Association between Interleukin-8 -251T/A Polymorphism and Endometriosis in Iranian Women

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Abstract

Background: Endometriosis is a disease of female genital system, which is defined by the presence of ectopic endometrial tissue outside the uterine cavity. IL-8 is an autocrine growth factor in the endometrium that contributes to the pathogenesis of endometriosis. The aim of this study was to investigate the association between -251T/A polymorphism in the IL-8 gene and risk of developing endometriosis in Iranian population.

Materials and Methods: This case-control study was performed in 100 endometriosis patients and 100 healthy individuals. The IL-8 -251T/A genotypes were determined using PCR-RFLP method. The association between genotypes of the -251T/A polymorphism and the risk of developing endometriosis was examined by odds ratios (OR) and 95% of confidence intervals (CIs).

Results: No statistically significant associations were observed between IL-8 -251 variants and the risk of developing endometriosis (χ^2: 1.02, P: 0.63). In addition, subgroup analysis (according to severity of disease) were unable to identify any association between IL-8 -251T/A polymorphism and endometriosis (P>0.05).

Conclusions: To our knowledge this is the first study investigating the association between -251T/A polymorphism and risk of developing endometriosis. Our results indicate that the presence of the -251T/A polymorphism in IL-8 gene is not associated with the risk of endometriosis.

Keywords: Endometriosis; Interleukin-8; Iranian population; Polymorphism

Introduction

Endometriosis is a chronic inflammatory disease defined as the presence of endometrial glands and stroma outside the uterine cavity. Typical symptoms consist of pelvic pain, dysmenorrhea, and infertility (1). It is estimated that >175 million women worldwide suffer from this disease (2). Though there are still unknowns as far as its pathogenesis is concerned, there is evidence showing that genetic, endocrine, immunological, and environmental factors play an important role in the genesis and development of endometriosis (3).

Inflammation is a key feature of endometriosis tissue and is associated with overproduction of prostaglandins, metalloproteinases, cytokines, and chemokines (4). The development of new blood vessels represents a crucial step during the establishment of endometriosis because endometriotic implants require neovascularization to guarantee oxygen and essential nutrient supply (5). IL-8 is a member of the CXC chemokine family, attracting and activating neutrophils during the immune reaction (6). It is a potent angiogenic agent (7) and has the capability of promoting endometrial cell growth (8). IL-8 is produced by several cell types including monocytes (9), fibroblasts (10), mesothelial cells (11), endometrial cells (12) and endometriotic cells (13). IL-8 gene is mapped on chromosome 4q13-q21 (14). It has been proposed that the -251T/A

(rs4073) polymorphism in the proximal promoter of IL-8 has a functional influence on IL-8 expression levels (15).

To the best of our knowledge, there are no studies regarding the impact of IL-8 -251T/A polymorphism on susceptibility to endometriosis. Thus, the present study aimed to evaluate the possible association between IL-8 -251T/A polymorphism and endometriosis in a sample of Iranian population.

Materials and Methods

Study populations

This case control study was done in 100 patients with endometriosis and 100 healthy control women attending Zeinabiyeh Hospital and Dr. Rostami Infertility Center, Shiraz, Iran. The diagnosis of endometriosis was made based on laparoscopy and patients were classified by histological criteria according to the American Society for Reproductive Medicine. Among the patients with endometriosis, 42% had minimal or mild (stage I/II) and 58% had moderate or severe (stage III/IV) endometriosis. The control group consisted of age-matched from the same ethnic area without any malignant disease and endometriosis, as confirmed by pelvic examination, ultrasound and laparoscopy. Women with leiomyoma, adenomyosis, fibroids, pelvic inflammatory disease, and invasive carcinoma of the uterine cervix or ovarian cancer were excluded from both cases and controls.

Genotyping

Genomic DNA was extracted from whole blood (16) and IL-8 genotyping was performed using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) technique (17). The primers used were 5´ -TCA TCC ATG ATC TTG TTC TAA-3´ (forward) and 5´-GGA AAA CGC TGT AGG TCA GA-3´ (reverse). The PCR reaction was carried out using commercially available PCR premix (Cinnagen, Iran) according to the manufacturer's instruction. Briefly, a final volume of 25 μL, contained 12.5 μL Master Mix, 1 μL of each primer (10 pmol/μL), 9.5 μL DNase free water, and 1 μL of DNA (100ng/ μL). Amplification was done with an initial denaturation step at 95 ℃ for 5 min, followed by 35 cycles of denaturation at 93 ℃ for 45 s, annealing at 54 ℃ for 1 min, and extension at 72 ℃ for 1 min and a final extension for 5 min at 72 ℃ followed by a final cooling at 4 ℃. The 542 bp PCR product was digested overnight at 37 ℃ with 5U of the restriction enzyme *MfeI*. The fragment of the IL-8 gene containing A variant was digested into 450 and 92 bp fragments, whereas the fragment containing the T allele remained intact (Figure 1). More than 15% of the samples were repeated, and the results of PCR
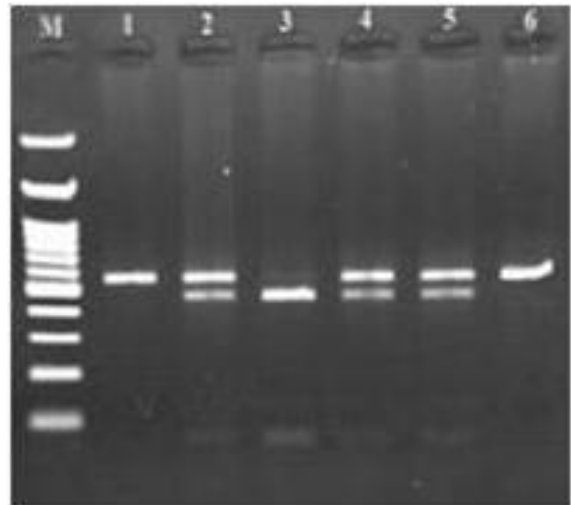
-RFLP was 100% concordant.

Figure 1. Electrophoresis pattern of PCR-RFLP reaction for detection of IL-8 -251T/A polymorphism: Lane M: 100bp DNA ladder. Lanes 1 and 6: TT genotype, Lanes 2, 4, 5: AT genotype, Lanes 3: AA genotype.

Statistical analysis

All statistical analyses were performed in SPSS version 19. Chi-square test was applied for the polymorphism to determine if the control sample demonstrated Hardy–Weinberg equilibrium. The association between genotypes and endometriosis was assessed by computing the odds ratio (OR) and 95% confidence intervals (CI) from logistic regression analyses. P-value <0.05 was considered statistically significant.

Ethics statment

The study was approved by the ethics committee of Shiraz University of Medical Sciences and informed consent was obtained from all individuals.

Results

To investigate the association of IL-8 gene polymorphism with endometriosis, this study included 100 patients (mean age: 31.8.±7.5 years) and 100 age-matched (±5) controls (31.7 ± 7.4 years). There were no significant differences between patient and control groups in mean age.

Detailed genotype distributions among cases and controls are summarized in Table 1. The genotypic frequencies of the -251T/A IL-8 polymorphism did not show significant deviation from Hardy-Weinberg equilibrium in control (χ^2: 0.01, df: 1, P: 0.9) and patient (χ^2: 2.23, df: 1, P: 0.13) subjects.

There were no differences in genotype frequencies of IL-8 -251T/A polymorphism between endometriosis cases and controls (Table 1). The frequency of the T and A alleles were 0.58, 0.42 in patients and 0.59,

0.41 in control group. There was no significant difference in allele frequency between patients and control group (OR: 1.02, 95%CI: 0.68-1.5, P:0.9). For further analysis, the endometriosis group was divided into subgroups; the genotype and allelic distribution was not significantly different between the control and case subgroups (Table 2).

Table 1. Association between -251T/A IL-8 gene polymorphism and endometriosis risk.

SNP	Endometriosis	Controls	OR(95%CI)	P-value
-251T/A				
Genotype				
TT	30	34	1	-
AT	56	49	1.2 (0.7-2.4)	0.42
AA	14	17	0.93 (0.4-2.2)	0.87
				*χ^2:1.02, P:0.63
Allele (n%)				
T	116 (58)	117 (59)	1	-
A	84 (42)	83 (41)	1.02 (0.68-1.5)	0.9

*Fisher's exact test

Discussion

Endometriosis is one of the most common gynaecological diseases (18). It has been postulated that one mechanism for the development of ectopic endometrial lesions is a defective immune response, which fails to clear the implants from the peritoneal surface (19). Alterations in the immune system, such as an increase in activated peritoneal immune cells and pro-inflammatory factors, are believed to be involved in the pathogenesis of endometriosis (20).

Table 2. Genotype and allele frequencies distribution in endometriosis case subgroups.

SNP		Genotype				Allele frequency			
-251T/A	No.	TT (%)	TA (%)	AA (%)	*P	T (%)	A (%)	OR (95% CI)	P
Stage I/II	42	15 (35.7)	20 (47.6)	7 (16.7)	0.98	50 (60)	34 (40)	0.96(0.57-1.61)	0.87
Stage III/IV	58	15 (25.9)	36 (62.1)	7 (12.1)	0.28	66 (57)	50 (43)	1.1(0.67-1.7)	0.78

*Evaluated using the χ^2 test to compare the case group with the control group.

IL-8 plays an important role in induction of inflammatory reaction (6). It is a cytokine with mitogenic and angiogenic activity (7, 8). The cytokine induces proliferation of endometrial stromal cell as a potential autocrine growth factor (8). Peritoneal fluid from women with endometriosis, as well as their eutopic and ectopic endometrial tissue, were found to have more angiogenic activity compared to normal controls (21, 22). IL-8 is one of the cytokine that promote angiogenesis and up regulated in endometriosis (23). In fact, IL-8 is an autocrine growth factor in the endometrium that contributes to the pathogenesis of endometriosis by encouraging endometrial cell attachment, invasion, cell growt and proliferation, immune protection, and IL-8 secretion in ectopic sites (24).

Arici et al showed that IL-8 was not only elevated in the peritoneal fluid of women with endometriosis compared with those without this disease, but the levels also correlated with the severity of the disease. They postulate that IL-8 may play a role in the growth and maintenance of ectopic endometrial tissue not only by stimulating leukocytes to secrete growth factors and cytokines, but also by directly stimulating endometrial cell proliferation (8). In another study, increased concentration of IL-8 was detected in patients with moderate/severe endometriosis as compared to healthy controls (25). These results

suggest that IL-8 may promote the progression of endometriosis. Several polymorphisms have been reported in the IL-8 gene. Interestingly, IL-8 production can be controlled by the -251 A/T in the promoter region of this chemokine. Recent data revealed that the IL-8 -251A allele is associated with a high expression level of IL-8 protein and a severe neutrophil infiltration (15). In several studies, the relationship between IL-8 -251T/A polymorphism and diseases such as cancer (26), Alzheimer's disease (27), acute pancreatitis (28) and post-stroke depression (29) was investigated. We investigated the frequency of -251T/A polymorphism in *IL-8* gene in Iranian patients with endometriosis. The distribution of different genotypes did not show any significant differences between patients and control subjects. The results of our study failed to show an association between the -251T/A variants and endometriosis susceptibility. The limitations of this study were due to focus on one SNP (-251T/A) we could not exclude the possibility of other SNPs within the IL-8 gene having an effect on endometriosis susceptibility and a small sample size. Further studies including much larger numbers of patients and controls are required to confirm these findings on the relationship of IL-8 genetic variation to pathogenesis of endometriosis.

In conclusion, we did not observe an association between the IL-8 -251T/A promoter polymorphism and the susceptibility to endometriosis in studied Iranian population. The small sample size limits the strength of this study, so to further confirm our findings, association studies with large sample sizes and different ethnicities are needed.

Authors' Contributions
Leila Kohan participated in study design, analysis and interpretation of data, revising the article and approving the content of manuscript. Farnoosh Rabbanizadeh participated in study by performing experimental methods, acquisition of data and drafting the article. Fatemesadat Najib also participated in acquisition of data and selecting the patients and control group, and critical revision of the manuscript for important intellectual content. All authors have read and approved the content of the manuscript.

Conflicts of interest
The authors declare no conflicts of interest.

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