Association of breast cancer and cytokine gene polymorphism in Turkish women

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ABSTRACT

Objective: To investigate the association of cytokine gene polymorphism with the development of breast cancer.

Methods: The study was carried out in Uludag University Medical School, Bursa, Turkey. The study included 38 patients with breast cancer admitted to the Medical Oncology outpatient clinic, and 24 healthy controls, age and sex matched, from the Internal Medicine Department between 2004 and 2005. All genotyping of tumor necrosis factor-α (TNF-α), tumor growth factor-β1 (TGF-β1), interleukin (IL)-10, IL-6, and interferon-γ (IFN-γ) experiments were performed using polymerase chain reaction sequence-specific primers.

Results: The frequencies of IL-6-174GC genotype and IL-10 (-1082, -819, -592) GCC/ATA haplotype were significantly higher in the patient group (p=0.0008) when compared with controls (p=0.020). Significantly lower frequencies of IL-10 (-1082, -819, -592) ACC/ATA haplotype were observed in the patient group in comparison to the controls (p=0.026). The distribution of IFN-γ +874, TNF-α 308, and TGF-β1 codon 10-25 genotypes failed to show any statistical significant association with the development of breast cancer.

Conclusion: Our data suggest that IL-10 (-1082, -819, -592) GCC/ATA haplotype and IL-6-174 GC genotype seem to be potential risk factors for the development of breast cancer. The presence of IL-10ACC/ATA haplotype may be protective for the oncogenesis of breast cancer.

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Breast cancer is the most common neoplasm in women worldwide. The role of cytokines and growth factors in tumor development and progression has been the focus of attention recently. Cytokines and growth factors can contribute directly to the proliferation of cancer cells and tumor progression in a number of ways. These include constitutive autocrine production of stimulatory factors by tumors, and increased sensitivity to autocrine or paracrine factors, or acquisition of resistance to cytokines and growth factors.[1-3] These factors have been reported to play significant roles in breast carcinoma progression.[4] Genetic polymorphisms potentially affecting the production levels of certain cytokines may be important determinants of disease risk, severity, or protection for several conditions in which the immune system plays significant roles, such as malignancies. The aim of the present study is to investigate whether there is any association between cytokine gene polymorphisms, and the risk of breast cancer development and severity of the disease.

Methods. The study was carried out at the Uludag University Medical School, Bursa, Turkey, between 2004 and 2005. Thirty-eight breast cancer patients were recruited from the outpatient clinic. A panel of cytokines related to tumor development and progression, tumor necrosis factor-α (TNF-α), tumor growth factor-β1 (TGF-β1), interleukin (IL)-10, IL-6, and interferon-γ (IFN-γ), that are known to be involved in tumor immunity were used. All of the cases were invasive ductal carcinoma on pathologic examination. The stage of the disease according to the tumor, node, metastasis system, histologic type, menopausal status, estrogen receptor (ER), and progesterone receptor (PR), and vascular and lymphatic invasion was recorded for each patient. Thirty-five patients were investigated for the presence of both ER and PR in the lymph node biopsies by using histopathological stainings.

Axillary lymph node involvement in breast cancer is a marker of recurrence risk. Patient groups were divided into 2 subgroups according to lymph node involvements. Group I included 18 cases without lymph node involvement and Group II was composed of 20 patients with at least 4 lymph node metastases. The control group (Group III) comprised of geographically and racially matched adult healthy blood donor volunteers (n=24). Patients with only breast cancer without any other neoplasm were included the study. Patient and control subjects were all born in Turkey, and shared the same geographic origin and culture. Ethical approval and written informed consent were obtained.

DNA isolation and cytokine genotyping. Genomic DNA was extracted from whole ethylenediamine tetraacetate-treated blood with the Machery Nagel DNA isolation kit (Duren, Germany) according to the manufacturer's instructions. Single nucleotide polymorphisms were analyzed in 5 cytokines for genotype assignment. The presence of a G or A nucleotide in position -308 of the promoter region was analyzed for TNF-α. Two single nucleotide mutations in the coding region were surveyed for TGF-β1 codon 10 can be either T or C, and codon 25, either C or G. Three different polymorphisms were analyzed for the IL-10 promoter region position -1082 (G versus [vs] A), position -819 (C vs T), and position -592 (A vs C). The presence of a single nucleotide modification in position -174 was examined for IL-6 promoter. An additional coding sequence mutation (T vs A) at position +874 was analyzed for IFN-γ. Cytokine genotypes were determined using the polymerase chain reaction (PCR) sequence-specific primers method by a commercially available kit (One lambda, Inc, Canoga Park, CA, USA) in accordance with the manufacturer's instructions. The DNA extractions and PCR amplifications were performed by a technician blinded to the clinical results.

Statistical analysis was performed by Epi Info Version 3.2.2 (Centers for Disease Control and Prevention, USA). The distribution of cytokine gene polymorphisms were compared between patients with breast cancers and healthy controls by χ^2 with Yate's correction, or Fisher's exact test and Kruskal-Wallis tests. The *p*-values smaller than 0.05 were considered significant. Odds ratios and 95% confidence intervals were also calculated if χ^2 with Yate's correction or Fisher's exact test was significant.

Results. Mean ages were as follows: Group I, 48.5 years (34-69), Group II, 45.5 years (35-70), and Group III, 39 years (20-57), with no statistically significant difference found ($p>0.05$). Fifty-three percent of the patients underwent modified radical mastectomy and 47% underwent breast-conserving surgery. All patients received postoperative adjuvant chemotherapy. Eighteen of the patients (47%) were at stage II, and 20 (53%) at stage III. Hormone therapy was also used according to the receptor status of the cases. Fifty-five percent of the patients were premenopausal (n=21), whereas 45% were postmenopausal (n=17). The ER-positive was found in 44% of Group I (n=8) and 55% of Group II (n=11), and PR-positive was observed in 39% of Group I (n=7) and 50% of Group II (n=10). The 2 groups when compared, showed no statistically significant difference in terms of menopausal situation, ER- and PR-positivity, and vascular, and lymphatic invasion ($p>0.05$). The distribution of cytokine genotypes among the patients with breast cancer and healthy control subjects are summarized in **Table 1**. The frequencies of IL-6 -174 GC genotype and IL-10 (-1082, -819, -592) GCC/ATA haplotype were significantly higher in the patient group when compared with control group. In contrast, significantly lower frequencies of IL-10 (-1082, -819, -592) ACC/ATA haplotype were observed in the patient group in comparison to control group. The distribution of IFN-γ +874, TNF-α 308, and TGF-β1 codon 10-25 genotypes failed to show any statistically significant association with the development of breast cancer. Risk and protective genotypes are summarized in **Table 2**.

Patients were divided into 2 subgroups according to lymph node involvements. First group (Group I) consisted of patients with poor prognostic factors, as they had axillary node involvement, and second group (Group II) without any. For the subgroups, as shown in **Table 3 and 4**, a significant difference was observed in the frequency of TGF-β1 codon 10-25 CC/GG genotype, which was significantly lower in node-positive patients when compared with node-negative patients. The IL-10 (-1082, -819, -592) GCC/ATA haplotype frequency between the patients with node-positive and healthy controls is significantly high. The frequencies of IL-6 -174 GC genotype were significantly higher in node-positive patients than controls. The frequency of TNF-α -308 GG genotype was significantly higher in patients with node-negative when compared with controls.

Discussion. Many studies have reported that there is an association between cytokine gene polymorphisms and the development of cancer.[5] In some studies, these genetic polymorphisms are shown to affect the overall expression and secretion of cytokines, both in vitro and sporadically in vivo systems.[6] In this study, we investigated the potential associations between cytokine gene polymorphisms and breast cancer in Turkish women. Homozygosity for TNF-α -308 GG is associated with low production, whereas the TNF-α -308 GA and

Table 1 - Cytokine gene polymorphisms among breast cancer patients and healthy controls.

Cytokine gene polymorphism	Genotype	Patients (n=38)	Controls (n=24)
TNF-α (-308)	G/G	30 (79.0)	15 (62.5)
	G/A	6 (15.7)	9 (37.5)
	A/A	2 (5.3)	0
TGF-β1 (codon 10-25)	T/T-G/G	20 (52.7)	9 (37.5)
	T/C-G/G	10 (26.3)	8 (33.3)
	T/C-G/C	0	1 (4.7)
	C/C-G/G	8 (21.0)	3 (12.5)
	T/T-G/C	0	0
	C/C-G/C	0	1 (4.7)
	C/C-C/C	0	0
	T/T-C/C	0	2 (8.3)
	T/C-C/C	0	0
IL-10 (-1082 -819, -592)	GCC/GCC	3 (7.9)	1 (4.1)
	GCC/ACC	9 (23.7)	5 (20.9)
	GCC/ATA*	13 (34.2)	2 (8.3)
	ACC/ACC	4 (10.5)	4 (16.7)
	ACC/ATA*	4 (10.5)	8 (33.3)
	ATA/ATA	5 (13.2)	4 (16.7)
IL-6 (-174)	G/G	15 (39.4)	14 (58.3)
	G/C*	17 (44.8)	3 (12.5)
	C/C	6 (15.8)	7 (29.2)
IFN-γ (+874)	T/T	8 (22.0)	4 (16.6)
	T/A	15 (39.0)	9 (37.5)
	A/A	15 (45.9)	11 (39.0)

Data were expressed as number and percentage (%).
TNF-α- tumor necrosis factor-alpha, TNF-α, TGF-β1 - tumor growth factor-β1, IL - interleukin, IFN-γ - interferon-gamma, *statistically significant for patients with breast cancer versus controls

Table 2 - *P*-values and odd ratios (ORs) of potential risk and protective factors for breast cancers.

Cytokine gene polymorphisms	*P*-values	ORs	95% CI
Risk Factors			
IL-10 (-1082,-819)GCC/ATA	0.020	5.7	1.04-41.3
IL-6-174 G/C	0.0008	5.7	1.28-28.7
Protective Factors			
IL-10 (-1082,-819)ACC/ATA	0.026	4.3	0.96-20.13

CI - confidence interval, IL - interleukin

TNF-α -308 AA genotypes are high producers.[7] In this trial, TNF-α -308 genotypic disassociation has showed dominance as GG genotype, as in prostate and pancreas cancer, whereas there is no significant difference in the control group.[8,9] We did not find a significant elevation in TNF-α -308 GG, contrary to Smith et al's trial.[10] In the subgroup analysis, the frequency of TNF-α -308 GG genotype was higher in node-negative patients, and may seem to increase the risk for the development of the disease. In our study, the TNF-α -308 AA genotype was only observed in the cancer group (5.3%), in comparison with previous studies by Jang et al[9] and Park et al.[11] The AA genotype was found higher in only the poor prognosed breast cancer group in the trial by Mestiri et al.[12] This observation suggests that the carriage of -308 A may predispose to a more aggressive disease, possibly as a result of increased levels of TNF protein.

The IL-6 G allele is associated with a more advanced stage and poorer survival.[13] After the previous trials on the relationship of IL-6 -174 gene polymorphism and disease in gastric carcinoma,[14] and ovarian cancer,[15] recent studies examined the role of IL-6 in breast cancer staging and mortality. DeMichele et al[16] studied 124 node-positive breast cancer patients, and found that survival was shorter in GG as compared to CG or CC individuals, and concluded that the -174G allele contributes to the reduced survival of patients with node-positive breast cancer. On the contrary, Iacopetta et al[17] found that the CC genotype is associated with poorer histological grade, a ductal histology, and a tendency towards larger size.

Table 3 - Cytokine gene polymorphisms among subgroups patients and healthy controls.

Cytokine gene polymorphisms	Genotype	Group I (n=18)	Group II (n=20)	Group III (n=24)
			n (%)	
TNF-α (-308)	G/G‡	17 (94.4)	13 (65)	15 (62.4)
	G/A	1 (6.6)	5 (25)	9 (37.5)
	A/A	0	2 (10)	0
TGF-β1 (codon 10-25)	T/T-G/G	7 (38.9)	13 (65)	9 (37.5)
	T/C-G/G	3 (16.7)	6 (30)	8 (33.0)
	T/C-G/C	0	0	1 (4.2)
	C/C-G/G*	7 (38.9)	1 (5)	3 (12.5)
	T/T-G/C	0	0	0
	C/C-G/C	0	0	1 (4.16)
	C/C-C/C	0	0	0
	T/T-C/C	0	0	2 (8.3)
	T/C-C/C	1 (5.5)	0	0
IL-10 (-1082 -819, -592)	GCC/GCC	2 (11.1)	1 (5)	1 (4.16)
	GCC/ACC	4 (22.1)	6 (30)	5 (20.9)
	GCC/ATA†	3 (16.7)	10 (50)	2 (8.3)
	ACC/ACC	3 (16.7)	1 (5)	4 (16.7)
	ACC/ATA*	3 (16.7)	1 (5)	8 (33.3)
	ATA/ATA	3 (16.7)	1 (5)	4 (16.7)
IL-6 (-174)	G/G	9 (50.0)	5 (25)	13 (54.1)
	G/C†	5 (27.8)	12 (60)	4 (16.7)
	C/C	4 (22.2)	3 (15)	7 (29.2)
IFN-γ (+874)	T/T	6 (33.3)	2 (10)	4 (16.6)
	T/A	8 (44.5)	7 (35)	9 (37.5)
	A/A	4 (22.2)	11 (55)	11 (45.9)

Group I - breast cancer with node negative, Group II - breast cancer with node positive, Group III - control cases, TNF-α- tumor necrosis factor-α, TGF-β1 - tumor growth factor-β, IL - interleukin, IFN-γ - interferon-γ, *Group I-II, †Group II-III, ‡Group I-III (p<0.05).

Table 4 - *P*-values and odd ratios (ORs) of potential risk and protective factors subgroups.

Cytokine gene polymorphisms	*P*-values	ORs	95% CI
Risk factors			
TNF-α -308 G/G (I-III)	0.017	10.2	1.28-285.5
IL-10 (-1082,-819, -592), GCC/ATA(II-III)	0.00059	11	1.72-89.9
IL-6-174 G/C (II-III)	0.007	7.5	1.57-40.0
Protective factors			
TGF-β1 codon 10-25, C/C G/G (I-II)	0.013	13.3	1.27-331.3

CI - confidence interval, TNF-α- tumor necrosis factor-α, IL - interleukin, TGF-β1 - tumor growth factor-β, Group I - breast cancer with node negative, Group II - breast cancer with node positive, Group III - control cases

The study by Smith et al[10] found the relationship with GC genotype and high grade. In our study, the higher frequency IL-6 GC (high producer)[18] genotype was found in cancer patients and also in the high-risk group, which suggests that the presence of this haplotype may be a risk factor for the oncogenesis of breast cancer and severe disease. Several features of the IL-6 gene polymorphism at nucleotide -174 indicated it to be a possible susceptibility factor underlying racial and ethnic disparities in breast cancer mortality. The different ethnic groups participating in the trials may cause the variable conclusions. Although the patient number is small, our trial shows the special features of Turkish breast cancer patients.

The IL-10 levels were found to be significantly higher in the sera from breast cancer patients, and correlated with the severity of neoplasia.[19] A relationship between IL-10 gene polymorphism and lung cancer has been shown,[20] whereas the same results could not be reached in gastric cancer,[21] and renal cell carcinoma.[22] In our study, the higher frequency of IL-10 (-1082 -819 -592) GCC/ATA haplotype, which is associated with high production,[23] was found in approximately all cancer patients and the high-risk group. The results

suggest that the presence of this haplotype may be a risk factor for the oncogenesis of breast cancer and severe disease. Cunningham et al,[24] showed that patients with aggressive non-hodgkin's lymphoma are more likely to have a low (ATA) and intermediate (ACC) IL-10 producing genotype; however, in our trial the frequency of IL-10 (-1082 -819 -592) ACC/ATA haplotype, which is associated with low production, was lower in patients with breast cancer with lymph node involvement when compared to controls. Therefore, the IL-10 ACC/ATA haplotype may have a protective effect on the development of breast cancer, especially in cases with poor prognosis.

The TGF-β1 has been implicated in both mammary development and mammary tumorigenesis,[25] and it can be concluded that functional genetic variations of the TGF-β1 gene may be involved in breast cancer progression. Basturk et al,[22] found TT/GC as a protective factor in renal carcinoma patients. Migita et al[26] showed the CC genotype as a protective factor in hepatocellular carcinoma. There is no correlation with breast cancer and codon 10.[27] In our study, the frequency of TGF-β1 codon 10-25 CC/GG genotype was significantly lower in node-positive patients suggesting that TGF-β1 codon 10-25 CC/GG genotype seems to be a potential protective factor and may have a prognostic value.

In a study performed in African women with cervical cancer, no significant difference was found in the frequencies of IFN-γ +879 gene polymorphisms.[28] Halma et al[29] found this significant in pancreatic cancer, whereas Lai et al[30] found no relation in Taiwanese patients. We did not demonstrate any statistically significant difference in IFN-γ +874 gene polymorphism in breast cancer patients in Turkish women.

In conclusion, we have demonstrated that there are some associations between certain cytokine gene polymorphisms and breast cancers, and these polymorphisms may be a valuable predictor determinants for the development of breast cancer. However, larger studies are necessary to investigate the independency of these polymorphisms involved in the oncogenesis and prognosis of breast cancer. This may not only provide opportunities for early prediction of the risk of developing breast cancer, and also bring new ideas to design individual-based treatment strategies.

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Statistics

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