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Association of Interleukin-16 Gene Polymorphisms rs11556218 T/G and rs4072111 C/T with Risk of Breast Cancer in an Iranian Population

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ABSTRACT

Background and objectives: Breast cancer is the leading cause of death in women. Cytokines regulate the intensity and continuity of immune response by balancing cell-mediated immunity and humoral responses. This study aimed to investigate the relationship between two polymorphisms of the interleukin-16 (*IL-16*) gene and risk of breast cancer.

Methods: Blood samples were collected from 80 breast cancer patients and 80 healthy individuals. Polymorphisms rs11556218 T/G and rs4072111 C/T were investigated by polymerase chain reaction-restriction fragment length polymorphism (RFLP-PCR). Then, genotype and allele frequency distributions were evaluated in each group using the SPSS software (version 24).

Results: The frequency of genotype rs11556218 differed significantly between the patients and controls (P=0.007). The TG genotype (odds ratio [OR]= 2.471, 95% confidence intervals [CI]: 1.229-4.965, P= 0.001) and total TG+GG genotypes (OR= 3.095, 95% CI: 1.624-5.899, P= 0.001) had a significant relationship with increased risk for breast cancer. The allele and genotype frequencies of rs4072111 C/T polymorphism did not differ significantly between the patients and controls.

Conclusion: Our findings suggest that the rs11556218 T/G polymorphism of the *IL-16* gene may be associated with susceptibility to breast cancer.

Keywords: Breast Neoplasms, Interleukin-16, Polymorphism, Single Nucleotide.

INTRODUCTION

Breast cancer is the most common cancer among women and a leading cause of death worldwide. Female breast cancer has now surpassed lung cancer as the leading cause of global cancer incidence in 2020, with an estimated 2.3 million new cases, representing 11.7% of all cancer cases (1).

Most cancers usually result from the interaction between several factors including genetic, environmental, reproductive and lifestyle factors (2, 3). Although breast cancer has not been traditionally considered as an immunogenic tumor, studies suggest that the immune system and its interaction with tumor cells and the tumor environment may play an important role in this malignancy (4).

Cytokines are small proteins (usually less than 30 kDa) that are released by various types of cells with important roles in immune processes (5). Interleukin-16 (IL-16) gene in humans is located on chromosome 15q25.1. The gene is 153 kb in length and consists of 22 exons and 21 introns (6). Today, one of the most interesting topics for scientists is the study of single-nucleotide polymorphisms (SNPs) and their association with cancer (7). These mutations can be found in different parts of the genome, such as the coding or non-coding regions (8) and can provide information on population histories and the form of gene selection (7). Several studies have shown that some of the *IL-16* polymorphisms are significantly associated with cancers such as gastric, colorectal, nasopharyngeal and hepatocellular cancers. Some important polymorphisms of the *IL-16* gene are rs11556218 and rs4072111, which might have a significant role in the development of cancer (9). In a study in Taiwan, the distribution of genotypic and allelic frequencies of *IL-16* rs11556218 differed significantly between oral cancer patients and healthy individuals (10). In another study, the distribution of genotypic and allelic frequencies of *IL-16* rs11556218 differed significantly between lung cancer patients and healthy individuals (11).

This study aimed to determine the SNPs of the *IL-16* gene (rs11556218 T/G and rs4072111 C/T) in breast cancer patients and healthy controls using polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP).

MATERIALS AND METHODS

In this study, peripheral blood samples (5 ml) were taken from 80 breast cancer patients and 80 healthy individuals who were referred to the Ayatollah Khansari Hospital in Arak, Iran. The study was approved by the Research Ethics Committee of Arak University (IR.ARAKMU.REC.1395.288). Written consent was obtained from all participants. Information about age, marital status, occupation, diet, number of children and Age at menarche were collected. All pariticpants were female and the mean age of the patients was 50 years.

DNA extraction was performed with the Iraizol DNA Extraction kit (RNA Biotechnology Company, Iran) according to the manufacturer's instruction. The quality of the extracted DNA was evaluated using a spectrophotometer (v-gene, USA). Finally, the extracted DNA was stored at -20 ˚C for next experiments.

Polymorphisms rs11556218 T/G and rs4072111 C/T were investigated by the RFLP-PCR technique using primers used in a previous study (12) (Table 1). The primers were checked in the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/tools/primer-blast). Finally, the cutting site, the type of restriction enzymes and the cutting parts were investigated by NEBcutter (http://nc2.neb.com/NEBcutter2/).

First, PCR reaction was carried out in a total volume of 25 µg containing 14 ng DNA, 18.9 µl distilled water, 0.5 µl of forward and reverse primers (TAG Copenhagen, Denmark), 1 µl $MgCl_2$, 0.4 µl dNTP (Sina Gene, Iran), 2.5 µl buffer and 0.2 µl Taq polymerase (Sina Gene, Iran). Thermal cycling conditions consisted of 35 cycles with an annealing temperature of 58˚C and 62˚C for rs11556218 T/G and rs4072111 C/T, respectively. PCR products (5 µl) were analyzed by agarose gel (4%) electrophoresis. Finally, the enzymatic digestion of the PCR products was performed using *Bsm*AI (Thermo Scientific) and *Nde*I (Jena Bioscience) according to the manufacturer's instructions (Table 2).

The Hardy–Weinberg Equilibrium (HWE) was used with the X^2 test to compare distribution of the observed genotype frequencies in the study groups with the expected genotype

frequencies. The SPSS software (version 24) was used for statistical analysis of data. The relationship between numeric variables (nonparametric) and cancer risk was evaluated using the Student's t-test. Also, genotype and allele frequency distributions were compared using odds ratios (OR).

The significance level was set at 0.05. Power analysis was performed using the G* Power software (version 3.1.9.2).

RESULTS

According to the HWE test, the observed genotypic frequency distributions were not significantly different from the expected frequency distributions (P>0.05). In the post hoc power analysis, based on the number of cases and controls, our sample had enough power (79%) when considering medium effect sizes (w =0.30) and significance level of 0.05.

The age at menstruation and family history of cancer were significantly associated with the risk of breast cancer (P <0.05) (Table 3). The analysis for SNPs was performed under the dominant model. The genotype and allele frequencies of polymorphisms rs11556218 T/G and rs4072111 C/T are shown in table 4. There was a significant relationship between the rs11556218 polymorphism and breast cancer. According to the statistical analysis, the allele and genotype frequencies of *IL-16* rs4072111 C/T polymorphisms did not differ significantly between the patients and healthy controls (P> 0.05).

Haplotype analysis was performed using the SNP Analyzer software. A total of four possible haplotypes (TC, GC, TT and GT) were obtained for the SNPs rs11556218 (T>G) and rs4072111 (C>T). The haplotypes were not significantly different between the patients and controls (P> 0.05). The haplotype frequencies were TC 0.43, GC 0.28, TT 0.21 and GT 0.09. The frequency of TC haplotype was significantly higher (43%) compared to other haplotypes (OR= 0.583, 95% CI: 0.282-1.207, P= 0.144).

Table 1- Primer sequences used in the study

Polymorphism	Annealing temperature (˚C)	Primer sequence (5'-3')	Product size (bp)
rs11556218 T/G	58	F 5'-GCTCAGGTTCACAGAGTGTTTCCATA-3' R 5'-TGTGACAATCACAGCTTGCCTG-3'	171
rs4075111 C/T	62	F 5'-CACTGTGATCCCGGTCCAGTC-3' R 5'-TTCAGGTACAAACCCAGCCAGC-3'	164

Table 2- RFLP conditions used in the study

Polymorphism	Enzyme	Incubation temperature (˚C)/Time	Fragment size (bp)
rs11556218 T/G	*Nde*I	37/15 minutes	TT: 171; GG:147+24 TG: 171+147+24 CC: 164; TT:140+24
rs4075111 C/T	*Bsm*AI	40/5 hours	CT: 164+140+24

Table 3- The association of variables with risk of breast cancer in the patients and controls

Variable	Cases (%) N=80	Controls (%) N=80	P-value
Ages	50 (62.5%)	52 (65%)	0.025
≤50	30 (37.5%)	28 (35%)	
>50			
Family history of cancer			0.013
No	57(71.25%)	72(90%)	
Yes	22(27.5%)	6(7.5%)	
Unknown	1	2	
Age at menarche (years)<14			0.000
≥14	62(77.5%)	32(40%)	
Unknown	18(22.5%)	39(48.75%)	
	0	9	

Student's t-test.

Table 4- The relationship between the two SNPs (T/G rs11556218 and rs4072111 C/T) in the *IL-16* gene and risk of breast cance

Genotype/allele	Cases (N=80)	Controls (N=80)	P-value	OR (95% CI)
rs11556218 T/G				
TT	28	50		1
TG	32	17	0.001	2.471 (1.229-4.965)
GG	20	13	0.171	1.718 (0.787-3.749)
TG + GG	52	30	0.001	3.095 (1.624-5.899)
(Dominant model)				
T	88	117		1
G	72	43	0.001	2.226 (1.394-3.555)
rs4072111 C/T				
CC	43	47		1
CT	25	26	0.908	0.962 (0.494-1.872)
TT	11	7	0.303	1.687 (0.618-4.602)
CT + TT	36	33	0.583	1.192 (0.636-2.234)
(Dominant model)				
C	111	120		
T	47	40	0.342	1.270 (0.775-2.083)

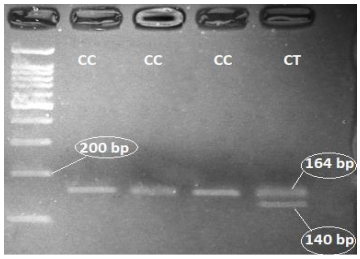

Figure 1-Results of digestion of IL-16 rs4072111 C/T polymorphism on 4% electrophoresis gel. Amplified fragment of rs4072111 polymorphism was digested by BsmAI. Lane 1: 100 bp DNA ladder (Sinaclon, Iran); CC: wild-type (164 bp); CT: heterogeneous type (164, 140 and 24 bp); TT: mutant type (140 and 24 bp) of rs4072111 polymorphism.

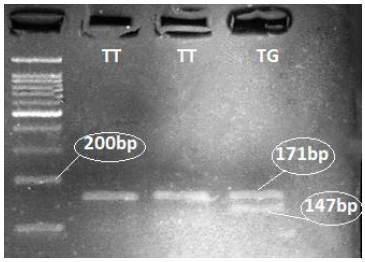

Figure 2- Results of digestion of IL-16 rs11556218 T/G polymorphism on 4% electrophoresis gel. Amplified fragment of rs4072111 polymorphism was digested by NdeI. Lane1: 100 bp DNA ladder (Sinaclon, Iran); TT: wild-type (171 bp); TG: heterogeneous type (171, 147 and 24 bp); GG: mutant type (140 and 24 bp) of rs11556218 polymorphism.

DISCUSSION

Although IL-16 has been identified as an important mediator in inflammatory diseases, there is little information about the association between IL-16 and cancer. In the present study, we investigated the relationship between two polymorphisms of *IL-16*(rs11556218 T/G and rs4072111 C/T) and the risk of breast cancer. The rs11556218 T/G polymorphism occurs in the coding region of exon 17. This is a missense mutation wherein asparagine is substituted by lysine. Statistical analysis showed a significant correlation between this polymorphism and breast cancer. Distribution of genotype and alleles frequencies of this polymorphism was significantly different between the patients and controls. In a previous study in China, the GG and TG genotypes were found to be associated

with an increased risk of breast cancer. Combination variants of the GG and TG were associated with a higher risk of breast cancer compared with the TT genotype (9), which is consistent with the results of our study. Another study in China showed that the rs11556218 T/G polymorphism was significantly associated with gastric and colorectal cancers. Both male and female patients carrying the G allele were at higher risk of developing colorectal cancer and gastric cancer compared with individuals carrying the T allele (13), which is in line with our findings. Contrary to these findings, a meta-analysis study by Zhou et al. (2019) in China reported that the rs11556218 polymorphism was not associated with the risk of renal cell carcinoma (14).

The rs4072111 C/T polymorphism is located in the coding region of exon 10. This is a missense mutation wherein proline is substituted by serine. We found no significant association between this polymorphism and breast cancer (P> 0.05). In a study in China, the allele and genotype frequencies of the rs4072111C/T polymorphism in breast cancer patients and healthy controls did not differ significantly (9), which is consistent with the results of the present study. In a study in Iran, Kashefi et al. claimed that gastric cancer had a significant association with the rs4072111 polymorphism. Individuals with the CT genotype had a significantly higher risk of developing gastric cancer. Also, there was a significant association for the T allele and increased risk of gastric cancer, which is not in line with our findings (15).

In this study, there was a significant association between positive family history of cancer and risk of breast cancer. Moreover, there was a significant relationship between the age of onset of menstruation and breast cancer, which is consistent with results of a study by Rojaas et al. (3).

According to the results of this study, there was a significant association between the rs11556218 polymorphism and breast cancer in the study population. Screening for presence of this polymorphism could contribute to prevention of breast cancer, particularly in at risk populations. However, no significant association was found between the rs4072111 polymorphism and the risk of breast cancer.

Our study population was relatively small, which may hide various gene-gene and gene-environment interactions. It is recommended to conduct more studies using a larger sample size to confirm our findings.

CONCLUSION

These findings suggest that the rs11556218 T/G polymorphism of the *IL-16* gene could be used as a biomarker for predicting breast cancer. However, further investigations with a larger sample size from different ethnicities may be required to confirm this finding.

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CONFLICT OF INTEREST

There is no conflict of interest regarding publication of this study.

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