Interleukin-10 Promoter Polymorphisms and Breast Cancer Risk in Iranian Women

Hossein Abdolrahim-Zadeh[1], Niloufar Hakkakian[1], Reza Asadollahi[1], Behrouz Gharesifard[1], Jamal Sarvari[1], Eskandar Kamali-Sarvestani[1], Abdolrasoul Talei[2]*

[1]Departments of Immunology and [2]Surgery, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran.

ABSTRACT

Background: IL-10 is an anti-inflammatory cytokine which is involved in tumorigenesis. Over production of IL-10 and elevated number of IL-10 generating mononuclear cells in breast tumor tissue has already been shown. **Objective:** To determine the association of IL-10 promoter polymorphisms with increased risk of breast cancer and its association with breast cancer prognostic factors. **Methods:** Peripheral blood samples from 275 female breast cancer patients and 320 cancer free controls were used to detect three single nucleotide polymorphisms in IL-10 promoter region (-1082, -819, -592) by PCR method. **Results:** The frequency of genotypes and alleles of three mentioned regions of IL-10 promoter and their haplotypes (GCC, ATA, and ACC) showed no statistically significant difference between patients and controls. In the case of prognostic factors, progesterone receptor (PR) status exhibited significant relation with -1082 genotypes (P=0.03) and haplotypes (P=0.02). -1082 AA genotype was associated with negative PR expression whereas AG and GG genotypes of this site were positively associated with PR expression. Similarly GCC haplotype correlated with positive PR expression and ATA and ACC with negative PR expression. **Conclusion:** The data of this study showed that IL-10 promoter gene polymorphisms may not be considered as one of the risk factors for breast cancer in Iranian patients.

Keywords: Breast Cancer, IL-10, Polymorphism, Progesterone Receptor

*Corresponding author: Dr. Abdolrasoul Talei, Department of surgery, Shiraz University of Medical Sciences, Shiraz, Iran. Tel: +98(711)2351090

INTRODUCTION

Breast cancer (BC) is the most common malignancy affecting women worldwide and a family history of BC is one of the most important risk factors (1). It is suggested that a number of prevalent, low-penetrance genes contribute to BC susceptibility in a large population of women (2). However, little is known about low-penetrance susceptibility genes which contribute to BC susceptibility and only a few have been identified. Cytokines are powerful modulators of the immune system and it has been shown that they are released by the tumor associated lymphocytes (TALs) present in breast tumors (3).

IL-10 is an important anti-inflammatory and immunosuppressive cytokoine produced by Th2 cells and affect many aspects of immune response (4). It is involved in the regulation of inflammatory response (5), infection progression (6), autoimmunity (7), transplantation tolerance (8) and tumorigenesis (9, 10). As a tumor-related inhibitory cytokine, IL-10 can inhibit class I major histocompatibility complex (MHC-I) expression (11), tumor antigen presentation to T lymphocytes and ultimately induces T-cell anergy (12), which consequently can contribute to tumor escape from immune response. Furthermore, elevated serum levels of IL-10 have been observed in several malignancies such as pancreatic and gastric adenocarcinoma (10), malignant melanoma (13), multiple myeloma (14), and breast cancer (15). In addition to increased IL-10 production in breast cancer, high prevalence of Th2 cells in peripheral blood and tumor microenvironment of patients (16), elevated numbers of IL-10 generating mononuclear cells (17) and IL-10 mRNA (18) in breast tumor tissue have been shown. This condition may lead to inappropriate function of tumor infiltrating lymphocytes (28). Therefore, it seems that factors affecting IL-10 production, mainly genetic factors, may have a potent role in development of breast cancer.

IL-10 gene is located on chromosome 1 at q31-32 (19). One microsatellite and three single nucleotide polymorphisms within its gene promoter at positions -1082, -819, and -592 in upstream region of the gene have been previously reported. From different combinations of the three mentioned SNPs, three functional haplotypes (GCC, ACC, ATA) are described in Caucasian population (20). GCC haplotype correlated with higher levels of IL-10 production (20).

Polymorphisms of IL-10 promoter region have been investigated in malignancies like cervical cancer (21) and multiple myeloma (17). Few studies have examined the association between these polymorphisms and breast cancer and only one has evaluated the correlation between these polymorphisms and the prognostic factors (22). In this study, we analyzed the three polymorphisms at positions -1082, -819, and -592 of IL-10 promoter region in breast cancer patients and compared the results with healthy controls. Then, we evaluated the correlation between these polymorphisms and important breast cancer prognostic factors.

SUBJECTS AND METHODS

Subjects

Our case-control study was conducted on patients who had come to the Motahari clinic, Shiraz, Iran. This clinic is a referral center for south of Iran, especially Fars province. 275 breast cancer patients (mean age = 48.3 ± 11 years) who were at stages

I-III with three to eighty-month follow up were selected. The study was performed with the informed consent of the patients. Demographic information including age, age at disease onset, menarche, menopause, past medical history and other important risk factors were questioned through face to face interview. 8 to 10 ml EDTA-treated blood was collected from each individual. Clinical tumor staging, lymph node involvement status, histological grade, tumor size and other prognostic factors were derived from their charts (Table 1). 320 ethnic, age and sex-matched women were chosen as control group. Patients had no history of autoimmune diseases and other cancers.

Table 1. Breast cancer patients' characteristics

Variables	Number (%)
Tumor size	
0-2	45 (17.6)
2-5	181 (70.7)
>5	30 (11.7)
Histological grade	
I	85 (35.9)
II	121 (51)
III	31 (13.1)
Stage	
I	47 (18.8)
II	166 (66.4)
III	37 (14.8)
ER status	
Positive	82 (36)
Negative	146 (64)
PR status	
Positive	119 (52.9)
Negative	106 (47.1)
Lymph node involvement	
0	89 (33.6)
1-3	46 (17.4)
>3	130 (49)

Methods

DNA Extraction and Polymerase Chain Reaction (PCR)

DNA extraction and polymorphism screening

Genomic DNA was extracted from peripheral blood leukocytes by salting out method. Cytokine gene polymorphisms were studied by Polymerase Chain Reaction (PCR) using a thermocycler (5530 Master cycler eppendroff, Germany). Allele specific PCR (AS-PCR) method was carried out for IL-10 (-1082) genotyping (22). Beta globin gene was used as the internal control. PCR-RFLP method in a final volume of 10 µl was employed for determining the polymorphisms of IL-10 -592 and -819 by using *Rsa* I and *Mae* III restriction enzymes, respectively (Table 2) (21). The sequence of primer sets have been shown in Table 2. The amplified products were run

Table 2. PCR conditions in IL-10 genotyping

Locus	PCR conditions	Reference
IL-10 -1082	30 cycles: 94°C 30 sec, 56°C 30 sec, 72°C 30 sec 250 ng DNA, 200 µmol dNTPs, 0.35 mM MgCl2	(Huaung et al., 1999)
IL-10 -819	30 cycles: 94°C 45 sec, 60°C 45 sec, 72°C 1 min 250 ng DNA, 200 µmol dNTPs, 1.3 mM MgCl2	(Roh et al., 2002)
IL-10 -592	35 cycles: 94°C 1 min, 63°C 70 sec, 72 °C 1 min 250 ng DNA, 200 µmol dNTPs, 2.7 mM MgCl2	(Roh et al., 2002)

Table 3. Primer sequences for IL-10 genotyping

Locus	Primers	Method
IL-10 -1082	Common primer, 5´-cag ccc ttc cat ttt act ttc-3´ G allele primer, 5´-tac taa ggc ttc ttt ggg ag-3´ A allele primer, 5´-cta cta agg ctt ctt tgg gaa-3´	´AS-PCR
IL-10 -819	5´-tca ttc tat gtg ctg gag atg g-3´ 5´-tgg ggg aag tgg gta aga gt-3´	*Mae*III based RFLP
IL-10 -592	5´-ggt gag cac tac ctg act agc-3´ 5´-cct agg tca cag tga cgt gg-3´	*Rsa* I based RFLP

on 2-2.5% ethidium bromide stained agarose gel and visualized under UV light (Fig 1).

Statistical Analysis

The genotype frequency between patient and control groups was compared and P values were calculated by Chi-square test. Odds ratio (OR) and 95% confidence intervals (CI) for relative risks were calculated by logistic regression analysis. Correlation between genotypes and demographic and clinical variables were assessed using Chi-square test except for age at onset for which we used Kruskl-Wallis H test. All statistical analyses were performed using the SPSS software version 11.5. $P<0.05$ was considered significant.

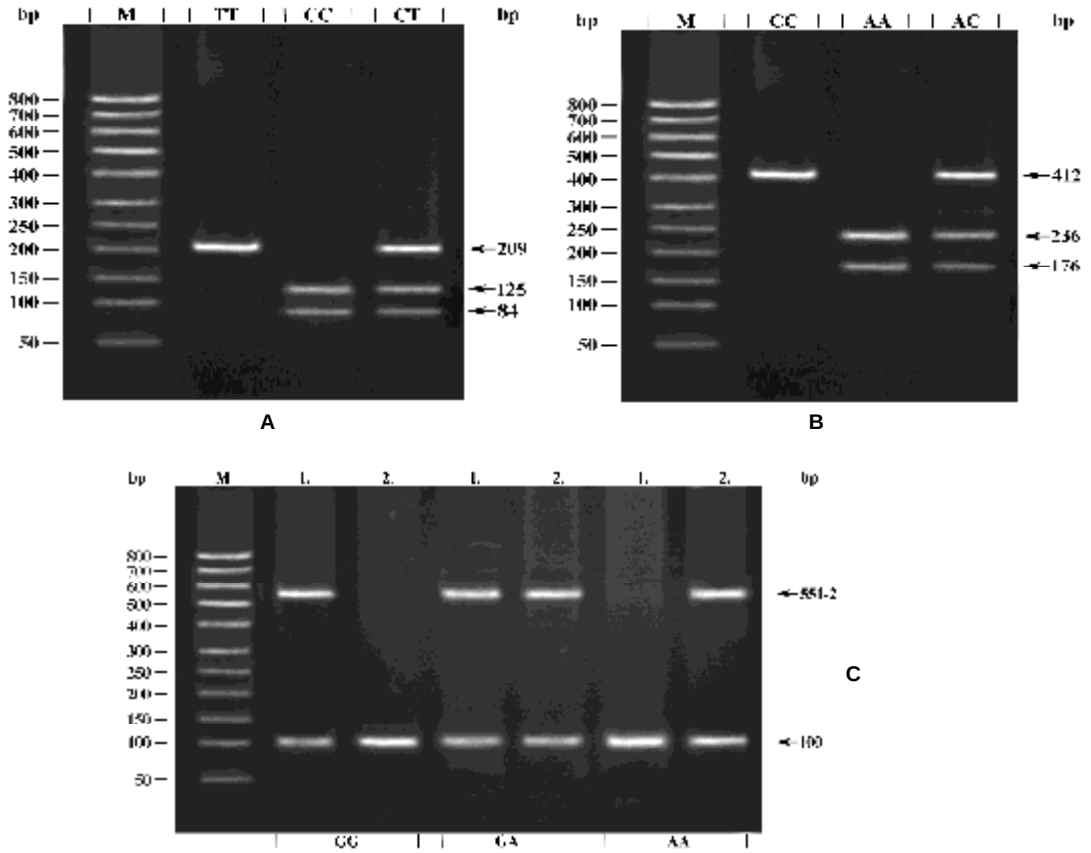

Figure 1. Electrophoresis patterns of the PCR products for IL-10 polymorphisms. M, DNA size marker. A) Determination of IL-10 (-819) genotypes by PCR-RFLP in three different individuals: 209 bp TT; 125, 84 bp CC and 209, 125, 84 bp CT genotypes. B) Determination of IL-10 (-592) genotypes PCR-RFLP in three different individuals: 412 bp CC; 236, 176 bp AA and 412, 236, 176 bp AC genotypes. C) Determination of IL-10 (-1082) genotypes by AS-PCR 100 bp internal control. Band of 551-2 bp present or absent. Lane 1: G allele, lane 2: A allele.

RESULTS

The genotypes and alleles frequency of three polymorphisms in IL-10 promoter region in patients and controls has been shown in Table 4. The genotypes frequency at each locus (-1082,-819,-592) was not statistically significant between patients and healthy controls (P>0.05). In addition, there was no statistically significant correlation between allele frequency in patients and healthy controls. The three defined haplotypes (GCC, ATA, and ACC) were present with six combinations of ACC/ACC, ACC/ATA, ACC/GCC, ATA/ATA, ATA/GCC and GCC/GCC (Table 5) but there was no statistically significant correlation between these haplotypes in breast cancer patients in comparison to healthy controls (P>0.05).

Table 4. Genotype and allele distribution of IL-10 promoter polymorphisms in breast cancer patients and controls

Genotype and Allele	Controls (%)	Patients (%)	P-value
-1082			
AA	45.8	43.4	0.75
AG	39	42.2	
GG	15.2	14.4	
-819			
CC	52	47	0.43
CT	38.2	43.6	
TT	9.8	9.4	
-592			
AA	9.9	9.2	0.52
AC	36.5	41.4	
CC	53.6	49.4	

Table 5. Association between IL-10 promoter haplotypes and breast cancer

Haplotype (-1082, -819, -592)	Controls n (%)	Patients n (%)	OR (95% CI;P value)
ACC/ACC	42(15.3)	36(18.4)	1.15 (0.72-1.86; 0.54)
ACC/ATA	55(20)	32(16.3)	1.81 (1.13-2.88; 0.09)
ACC/GCC	61(22.2)	39(19.9)	1.64 (1.06-2.53; 0.07)
ATA/ATA	32(11.6)	18(9.2)	1.81 (0.99-3.29; 0.10)
ATA/GCC	45(16.4)	45(22.9)	0.97 (0.62-1.51; 0.90)
GCC/GCC	40(14.5)	26(13.3)	1.56 (0.93-2.63; 0.08)

In addition, the relationship between mentioned polymorphisms and important prognostic factors in breast cancer was also assessed. As a result, IL-10 (-1082) genotype exhibited significant difference in PR positive and PR negative patients (Table 6, p=0.03). AA genotype was significantly higher in patients with negative PR compared to those with positive PR expression (51.7% *vs.* 30.8%) while AG and GG genotypes were higher in patients with positive PR expression than those with negative PR expression (54.3% *vs.* 37.1% and 14.9% *vs.* 11.1%, respectively). PR status was also correlated with haplotypes (P=0.02); GCC was higher in patients with positive PR status than those with negative ones (15.5% vs. 10%). Conversely, ATA or ACC haplotypes were higher in patients with negative PR status than those with positive ones (12.9% vs. 7% and 24.2% vs. 7%, respectively). Statistical analysis between different genotypes and haplotypes with age at onset, stage and other prognostic factors did not show any significant difference.

Table 6. Comparison of IL-10 promoter polymorphisms genotype and haplotype with PR status

IL-10promoter polymorphisms genotype	PR Status (%)		P-value
	+ve	-ve	
-1082			
AA	29(30.8)	46(51.7)	0.03
AG	51(54.3)	33(37.1)	
GG	14(14.9)	10(11.1)	
-819			
CC	41(41)	45(50.6)	0.27
CT	51(51)	33(37.1)	
TT	8(8)	11(12.3)	
-592			
AA	7(7.4)	11(12.8)	0.16
AC	47(49.5)	29(33.7)	
CC	41(43.1)	46(53.5)	
ACC/ACC	5(7)	17(24.2)	
ACC/ATA	10(14.1)	13(18.6)	0.02
ACC/GCC	18(25.4)	13(18.6)	
ATA/ATA	5(7)	9(12.9)	
ATA/GCC	22(31)	11(15.7)	
GCC/GCC	11(15.5)	7(10)	

DISCUSSION

In the present study, we analyzed the polymorphism at the three defined loci in the promoter region of the IL-10 gene in 275 breast cancer patients and 320 healthy individuals as the control group.

It has been proposed that IL-10 might contribute to the tumorigenesis of various types of cancers such as lymphoma (24), colon (25), and ovarian (26) cancer. Moreover, Kozlowski and co-workers reported higher amount of IL-10 in breast cancer patients compared to healthy women, and also a correlation between IL-10 levels and clinical stages of the disease (31). However, anti-tumor effects of IL-10 via mechanisms such as inhibition of angiogenesis and enhancing anti-tumor activity of natural killer cells have also been described (27-28). Therefore, it is possible that the genetic variation affecting IL-10 production might determine the susceptibility to breast cancer. According to this hypothesis, several studies have been performed in order to investigate the association of the IL-10 promoter polymorphisms with breast cancers (22, 29-30). In this respect, while Smith et al. reported no association between IL-10 -1082 A/G polymorphism and susceptibility to breast cancer (30), Giordani et al. have reported an increase in IL-10 -1082 AA genotypes frequency (low IL-10 producer genotype) in breast cancer patients compared with those in normal controls (29). Furthermore, Langsenlehner et al. have shown the association of IL-10 -592 C/A promoter polymorphism with the reduced risk of breast cancer (22). They did not find any association between IL-10 -592 C/A promoter polymorphism and clinicopathological data such as tumor size, histological grade, and steroid receptors status (22). In present study, we did not observe any difference in distribution of three single nucleotide polymorphisms in the promoter of IL-10 gene in breast cancer patients compared to normal controls. According to this finding, polymorphisms in IL-10 promoter gene could not contribute to the susceptibility of Iranian patients to breast cancer. However, when the patients were stratified according to the PR expression, we found a significant increase in the frequency of IL-10 -1082 AA genotype in patients with negative PR status. Also, GCC haplotype was associated

with positive PR status and ATA or ACC haplotype was associated with negative PR status. PR status is widely accepted as prognostic factor and in many analyses appears to be superior to ER in predicting the prognosis of primary breast cancer (32). Therefore, we concluded that IL-10 -1082 AA genotype could negatively affect the breast cancer prognosis. The association of IL-10 -1082 AA genotype and negative progesterone status remains to be explained at the molecular level.

In conclusion the results of the present study show that these three single nucleotide polymorphisms in promoter region of IL-10 gene may not be associated with breast cancer susceptibility in Iranian patients while Il-10 -1082 A/G polymorphism directly, or through linkage disequilibrium with other genes may involve in breast cancer prognosis. Regarding to the controversy that exists for the association of IL-10 -1082 A/G polymorphism with breast cancer, further investigation is recommended.

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