gene and increased risk of breast cancer has been reported by Mansouri Bidkani et al in 2018 (17), Bagheri et al in 2016 (18), Salimi et al. (19) and Kurppa et al. in 2014 (20).

The rs1972820G>A single nucleotide polymorphism is located in the 3′UTR region of the ERBB4 gene. Zabihi et al. studied the association between rs1972820 polymorphism of ERBB4 gene and the risk of breast cancer in the female population of Isfahan. In this study, no significant relationship was observed between clinical symptoms and polymorphism. However, rs1972820 polymorphism was associated with a reduced risk of breast cancer and could be a potential marker for the prognosis of breast cancer (16).

Considering the lack of previous reports in this regard, the association between rs1972820G>A polymorphism in the 3′UTR region of ERBB4 gene and the risk of breast cancer in northwestern Iran was investigated in this study.

Methods

This case-control study was conducted after approval by the ethics committee of Tabriz University of Medical Sciences with the ethics code IR.TBZMED.REC.1399.889. For this purpose, blood samples of 89 people with breast cancer were collected from among patients who referred to Noor-e Nejat Hospital in Tabriz. The control group of the study included 98 healthy individuals without a family history of cancer in their first- and second-degree relatives. Clinical characteristics of patients such as age, tumor size, tumor grade, lymph node involvement, involvement side (right or left) and tumor type and age of the control group were collected in a questionnaire with their permission.

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DNA extraction: DNA extraction from venous blood was performed by proteinase K or salting out method. After evaluating the quality and quantity of extracted DNA using 1% agarose gel electrophoresis and spectrophotometer, the extracted DNAs were diluted with sterile distilled water and kept at -20 °C until the time of experiment.

TETRA-ARMS-PCR technique: Evaluation of polymorphism of rs1972820 located in the 3'UTR of ERBB4 gene in the study population was performed using TETRA-ARMS-PCR technique. The sequence of primers was designed and synthesized by Metabion (Germany) based on the study of Zabihi et al. (16). Primer sequences included the forward inner (FI) (allele A) with sequence 5'CCACTGATAATGATCTTTTAAAATTCTA3', reverse inner (RI) (allele G) with sequence 5'ACAAATATTATACAGATGAGATCATGC3', forward outer (FO) with sequence 5'GATCCTACATTTTTGGACCTCTAC3' and reverse outer (RO) with sequence 5'GGGGCAGAGGATCTTACTGTG3'. The binding site of the primers to the ERBB4 gene sequence is shown in Figure 1.

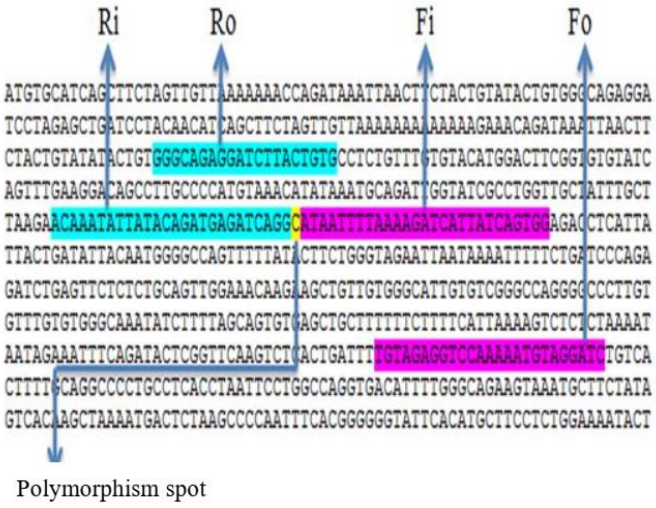

Figure 1. The binding site of the primers to the ERBB4 gene sequence

ARMS-PCR reaction was accomplished at a final volume of 15 µl with a composition of outer forward and reverse primers at an amount of 1 µl, inner forward and reverse primers at an amount of 2.5 µl, DNA template at an amount of 1 µl, Master mix (AMPLIQON) at an amount of 5 µl and 5.5 µl water. Thermal cycles of PCR reaction included initial denaturation at 95 °C for 5 minutes, 35 denaturation cycles at 95 °C for 30 seconds, binding of primers at 56 °C for 40 seconds and expansion at 72 °C for 50 seconds and ultimately, a final expansion cycle at 72 °C for 10 minutes. Finally, the products of PCR reaction were electrophoresed on 2% agarose gel.

Statistical analysis: To investigate the relationship between the frequency of genotypes and alleles between the control and patient groups, Pearson's chi-squared test was used and in cases where the number of observed data was less than five, Fisher's exact test at 95% confidence level was used and $p<0.05$ was considered as the significance level. The severity of the relationship between polymorphisms and risk for cancer was calculated using the odds ratio with a 95% confidence interval. The statistical tests mentioned for genotypes and alleles were calculated using the JavaStat online statistics package (http://statpages.org/ctab2x2.html). SPSS software version 24 was used to examine the relationship between genotypes and clinical characteristics of patients as well as the mean index. Spatial structure changes due to rs1972820 polymorphism of ERBB4 gene were examined by RNAsnp online software.

Results

In this study, 89 patients with breast cancer were examined. The age range of patients and controls was between 25-81 years (Table 1). The size of PCR products on agarose gel were 469 bp for control strip, 340 bp for G allele, and 183 bp for A allele. The banding pattern of these alleles on agarose gel is shown in Figure 2.

Frequency distribution of genotypes and alleles between case and control groups: The highest frequency was related to AA genotype which was observed in 66% of patients and 56% of healthy individuals. AG genotype was observed in 25% of patients and 38% of healthy individuals. GG genotype was also observed in 9% of patients and 6% of healthy individuals. There was no significant difference between the case and control groups in the frequency distribution of genotypes. The frequency of A allele in patients and healthy individuals was 79% and 75%, respectively, and the frequency of G allele in patients and healthy individuals were 21% and 25%, respectively. There was no significant relationship between the frequency of A and G alleles in the case and control groups (Table 2).

Frequency distribution of genotypes and clinical signs: Evaluation of the relationship between rs1972820 polymorphism of ERBB4 gene and clinical

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signs based on the conducted analyses show no significant relationship between the distribution of genotypic frequencies of this SNP and the studied clinical signs, including tumor type, tumor grade, age, involved side, tumor size and lymph node involvement (Table 1).

Table 1. Clinical characteristics of patients and distribution of single nucleotide polymorphism rs1972820 G allele in relation to clinical characteristics of patients (n=89)

Characteristics	Number(%)	Carrier of the G allele Yes	Carrier of the G allele No	Total	p-value
Age					
>49	40(44.95)	13	27	40	1.00
≤49	41(46.07)	14	27	41	
unknown	8(8.98)				
Involved side					
Right	44(49.43)	14	30	44	
Left	34(38.21)	12	22	34	0.822
Both sides	3(3.38)	1	2	3	
unknown	8(8.98)				
Tumor type					
ILC	5(5.62)	2	3	5	
IDC	51(57.31)	13	38	51	0.633
Other types	16(17.97)				
unknown	17(19.10)				
Tumor grade					
I	31(34.84)	9	22	31	
II	24(26.97)	7	17	24	0.767
III	1(1.12)	0	1	1	
unknown	33(37.07)				
Tumor size *					
T_1 **	8(8.98)	15	33	48	
T_2	56(62.93)	8	16	24	0.544
T_3	8(8.98)				
unknown	17(19.11)				
Lymph involvement					
N_0 ***	39(43.82)	13	26	39	
N_1	17(19.11)	7	10	17	0.494
N_2	9(10.11)	0	9	9	
N_3	1(1.12)	0	1	1	
unknown	23(25.84)				

*In calculations of the G allele polymorphic distribution in relation to tumor size, individuals were categorized into two groups of <3 (top row) and 3≤ (bottom row).

$T_1 \leq 2$ cm, T_2: 2-5 cm, $T_3 > 5$ cm, *N_0: 0, N_1: 1-3 cm, N_2: 4-9 cm, $N_3 \geq 10$ cm

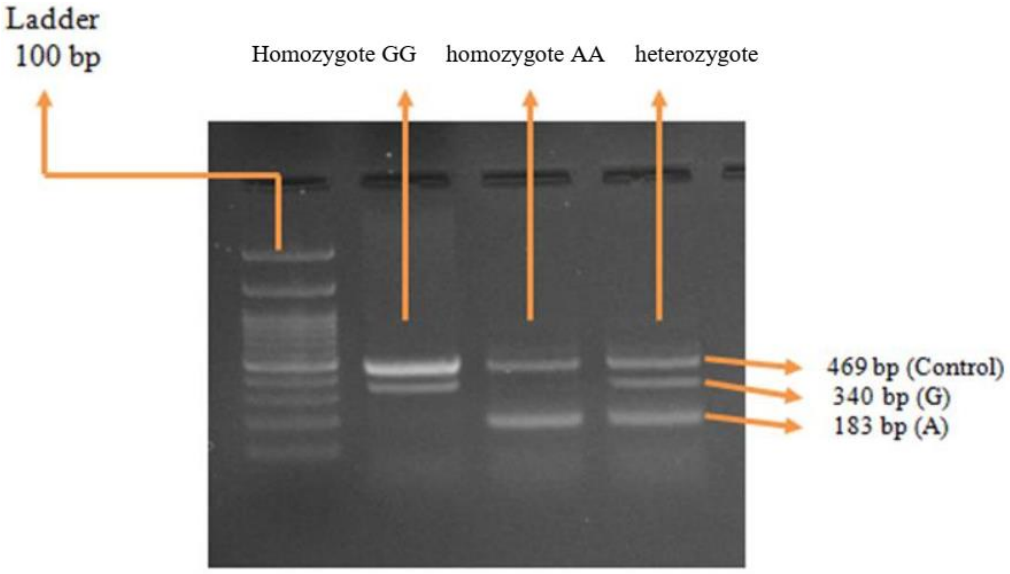

Figure 2. Electrophoresis of PCR products related to AA, AG and GG genotypes of patients on 2% agarose gel

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Table 2. Frequency distribution of genotypes and alleles in healthy and sick individuals

Genotype/allele	Case (n=89)	Control (n=98)	p-value (Chi square)	p-value (Fisher)	OR (CI- 95%)
AA	59	55	0.155	0.178	1.538 (0.849-2.784)
AG	22	37	0.055	0.060	0.541 (0.288-1.018)
GG	8	6	0.457	0.581	1.514 (0.504-4.549)
A	140	147	0.404	0.463	1.228 (0.758-1.990)
G	38	49	0.404	0.463	0.814 (0.502-1.320)

Discussion

Based on the results of this study, no significant relationship was observed between the frequency distribution of genotypes and alleles in the rs1972820G>A polymorphism of ERBB4 gene and breast cancer in women in northwestern Iran. In the study of Zabihi et al., the frequency of A allele in patients and healthy individuals was 158 and 164, respectively, and the frequency of G allele in patients and healthy individuals were 14 and 28, respectively. The frequency of AA, AG and GG genotypes was 76, 6 and 4 in patients and 74, 16 and 6 in healthy individuals, respectively. Based on the results of this study, there is a significant relationship between rs1972820 G allele of ERBB4 gene with reduced risk of breast cancer and can be introduced as a potential marker for breast cancer prognosis (16).

The difference in the frequency of A and G alleles and the lack of correlation between the results of these two studies can be related to the difference in size and genetic background of the two populations, for reasons such as differences in geographical area and type of nutrition (21). According to Zabihi et al., the tendency of miR-3144-3p binding, which is involved in the negative regulation of ERBB4 protein expression, to the 3′UTR region of the ERBB4 gene in a state of mutation is different from the normal state (16). This change could affect the regulation of the target gene and be associated with a higher risk of breast cancer. The G allele reduces the tendency of miRNA to bind to the 3′UTR of the ERBB4 gene and may thus increase the expression of ERBB4 as a tumor suppressor gene, which is associated with a reduced risk of breast cancer. Furthermore, based on the results of this study, no significant relationship was observed between any of the polymorphic genotypes of single nucleotide polymorphism rs1972820 in 3′UTR regulatory region of ERBB4 gene and tumor type, tumor grade, age, involved side, tumor size and lymph node involvement. The study of Zabihi et al. on this SNP also showed no association between clinical symptoms and polymorphism rs1972820 in the population of Isfahan (16).

None of the AA, AG and GG genotypes of rs1972820 polymorphism of ERBB4 gene in the study population were significantly associated with breast cancer. Furthermore, none of the genotypes were associated with clinical signs of patients such as age, tumor size, tumor grade, lymph node involvement, side of involvement (right or left) and type of tumor. Considering that this is the first report of the association between rs1972820 polymorphism of ERBB4 gene with the possibility of breast cancer in northwestern Iran, it may indicate that the studied polymorphism is not associated with breast cancer in women in this region. To confirm this, evaluating the association between rs1972820 polymorphism and the risk of breast cancer in a larger statistical population of sick and healthy individuals, studying the differential expression of this gene in sick and healthy individuals, as well as in silico analyses using multiple databases are recommended.

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