ENDOMETRIOSIS

Polymorphisms for Interleukin-1 β (IL-1 β)-511 Promoter, IL-1 β Exon 5, and IL-1 Receptor Antagonist: Nonassociation with Endometriosis

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Purpose: We aimed to investigate if interleukin-1 β (IL-1 β) and IL-1 receptor antagonist (IL-1Ra) gene polymorphism could be used as markers of susceptibility in endometriosis.

Materials and Methods: Women were divided into two groups: 1) endometriosis (n = 120); 2) nonendometriosis groups (n = 103). Polymorphisms for IL-1 β -511 promoter, IL-1 β exon 5, and IL-1Ra were detected by polymerase chain reaction. Genotypes and allelic frequencies for these polymorphisms in both groups were compared.

Results: Proportions of different IL-1 and IL-1Ra polymorphisms in both groups were nonsignificantly different. Proportions of C homozygote/heterozygote/T homozygote for IL-1 β -511 promoter in both groups were 1) 21.6/59.1/19.1% and 2) 26.2/50.5/23.3%. Proportions of E1 homozygote/heterozygote/E2 homozygote for IL-1 β exon 5 in both groups were 1) 91.6/5/3.3% and 2) 95.15/4.85/0%. Allele I/II/IV/V for IL-1Ra in both groups were 1) 92.5/5.4/1.6/0.4% and 2) 95.1/3.9/1/0%.

Conclusions: Association of endometriosis with IL-1 β -511 promoter, IL-1 β exon 5, and IL-1 receptor antagonist gene polymorphisms doesn't exist. These polymorphisms

are not useful markers for prediction of endometriosis susceptibility.

KEY WORDS: endometriosis; gene polymorphism; IL-1 β ; IL-1 β exon 5; IL-1 β -511 promoter; IL-1 receptor antagonist.

INTRODUCTION

Endometriosis, a complex disease, is associated with immunologic changes (1). Endometriosis is related with the changes of several cytokines in serum and peritoneal fluid (2). Numerous cytokines were associated with endometriosis, including interleukin-1 (IL-1), IL-6, and IL-8 (3). IL-1 belongs to a cytokine family modulating cellular proliferation and has the capacity to induce other cytokines. IL-1, the major proinflammatory cytokines, plays an important role in the pathogenesis of endometriosis (4,5). Abnormal expression of IL-1 may contribute to the endometrioma development (6).

IL-1 genes are associated with several immunoinflammatory diseases (7). IL-1 β polymorphisms are associated with the enhancing production of IL-1 β and the increased risk of both hypochlorhydria induced by *H. pylori* and gastric cancer (8). Allele E2 in IL-1 β exon 5 was related to an increased risk of erosive arthritis (9). IL-1 is a primary mediator of the inflammatory response and has been shown to induce prostaglandin synthesis (10). IL-1 action is complex and regulated in part by its naturally occurring inhibitor, the IL-1 receptor antagonist (IL-1Ra). IL1 β

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and IL-1Ra polymorphisms were related with the regulation of cytokine and growth factor expression in articular chondrocytes and the final development of osteoarthritis (11).

Genetic studies of these multifactorial diseases such as endometriosis are difficult to approach due to the uncertainty of a polygenic trait. In our laboratory, we have chosen 3 polymorphisms (IL-1 β promoter, IL-1 β exon 5, and IL-1Ra) to screen candidate genes for several diseases. We observed that the IL-1 β -511*T allele could be used as a genetic marker of susceptibility to Kawasaki disease (Shi *et al.*, unpublished data). In contrast, we also noted that the IL-1 β (IL-1 β -511 promoter, IL-1 β exon 5) and IL-1Ra were not useful markers to predict the susceptibility of rheumatoid arthritis (Huang *et al.*, unpublished data). On the basis of these experiences, we tried to evaluate whether these polymorphisms are useful markers for predicting the susceptibility of endometriosis or not. To our knowledge, this is the first survey in this aspect.

PATIENTS AND METHODS

Premenopausal Taiwan Chinese women with surgically diagnosed endometriosis and nonendometriosis were included. All women were divided into two groups: 1) endometriosis ($n = 120$); 2) nonendometriosis groups ($n = 103$). The nonendometriosis individuals have been confirmed by the sonography and cesarean section. All women accepted the peripheral blood sampling for genotype analyses. Genomic DNA was prepared from peripheral blood by DNA extractor WB kit (Wako, Tokyo). This series was approved by the Ethical Committee of the China Medical College Hospital. Informed consents were signed by all women who donated their blood. There were nonsignificant differences between both groups in age, weight, and height.

Six polymerase chain reaction (PCR) primers were used to amplify the correlated gene. The sequences of the 6 primers were as following (from 5' to 3' end):

IL-1 β promoter

Upstream, TGGCATTGATCTGGTTCATC
Downstream, GTTAGGAATTCTCCCACTT

IL-1 β exon 5

Upstream, GTTGTCATCAGACTTTGACC
Downstream, TTCAGTTCATATGGACCAGA

IL-1Ra

Upstream, CTCAGCAACACTCCTAT
Downstream, TCCTGGTCTGCAGGTAA

About 50 ng of genomic DNA was mixed with 20 pmole of each PCR primer in a total volume of 25 μ l containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.0 mM MgCl₂, 0.2 mM each deoxyribonucleotide triphosphate, and 1 unit of Amplitaq DNA polymerase (Perkin-Elmer). PCR conditions were as follows: 35 cycles at 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min, then stand at 72°C for 30 min and hold at 4°C. Two biallelic base polymorphisms in the IL-1 β gene were detected. One is in the promoter region at position -511 (IL-1 β -511); the other is in exon 5 at position +3953 (IL-1 β + 3953). IL-1 β -511 promoter polymorphism was analyzed by PCR amplification followed by *Ava* I restriction analysis (12). IL-1 β + 3953 exon 5 polymorphism was analyzed by PCR amplification followed by *Taq* I restriction analysis (13).

PCR products were directly analyzed for IL-1Ra by electrophoresis on agarose gel and each allele was recognized according to its size. Allelic frequencies are expressed as a percentage of the total number of alleles. Genotypes and allelic frequencies for IL-1 β and IL-1Ra polymorphisms in both groups were compared. The SAS system with χ^2 and Fisher's exact test were utilized for statistical analyses. A p -value < .05 was considered statistically significant.

RESULTS

Genotypes proportions of different IL-1 polymorphisms (IL-1 β -511 promoter, IL-1 β exon 5) and IL-1Ra in both groups were nonsignificantly different (Table I). Proportions of -511*C homozygote/heterozygote/-511*T homozygote for IL-1 β promoter in endometriosis and nonendometriosis populations were 1) 21.6/59.1/19.1% and 2) 26.2/50.5/23.3%, respectively. Proportions of E1 homozygote/heterozygote/E2 homozygote for IL-1 β exon 5 in both groups were 1) 91.6/5/3.3% and 2) 95.15/4.85/0%. Genotypes for IL-1Ra between both groups were also nonsignificantly different. Most genotypes for IL-1 gene in both groups were I/I and I/II. Proportions of I/I and I/II for IL-1Ra in both groups were 1) 85.8/10.8 and 2) 92.2/5.8% (Table I).

Allelic frequencies for different IL-1 polymorphisms (IL-1 β -511 promoter, IL-1 β exon 5) and IL-1Ra between both groups were also nonsignificantly

Table I. Genotypes for IL-1 β Polymorphisms and IL-1 Receptor Antagonist in Women with and Without Endometriosis

Genotype ^a	Endometriosis <i>n</i> = 120 (%)	Nonendometriosis <i>n</i> = 103 (%)	<i>p</i> -value
IL-1 β promoter			0.430 ^b
–511*C/–511*C	26 (21.6)	27 (26.2)	
–511*C/–511*T	71 (59.1)	52 (50.5)	
–511*T/–511*T	23 (19.1)	24 (23.3)	
IL-1 β exon 5			0.229 ^c
E1/E1	110 (91.6)	98 (95.15)	
E1/E2	6 (5.0)	5 (4.85)	
E2/E2	4 (3.3)	0	
IL-1 receptor antagonist			0.324 ^c
I/I (410/410)	103 (85.8)	95 (92.2)	
I/II (410/240)	13 (10.8)	6 (5.8)	
I/IV (410/325)	2 (1.6)	0	
I/V (410/595)	1 (0.8)	0	
IV/IV (325/325)	1 (0.8)	1 (1.0)	
II/II (240/240)	0	1 (1.0)	

^a Allelic size (bp) after enzyme digestion were as following: IL-1 β -511*C (190 + 114 bp), IL-1 β -511*T (304 bp); IL-1 β exon 5 E1 (135 + 114 bp), E2 (249 bp); IL-1 Ra allele I (410 bp), II (240 bp), IV (325 bp), V (595 bp).

^b *p*-value calculated by χ^2 test.

^c *p*-value calculated by Fisher's exact test.

different (Table II). Allele C and T for IL-1 β promoter in both groups were 1) 51.2/48.7% and 2) 51.5/48.5%. Allele E1 and E2 for IL-1 β exon 5 in both groups were 1) 94.1/5.8% and 2) 97.6/2.4%. Allele I/II/IV/V for IL-1Ra in both groups were 1) 92.5/5.4/1.6/0.4% and 2) 95.1/3.9/1/0% (Table II).

DISCUSSION

Endometriosis is associated with various immunoinflammatory process and cytokines activation (14). Cytokines are proteins that play a role in the

communication link between the immune and endometriosis tissues. Endometriosis is associated with activation of peritoneal macrophages and their production of IL-1 (15). Cytokines also reach the circulations and act locally as paracrine or endocrine signals. IL-1 plays a central role in the cytokine family. It may augment the production of other cytokines, such as IL-6 and IL-8, and trigger the complex immunological processes (16,17). IL-1 is related with adhesion process of endometriosis (18). IL-1 production was altered in different stages of endometriosis (19). In endometriosis patients, serum IL-1 level decreased progressively after the danazol administration (20).

Table II. Allelic Frequencies for IL-1 β Polymorphisms and IL-1 Receptor Antagonist in Women with and Without Endometriosis

Allele frequencies	Endometriosis <i>n</i> = 120 (%)	Nonendometriosis <i>n</i> = 103 (%)	<i>p</i> -value
IL-1 β promoter			0.965 ^a
Allele C	123 (51.2)	106 (51.5)	
Allele T	117 (48.7)	100 (48.5)	
IL-1 β exon 5			0.076 ^a
E1	226 (94.1)	201 (97.6)	
E2	14 (5.8)	5 (2.4)	
IL-1 receptor antagonist			0.691 ^b
I (410)	222 (92.5)	196 (95.1)	
II (240)	13 (5.4)	8 (3.9)	
IV (325)	4 (1.6)	2 (1.0)	
V (595)	1 (0.4)	0	

^a *p*-value calculated by χ^2 test.

^b *p*-value calculated by Fisher's exact test.

IL-1 exists in two forms, IL-1 α and IL-1 β , which was encoded by distinct genes but share the same receptors and biological properties (21). The loci for the IL-1 α , IL-1 β , and IL-1Ra are all located on the proximal region of the long arm of chromosome 2 (22). Different polymorphisms have been described in the IL-1 β gene, and at least two of them could influence the protein production: one located in the promoter region at position -511 (IL-1 β -511) (11), the other in exon 5 (13). These genes code several proteins that may be the key components in endometriosis pathogenesis. IL-1 β polymorphism was correlated with IL-1 β expression (23). IL-1 β -511*T/C polymorphism influences IL-1 β protein production (24,25). IL-1 β -511*T carriers have higher producers of IL-1 β than IL-1 β -511*C carriers (26). These results indicate that the genotype of the IL-1 β and IL-1Ra polymorphisms may affect IL-1 β production in an intricate and complicated manner.

The level of IL-1 β mRNA showed a positive correlation with that of IL-1 β and a negative correlation with the level of IL-1Ra mRNA (27). Peritoneal macrophages may secrete IL-1Ra protein that modulates the effects of IL-1 (28). The regulation of IL-1 β and IL-1Ra may be coordinated during inflammation (28). Macrophages express IL-1Ra mRNA rather than IL-1 β mRNA with the progress of endometriosis (27). IL-1Ra is structurally related to IL-1 α and IL-1 β and competes with these molecules for occupancy of IL-1 cell surface receptors. IL-1Ra allele II is associated with a variety of epithelial-related chronic inflammatory diseases including SLE (29), psoriasis (30), alopecia areata (31), lichen sclerosus (32), and ulcerative colitis (33). The presence of the IL-1Ra allele II was associated with enhanced IL-1 β production in vitro (24). IL-1Ra allele II was more often present in the IL-1 β -511*T allele carriers than in the noncarriers (28).

IL-1 or IL-1Ra polymorphisms were related with the susceptibility or disease activities for individual diseases, including Alzheimer's disease (34), Parkinson's disease (35), temporal lobe epilepsy (36), schizophrenia (37), erosive arthritis (9), polymyositis and dermatomyositis (38), multiple sclerosis (39), lymphocytic leukemia (40), atherosclerosis (41), coronary artery disease (42), alcoholic liver disease (43), idiopathic pancreatitis (44), inflammatory bowel disease (45), and IgA nephropathy (46). In contrast, some investigators disagreed with these associations. There was no correlation between genetic polymorphisms of IL-1 β and IL-1Ra and susceptibility to rheumatoid arthritis (9), COPD (47),

multiple myeloma (48), diabetes mellitus (49), postmenopausal osteoporosis (50), and ischemic heart disease (51). Furthermore, IL-1 genes tend to have a role in the severity of the disease rather than in susceptibility to the disease itself (7). In our previous study, we also observed an association between disease activity of RA and a polymorphic IL-1 β -511*C gene sequence (Huang *et al.*, unpublished data).

In this study, the genotype distributions and allelic frequencies for IL-1 β -511 promoter, IL-1 β exon 5, and IL-1Ra polymorphisms in endometriosis and nonendometriosis patients were not statistically different. This suggested that these polymorphisms were not the candidate genetic markers in the susceptibility of endometriosis in Taiwan Chinese. We also observed the nonassociation between the IL-1Ra allele II and the IL-1 β -511*T allele carriers. The discrepancy may be due to the racial or disease variations. This was compatible with previous report in which they observed that IL-1 β promoter polymorphism did not influence the susceptibility to or severity of rheumatoid arthritis (9).

In conclusion, association of endometriosis with IL-1 β -511 promoter, IL-1 β exon 5, and IL-1 receptor antagonist gene polymorphisms doesn't exist. The genotype and allele frequencies of these polymorphisms are not useful markers for the prediction of endometriosis susceptibility. This could provide the database for the further survey of the IL-1 and IL-1Ra polymorphisms. However, the real roles of the IL-1 polymorphisms in the endometriosis remain to be clarified. Furthermore, other cytokine polymorphisms in endometriosis development merit further surveys.

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