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Interleukin-1 β Gene polymorphisms in Iranian Patients with Uterine Fibroid, A Case- Control Study

Fatemeh Taghizade Mortezaee[1], Morteza Hashemzadeh Chaleshtori[1], Soleiman Kheiri[2], Effat Farrokhi[1], Laleh Shariati[3], Mohammad Amin Tabatabaiefar[1, 4] and Sepideh Miraj[5]*

[1]Cellular and Molecular Research Center, Shahrekord University of Medical Sciences,Shahrekord, Iran.
[2]Clinical Biochemistry Research Center, Shahrekord University of Medical Sciences,Shahrekord, Iran.
[3]Department of Molecular Medicine, School of Advanced Medical Technologies, Tehran University of Medical Sciences, Tehran, Iran.
[4]Department of Medical Genetics, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
[5]Deparmment of Obstetrics of Gynecology,Hajar Hospital, Shahrekord University of Medical Sciences, Shahrekord, Iran.

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Uterine leiomyoma (UL) or fibroid is the most common estrogen- dependent tumor of the reproductive system. Almost a quarter of women at reproductive age are affected with this benign tumor. The purpose of the present study was to investigate the possible association of IL-1β-511and IL-1β 3954 polymorphisms with UL in the women of Charmahal & Bakhtiari province of Iran. Totally, 276 patients with UL and 157 healthy control women were studied. The genetic polymorphisms for IL-1β-511and IL-1β 3954 were analyzed by PCR-RFLP method. The results were analyzed with SPSS software using χ^2 test. The TC genotypes of the IL-1β -511C/T polymorphism showed a decreased risk of UL (OR = 0.232, P = 0.01, 95 % CI = 0.11 – 0.48). A significant difference was found for the C allele frequencies of the IL-1 β -511 C>T polymorphism between the two groups (OR = 0.232, P = 0.01, 95 % CI = 0.11 – 0.48). However, no significant difference was found for the IL-1 â -3954 polymorphism between the two groups. Our findings indicated that IL-1 â -511C>T promoter polymorphism affects the risk of UL in the women of our study and this polymorphism might be involved in the pathogens of this disease.

Key word: Leiomyoma, polymorphism, IL-1β, Iran

Uterine leiomyoma (UL) or fibroid is the most common pelvic benign tumor with a prevalence of 25% among the women in the reproductive age and 50% in autopsy report[1]. These tumors are the most frequent cause of hysterectomy and uterine surgery[2]. UL is the case in about one third of bedridden cases due to abnormal uterine bleeding. It is the common reason of menstrual abnormalities, pelvic pain and other symptoms which negatively affect the life quality including infertility, recurrent abortions and adverse prenatal outcomes[1]. Despite a wide research on the factors involved in the initiation and growth of UL, the main causes of these tumors remain unknown. The genetic factors underlying the development and expansion of UL are being extensively investigated. As to the cause of these tumors, the hypothesis has been put forth based on which immunological and inflammatory processes may play a role[3, 4]. Possible associations

* To whom all correspondence should be addressed.
Tel: +98 912 650 99 52,
E- mail: Miraj_sepideh@yahoo.com

of certain cytokine gene polymorphisms with the the UL have been suggested in numerous preceding studies[5-8]. Based on the hypothesis that cytokines may increase the production of matrix metalloproteinases, which, in turn, may induce UL development, the associations of several single nucleotide polymorphisms (SNPs) in cytokine genes and UL have been sought and confirmed[9]. Interleukin (IL) - 1 family gene encodes three homologous polypeptides related together structurally and functionally[10]. The IL family gene is located in a cluster on 2q14 chromosome and includes two distinct but functionally similar molecules, IL-1α and IL-1 β [11] which are produced by monocytes, macrophages and epithelial cells during both acute and chronic inflammatory responses[12]. It has been shown that a gene in this cluster is associated with increased production of IL-1 protein which leads to tissue destruction[13, 14]. Several association studies have reported the association between IL-1β and such diseases as atherosclerosis, rheumatoid arthritis, peritonitis and bowel disease[15]. Two polymorphisms in IL-1βgene, one in the promoter (IL-1β-511) and the other in the coding region (IL-1β3954) have been mostly studied. Pietrowski et al. reported a significant association between IL-1β-511 gene polymorphism and UL development[8]. Thus, there is lot of evidence showing the involvement of genetic determinants in the etiology of UL. The present study was launched to investigate the association between the IL-1β-511 and IL-1β3954 gene polymorphisms with UL in a series of Iranian women.

MATERIALS AND METHODS

Patients and control samples

In this case–control study, we investigated 276 women with diagnosed UL at the Department of Gynecology at Shahrekord Hajar Hospital from 2010 to 2011. UL diagnosis was based on transvaginal sonographic examination and the following confirmation by the histological testing using either myomectomy or hysterectomy. All the participants were of reproductive age. The control group also included 157 women without any evidence of UL, based on the ultrasound exam. There were no significant differences in age, ethnic and menarche between the two groups. Cases with adenomyosis, reproductive tract neoplasm, pregnancy, ERá dependent cancers, rheumatoid arthritis and with smoking and alcohol intake habits were excluded from the study. Informed consent was obtained from all participants before their inclusion in the study. All the procedures were approved by the Ethics Committees of the Shahrekord University of Medical Sciences. Blood was taken from all participants and stored at -20°C until the analyses were carried out.

Genotyping and statistical analysis

Genomic DNA was extracted from EDTA blood samples by using a standard phenol/ chloroform extraction method. The quality of DNA was evaluated by spectrophotometer in all samples. Genotyping of the IL-1β-511C>T (promoter) and IL-1β 3954 C>T (exon 5) was performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The PCR primers were designed previously[8]. PCR primer sequences and restriction anzymes are shown in Table 1. The 25 µl reaction mix for each PCR product comprised: 50–100 ng of genomic DNA, 1.5 µl MgCl2 (50 mM), 2.5 µl PCR Buffer (10×), 0.3 µl of each primer (10 pM), 0.5 µl dNTP(10 mM), 0.1 µl Taq DNA polymerase (5 U/ µl) (Cinagen) and ddH2O added to 25 µl reactions. PCR conditions for the amplification of both PCR products in a Techne TC-412 thermocycler (Barloworld Scientific, Staffordshire, UK). included an initial denaturation step at 94°C for 5 min, 34 cycles including 94°C for 30 s, 55°C for 30 s, and 72°C for 45 s and a final extension at 72°C for 7-min. Digestion of 4 µl of each PCR amplification product was carried out with 1 U of the specific restriction enzyme (Fermentas) at 37°C for 16 h according to manufacturer's instructions. Both PCR and digestion products were separated by vertical non-denaturing 8%polyacrylamide gel and visualized by silver staining. Expected fragment lengths of the IL1 β -511 polymorphism (AvaI digested ; heterozygotes (CT)) were 305, 190, and 115 bp, and for the IL1 β 3954 polymorphism (Taq I digested; heterozygotes (CT)) were 250, 136, and 114 bp. To confirm the genotyping results, a subset of PCR-amplified DNA from heterozygous samples were examined by DNA sequencing, and the results were 100% concordant. Genotypes and allelic frequencies for individual polymorphisms were compared between cases and controls using the

χ^2 test. P<0.05 was considered statistically significant. The associations between alleles and genotype and disease risks were calculated by odds ratios (OR) with a 95% confidence interval (CI).

RESULTS

Association between polymorphisms of IL-1 gene and the risk of UL

The allele and genotype frequencies of IL-1â-511 and IL-1â3954 in the healthy and UL groups are shown in Table 2. Patients with UL were more likely to be homozygous for C allele at position -511 than the healthy women (19.9% vs 5.7%, respectively). While, the healthy group showed a higher frequency of the TC genotype (74.6% versus 60.2%). Thus, the genotype conferred a significant protection against UL (OR = 0.232, P = 0.01, 95 % CI = 0.11 – 0.48).

Allele frequencies of the IL1 â 3954 polymorphism were 25% and 26% for the T allele and 75% and 74% for the C allele in patients and controls, respectively (P > 0.05). Thus, there were no significant differences in IL1 â 3954 genotype or allele frequencies between women with UL and controls.

DISCUSSION

In the current study, we investigated the association of two polymorphisms in IL-1β-511C>T (promoter) and IL-1 β 3954 C>T (exon 5) gene with UL in 276 patient compared to157 normal women as the control group. Studies have shown that polymorphism in various cytokines cause

Table 1. Primer sequences for IL-1â-511 promoter, IL-1â 3953 exon 5

SNP	Location	Primer sequence	DNA fragment Size (bp)	Restriction Anzymes
IL-1â-511C>T	promoter	F 5´-TGG CAT TGA TCT GGT TCA TC-3´ R 5´-GTT TAG GAA TTC TCC CAC TT-3	TT 305 CT 190 +115 + 305 CC190 + 115	AvaI
IL-1â3954C>T	Exon5	F5´-GTT GTC ATC AGA CTT TGA CC-3´ R 5´-TTC AGT TCA TAT GGA CCA GA-3	TT250 CT136 + 114 + 250 CC136 + 114	TaqI

Table 2. Genotype and allele frequencies

IL-1 â -511 AvaI	Myoma(276) No.(%)	Control(157) No.(%)	OR (95% CI)	P-value
Genotypes				
CC	55(19.9)	9(5.7)	1.00	
CT	166(60.2)	117(74.6)	0.232(0.11- 0.48)	0.01
TT	55(19.9)	31(19.7)	0.290(0.57 -0.99)	0.03
Alleles				
C	276(0.5)	135(0.43)	1.00(ref0	
T	276(0.5)	179(0.57)	0.754(0.57 – 0.99)	0.04
IL-1 â 3954TaqI	Myoma(276) No.(%)	Control(157) No.(%)	OR (95% CI)	P-value
Genotypes				
CC	158(57.2)	87(55.4)	1.00	
CT	98(37.6)	59(35.5)	0.915(0.60 – 0.38)	0.674
TT	20(7.3)	11(7)	1.001(0.45 – 2.18)	0.998
Alleles				
	138(0.25)	81(0.26)	1.00(ref)	
CT	414(0.75)	233(0.74	0.959(0.69 – 0.31)	0.796

susceptibility to some diseases[16, 17]. Polymorphisms in the regulatory region of a cytokine gene can alter its expression level related to immunologic reactions[18, 19]. It seems that the susceptibility genes interact both with other genes and peripheral factors to accelerate the disease development[20, 21]. Studies have shown that interleukins and other cytokines may be involved in susceptibility to develop various gynecological neoplasms[7, 22, 23]. Because IL-1b may exert a detrimental effect on leiomyoma development, we hypothesize that its gene polymorphisms may be associated with the pathogenesis of UL. In this study, IL-1B -511 TC heterozygotes were significantly lower in the patient group, suggesting that the -511TC genotypes might have a protective effect in the development of UL (OR = 0.232, P = 0.01, 95 % CI = 0.11 – 0.48). Also, we found that women homozygous for the -511CC genotype had a greater risk of UL.

This is the first study in the Iranian population to show a specific genotype of the IL-1B at -511TC is protective against UL. Studies have demonstrated the increased production of IL-1b when the T allele is present at IL-1b-511[24, 25]. We could infer that the CC (-511) genotype in the promoter region is associated with reduced IL-1b levels of the proinflammatory signals in response to stimulation. This is consistent with the findings of other studies on Asian women[8]. Our finding is also consistent with the results of Pietrowski and coworkers[8]. Interplay between IL-1β-511genotypes and some peripheral factors might underlie the discrepancies found among studied populations. It is well possible that IL-1β gene polymorphism affects the susceptibility to UL in those populations which are exposed to specified peripheral factors. A meta-analysis on the associations of Interleukin-1beta and interleukin-1 receptor antagonist gene polymorphisms and gastric cancer has confirmed these differential effects observed between two Caucasian and Asian populations[26].

In our study, no differences were found in either genotype or allele frequencies of the IL-1β3954C D T polymorphism between patient and control groups. The result of the present study corresponded well with those of other studies on the IL-1β3954C/T polymorphism and the risk of UL. In study that carried out by Hsieh et al. in Taiwan, no association was found between IL-1β3954 gene polymorphism and UL[6]. Likewise, Pietrowski et al. did not find any association between IL-1β3954C/T gene polymorphism and UL[8]. This study has been conducted with a larger sample size to confirm our previose report[27].

In summary, the obtained result from this study indicated that Iranian patients with IL-1b-511 CC genotype have an increased risk of leiomyoma. Interestingly, we also found that the IL-1b-511CT genotype had a protective effect on the development of UL in our study population. Therefore, this SNP could serve as a useful marker, together with other risk factors, to predict the susceptibility to UL. Further studies are needed to clarify the exact role of the IL-1β gene polymorphism in the pathogenesis of UL.

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