Association of interleukin-8 (IL-8 or CXCL8) -251T/A and CXCR2 +1208C/T gene polymorphisms with breast cancer

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A case-control study involving 257 breast cancer patients with invasive ductal carcinoma and 233 healthy women was carried out to explore if the IL-8 -251T/A polymorphism and the CXCR2 +1208 C/T polymorphism have a role in breast cancer susceptibility. Genotypic analysis showed an increased frequency of high producer IL-8 -251AA genotype (p=0.016) in the patient group as compared to controls while CXCR2 +1208 C/T polymorphism did not show any differences between studied groups. However, in contrast to IL-8 -251 polymorphism, the percentage of CXCR2 +1208 TT genotype was significantly lower in patients with NPI≤3.4 compared to NPI>3.4 (2% and 12%, respectively; p=0.03). Also, ER^- tumors showed an approximately significant higher CXCR2 +1208 TT genotype compared to ER^+ tumors (18.6% and 7.1%, respectively; p=0.07). In conclusion, IL-8 -251T/A polymorphism is associated with development of invasive ductal carcinoma type of breast cancer while CXCR2 +1208C/T polymorphism may affect the disease progression.

Key words: Breast Cancer; IL-8; CXCR2; Polymorphism.

IL-8 (designated CXCL8 in current chemokine nomenclature) is a small basic protein that belongs to the structural subgroup of ELR^+-CXC chemokines [1]. IL-8 is expressed in many different cell types as well as in several human tumor cell lines [2, 3]. This chemokine was first characterized for its ability in recruitment and activation of neutrophils at inflammatory sites [1, 4]. IL-8 also promotes inflammatory processes by inducing cytokine production [5] and release of tissue damaging mediators by neutrophils [4, 6]. Therefore, it could be supposed that IL-8 may play a role in immunity to tumors by mobilizing and activating neutrophils. However, it is known that the inflammatory response is involved in the progression of tumors and therefore, IL-8 might promote tumor cell proliferation by amplification of inflammation in the tumor microenvironment [7–11]. In agreement with this hypothesis, it has been shown that high serum levels of the IL-8 is a negative prognostic indicator in several tumors [12–15], including breast cancer tumors [16]. Furthermore, IL-8 possesses potent pro-angiogenic properties [17]. This property along with induction of matrix metalloproteinase transcription [18] is believed to contribute to the metastatic potential of breast cancer tumor cells ectopically releasing large amounts of this chemokine [19]. Consistent with this opinion, the metastatic breast cancer cell lines not only produce substantially higher quantities of IL-8 constitutively, but their response to inducers of IL-8 is also considerably high when compared to the non-metastatic cell lines or normal breast epithelial cells [19, 20]. CXCR2 is an IL-8 receptor expressed on endothelial cells and mediates ELR^+-CXC chemokine-induced angiogenesis [21, 22]. By considering the expression of IL-8 receptors by breast cancer cells and vessel endothelial cells, an autocrine effect for this chemokine has been suggested [23].

Above mentioned data indicate that the host ability to produce IL-8 may play an important role in susceptibility to and/or prognosis of breast cancer. Interestingly, production of IL-8 can be controlled by the -251 T/A polymorphism in the promoter region of this chemokine. The A-allele in this single nucleotide polymorphism was found to be related to higher in vitro levels of IL-8 production after stimulation with lipopolysaccharide or cytokines such as IL-1 β and TNF-α [24,

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25]. Several studies have shown the association of IL-8 -251T/A polymorphism with different infectious, neurological and malignant diseases [24, 26–28]. Three single nucleotide polymorphisms at position + 785 C/T, + 1208 T/C and + 1440 G/A of CXCR2 gene were also reported [29]. The CXCR2 +1208 C/T is located in the non-coding region of CXCR2 gene and there are several reports indicating that +1208 C/T variant might provide valuable information for the pathogenesis of and susceptibility to chronic inflammatory diseases [29, 30]. Because of the critical role of IL-8 is supposed to play in the pathogenesis of breast cancer, polymorphisms in either the ligand IL-8 or its receptor, CXCR2, might be linked to disease progression. Therefore, this study evaluated the association of CXCR2 +1208 C/T and functional IL-8 -251 T/A gene polymorphism and susceptibility to invasive ductal type of breast cancer.

Material and Methods

Patients. Eligible cases (n=257) were women who underwent mastectomy or breast conservative surgery with full axillary lymph node dissection at Faghihi hospital of Shiraz university of medical sciences, during the period of February 1999 and July 2003. The patients median age was 49.4 years (ranging 28-85 year). Only infiltrating ductal carcinomas were included in the study. A number of pathological data were recorded from pathology reports for these patients, including tumor size, histologic tumor type, tumor stage, tumor grade and nodal status. The levels of steroid receptors were determined as previously described [31]. In the present study the Nottingham Prognostic Index (NPI) was substituted for prognosis as it accurately forecast survival in breast cancer patients. The NPI was calculated for each patient according to Galea et al. [32]. The patients were divided into three prognostic groups. Patients with an NPI of ≤ 3.4 were placed in the good prognosis group while those with 3.4<NPI≤5.4 and NPI>5.4 were placed in the moderate and poor prognosis group, respectively. Control group consist of 233 healthy females who had no past history or a family history of breast cancer. They were recruited from Shiraz Blood Transfusion Organization with the age older than 50 years. Patients and controls were residents of the same area (fars province). The present study was approved by the local ethics committee and sampling was done after the study was explained and the patient's consent was obtained.

Determination of IL-8 and CXCR2 genotypes. Genomic DNA was extracted from peripheral blood leukocytes by a salting out procedure [33]. An allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) was used to detect the polymorphism at position -251 of IL-8 gene and +1208 of CXCR2 gene [24, 29]. As an internal control, the b-globin specific primers were included in the ASO-PCR (Table 1). For IL-8 genotyping, 10 µl of PCR reaction mixture consisting of 250 ng of genomic DNA, 200 mmol/L dNTPs, 2.25 mM MgCl2, 1X Taq DNA polymerase buffer, 2 units of Taq DNA polymerase (Bohringer Manheim, Germany), 10 pmol of each test primer and 5 pmol of internal control primers were employed. Then, a touch-down procedure followed that consisted of 25 s at 95°C, annealing for 45 s at temperatures decreasing from 68°C (four cycles) to 61°C (20 cycles), and an extension step at 72°C for 40 s. The annealing temperature for the remaining 5 cycles was 58°C for 40 s. Determination of CXCR2 gene polymorphism was carried out in the same PCR reaction mixture except that the concentration of MgCl2 was 1.7 mM. In addition, the touch-down procedure was similar to IL-8 genotyping except that annealing temperature in three consecutive steps were 70°C, 65°C, and 55°C, respectively. Reaction products of IL-8 and CXCR2 gene amplification were separated on 2.5% agarose gel and stained with ethidium bromide.

Table 1. Primer sequences for determination of IL-8 (-251) and CXCR2 (+1208) gene polymorphisms.

Locus	Primers	Reference
IL-8 (-251)	common primer, 5'-tgc ccc ttc act ctg tta ac-3'	24
	A allele primer, 5'-cca caa ttt ggt gaa tta tca at-3'	
	T allele primer, 5'-cca caa ttt ggt gaa tta tca aa-3'	
CXCR2 (+1208)	common primer, 5'-gtc ttg tga ata agc tgc tat ga-3'	29
	C allele primer, 5'-cca ttg tgg tca cag gaa gc-3'	
	T allele primer, 5'-cca ttg tgg tca cag gaa gt-3'	
Beta globin	5'- aca caa ctg tgt tca cta gc -3'	39
	5'- caa ctt cat cca cgt tca cc -3'	

Statistical methods

Data were analyzed using chi-square test and Fisher's exact test if the number of subjects was less than five. All tests were performed two tailed with a confidence interval (CI) of 95%. Similarly, genotype frequencies were compared within the patient series, stratified according to prognostic group, using the Chi-square analysis or the Fisher's exact test when appropriate. Association of polymorphisms with age at disease onset, number of involved lymph nodes and greatest tumor size were analyzed using non-parametric Kruskal-Wallis test. Statistical calculations were carried out using the Epi Info 2000 software.

Results

IL-8 -251 T/A and CXCR2 +1208 C/T polymorphisms were genotyped in breast cancer patients and controls. IL-8 -251 T/A and CXCR2 +1208 C/T polymorphisms were in Hardy-Weinberg equilibrium in patients (p=0.16 and p=0.26, respectively) and controls (p=0.34 and p=0.88, respectively). The allelic frequencies of these polymorphisms and genotype distributions are given in table 2. As shown in this table, while no significant differences were demonstrated for CXCR2 +1208 C/T polymorphism, the IL-8 -251AA genotype frequency was increased significantly in the patient group compared with those

Table 2. Comparison of genotypes and allele frequencies of IL-8 T-251A and CXCR2 C+1208T gene polymorphisms between breast cancer patients and controls.

Gene	Patient group number (%)	Control group number (%)	P	OR (95% CI)
IL-8 (-251)				
AA genotype	79 (30.7%)	48 (20.6%)	0.016	
AT genotype	114 (44.4%)	106 (45.5%)		
TT genotype	64 (24.9%)	79 (33.9%)		
A allele	272 (52.9%)	202 (43.3%)	0.003	1.47 (1.13-1.91)
T allele	242 (47.1%)	264 (56.7%)		
CXCR2 (+1208)				
CC genotype	106 (48.6%)	120 (46%)	0.54	
CT genotype	85 (39%)	114 (43.7%)		
TT genotype	27 (12.4%)	27 (10.3%)		
C allele	297 (68.1%)	354 (67.8%)	0.97	1.01 (0.76-1.34)
T allele	139 (31.9%)	168 (32.2%)		

in the control group, indicative of a strong association with breast cancer (30.7 vs. 20.6%, p = 0.016). However, there were no significant differences between clinicopathological parameters (including the NPI, number of involved lymph nodes, size of tumor, tumor grade, T-staging, estrogen and progesterone receptor expression levels and the age of disease onset) and different genotypes of IL-8 -251 T/A polymorphism in patients. As for the IL-8 -251 T/A polymorphism, there existed no significant difference between the CXCR2 genotypes frequency and progesterone receptor expression levels (p=0.32) or the age of disease onset (p=0.4). Nonetheless, analysis by non-parametric Kruskal-Wallis test showed a significant difference in NPI between different CXCR2 genotypes (p=0.006). This difference was due to the higher mean rank of NIP in patients with TT genotype (106.78) compared to CC (70.06) or CT (77.25) genotype. According to this finding, when the CXCR2 genotypes of patients were categorized to TT and CC+CT, significant differences were found among patients of good (NPI≤3.4) and moderate + poor (NPI>3.4) prognosis (p=0.017). Also, comparing the frequency of TT and CC+CT genotypes of CXCR2 polymorphism, a significant difference appeared between cases with positive and negative estrogen receptor status (p=0.04; OR=2.96, 95% CI=1.04-8.86) and different T-staging (p=0.021). Results from IL-8 and CXCR2 SNPs comparisons with estrogen receptor status and NPI are presented in the table 3.

Discussion

Chemokines are a subclass of cytokines that direct the migration of leukocytes to the site of inflammation. IL-8, the prototype of ELR$^+$-CXC subgroup, along with other chemokines was shown to be highly expressed in breast carcinoma by the tumor cells as well as by stromal cells [23, 33, 34]. Moreover, IL-8 levels are significantly higher in patients with breast cancer compared with healthy volunteers [16]. Receptors for IL-8 were also detected on breast tumor cells and endothelial cells of tumor vessels [23]. Thus it should not come as a surprise that, aside from their role in mediating the recruitment of the tumor-infiltrating leukocytes to tumor sites, IL-8 and CXCR2 expression may also affect neoplastic proliferation and metastasis. In concordance with this idea, Ben-Baruch et al. has been reported the pro-malignant activity of IL-8 which is produced by tumor-associated macrophages [35]. As a matter of inter-

Table 3. Genotype frequency of the IL-8 T-251A and CXCR2 C+1208T gene polymorphisms in various clinicopathological conditions of the breast carcinoma patients.

Characteristics	IL-8 genotypes Number (%)			CXCR2 genotypes Number (%)		
	AA	AT	TT	CC	CT	TT
NPI[a]						
NPI≤3.4	16(30.2)	24(30.8)	13(28.9)	25(34.7)	21(33.3)	1(5)
NPI≥3.4	37(69.8)	54(69.2)	32(71.1)	47(65.3)	42(66.7)	19(95)
ER[b]						
+ 30(45.5)	46(48.9)	24(44.4)	43(48.9)	35(50.7)	6(25)	
_	36(54.5)	48(51.1)	30(55.6)	45(51.1)	34(49.3)	18(75)
PR[c]						
+	33(50)	57(60)	26(48.1)	51(58)	33(47.8)	15(62.5)
_	33(50)	38(40)	28(51.9)	37(42)	36(52.2)	9(37.5)
T staging[d]						
I	12(22.2)	18(22.5)	9(23.1)	19(25.3)	16(26.2)	0(0)
II	38(70.4)	46(57.5)	25(64.1)	51(68)	33(54.1)	11(68.8)
III	4(7.4)	16(20)	5(12.8)	5(6.7)	12(19.7)	5(31.3)

a) $x^2 = 0.05$, P=0.97 for IL-8 genotypes; $x^2 = 7$, P=0.03 for CXCR2 genotypes ($x^2 = 5.66$, P=0.017 for CXCR2 TT vs CC+CT genotypes) b) $x^2 = 0.34$, P=0.84 for IL-8 genotypes; $x^2 = 5.15$, P=0.07 for CXCR2 genotypes ($x^2 = 4.15$, P=0.04 for CXCR2 TT vs CC+CT genotypes) c) $x^2 = 2.55$, P=0.28 for IL-8 genotypes; $x^2 = 2.28$, P=0.32 for CXCR2 genotypes ($x^2 = 0.37$, P=0.55 for CXCR2 TT vs CC+CT genotypes) d) $x^2 = 4.49$, P=0.34 for IL-8 genotypes; $x^2 = 12.69$, P=0.013 for CXCR2 genotypes ($x^2 = 7.71$, P=0.02 for CXCR2 TT vs CC+CT genotypes).

est, the IL-8 -251 T/A polymorphism affects the host ability in IL-8 production and it has been shown that the A allele is associated with enhanced promoter activity in response to TNF-α or lipopolysaccharide [24, 25]. Therefore, it could be concluded that individuals with higher ability in IL-8 production (carriers of A alleles) have more susceptibility risk to breast cancer development. Thus we investigated the CXCR2 +1208 C/T polymorphism along with functional polymorphism in the gene of IL-8 in a population of Iranian breast cancer patients.

Recently, the association of IL-8 -251 T/A polymorphism with several types of tumors, including gastric and prostate cancer, has been shown by investigators [25, 28, 36]. However, Smith et al. in a study on 144 female breast cancer patients did not detect any differences in allele or genotype frequencies of IL-8 -251 T/A polymorphism between cases and controls [37]. It is of interest that our results on the association of polymorphism in the -251 IL-8 gene with breast cancer show that like to the association of this polymorphism with other tumors [25, 28], there is a significant higher frequency of AA genotype in breast cancer patients compared to controls (p=0.01; OR=1.71, 95% CI=1.11-2.64). However, the results of the present study did not show any association between IL-8 -251 T/A polymorphism and different clinicopathological data, including steroid hormone status and NPI. This finding may indicate that while affecting the susceptibility to breast cancer, IL-8 -251 T/A polymorphism does not influence the progression of breast cancer. The contradictory results of the present study and Smith et al. study on association of IL-8 -251 T/A polymorphism and breast cancer development could not be explained by different genetic backgrounds of Iranian populations compared to those of the English population. In fact, the genotype frequency of the IL-8 -251 T/A polymorphism in our controls showed no significant difference from that of English controls reported by Smith et al. (p=0.81). However, the low number of cases in Smith et al. study could results in a weak power of statistical analysis to detect the effects of the IL-8 -251 A allele, or another linked polymorphism, on breast cancer development. Similar studies with more cases would certainly help to clarify whether a relation between IL-8 -251 T/A polymorphism and breast cancer can be established even in other populations.

CXCR2, though widely expressed in the immune system, is also expressed on endothelial cells and mediates ELR^+-CXC chemokine-induced angiogenesis in tumor microenvironment [21]. Despite the great interest in CXCR2 biological properties, little is known about the functional importance of single nucleotide polymorphisms in its gene. Therefore, in the present study, we asked whether the CXCR2 +1208 C/T gene polymorphisms could influence the susceptibility to/or progression of breast cancer. Our previous results show that in contrast to the study performed on this polymorphism in systemic sclerosis [29], no significant differences in distribution of CXCR2 C+1208T genotypes were found between breast cancer patients and normal controls (p=0.54). This result indicates that CXCR2 C+1208T gene polymorphism could not be considered as a susceptibility gene in breast cancer development. Given the importance of CXCR2 in angiogenesis [21], in the present study we also investigated the association of diallelic +1208 C/T polymorphism within the gene for CXCR2 with markers of tumor progression such as NPI and the level of steroid receptor expression. We did not find any association between CXCR2 C+1208T genotypes and Clinicopathological markers such as tumor grade (p=0.41), involved lymph nodes (p=0.2), the levels of progesterone receptor expression (p=0.32) and the age of disease onset (p=0.4). However, Our results showed a significant association between the CXCR2 +1208 TT genotype and reduced number of patients with good prognosis according to NPI ($p<0.02$). Indeed, only 5% of patients with CXCR2 +1208 TT genotype had NPI≤3.4 while 34% of patients with CC+CT genotypes had NPI≤3.4 (Table 3). This result indicated that the presence of CXCR2 +1208 C allele is somehow associated with good prognosis in breast cancer. Moreover, the lower percentage of estrogen receptor positive tumors were present in patients with CXCR2 +1208 TT genotype compared to patients with CXCR2 +1208 CC+CT genotypes (25% and 49.8%, respectively; p=0.04; OR=2.96, 95% CI=1.04-8.86). Considering that the presence of estrogen receptors in tumor tissue correlates with higher survival rates and lower risk of relapse [38], this finding also indicated the CXCR2 +1208 C allele is associated with good prognosis in breast cancer. Given the CXCR2 T1208C polymorphism is located in the noncoding region of CXCR2 gene, intriguing question raised regarding how the estrogen receptor expression is associated with CXCR2 T1208C polymorphism. Altogether, by considering the higher percentages of NPI≤3.4 and ER^+ tumors in carriers of the CXCR2 +1208 C allele, it could be concluded that the presence of C allele is associated with good prognosis in breast cancer.

In conclusion, the results of the present study show that IL-8 -251 T/A polymorphism may be related to the breast cancer development while CXCR2 C+1208T polymorphism directly, or through linkage disequilibrium of another gene(s), may be involved in breast cancer progression. Regarding the lack of agreement among the results obtained for IL-8 -251 T/A polymorphism in Iranian and English patients, and the absence of similar studies on CXCR2 C+1208T polymorphism in breast cancer, further investigation even of other populations are recommended.

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