Association of Interleukin-1β (IL-1β) Gene Polymorphisms with Uterine Leiomyma

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**Introduction**

Uterine leiomyomas is the most common benign uterine smooth muscle tumor in women. According to pathologic specimens, the incidence of this tumor has been reported approximately 77% [1]. So far, the factors of initiation, survival and growth of uterine leiomyomas have not been fully characterized. Symptoms include pelvic pain, bleeding, infertility, spontaneous abortions and pregnancy complications and adverse effects and, therefore, have significant effects on women's health [2]. Like breast and ovarian cancer, uterine leiomyomas tend to grow affected by estrogen and when estrogen levels are reduced, these tumors size will decrease [3, 4]. When estrogen levels are high, like during pregnancy, tumor size increases and after delivery tumors size will decrease [5].

One hypothesis about the cause of these neoplasms is the aspect of immunological and inflammatory processes, which may have a role in creating these tumors [6, 7]. IL-1 gene (IL-1) encodes three polypeptides that are structurally and functionally correlated [8]. Interleukin family gene is located on chromosome 2q14 as cluster and contains two is forms of interleukin-1alpha (IL-1α) and interleukin-1beta (IL-1β) [9] which is produced through monocytes, macrophages and epithelial cells and are involved in response to microbial invasion, inflammation and immune modulation [10]. It has been shown that, specific locus in this gene cluster are related with increased production of interleukin-1 proteins which leads to tissue destruction [11, 12].

The relationship between IL-1β and atherosclerosis, rheumatoid arthritis, peritonitis and crone diseases has been reported [13, 14]. Two polymorphisms in genes IL-1β, one in promoter region IL-1β-511 (rs 16944) and the other in the coding region IL-1β3954 (rs 11436334) is associated with various diseases. In a study conducted by Pietrowski, they suggested that there is a significant relationship between IL-1β-511 polymorphisms and the incidence of leiomyomas [15]. Considering high prevalence of this disease in this province and serious complications and effects these tumors have on health of women and given that the identification of genetic mechanism of this disease can be useful in designing...
screening tests, clinical diagnosis, treatment and presenting its inheritance model. This study has been conducted to evaluate two IL-1β-511 and IL-1β3954 polymorphisms and their relationship with the occurrence of uterine leiomyomas in women population.

Materials and Methods

Having been approved by the Ethics Committee for of Shahrah-e-Kord University of Medical Sciences, this case-control study was conducted in 2010 in Cellular and Molecular Research Center of Shahrah-e-Kord University of Medical Sciences. 159 women with uterine leiomyomas who had inclusion criteria and 157 women in the control group, who were identical racially and in terms of inclusion and exclusion criteria, were studied. Patients were those who were admitted and examined by gynecologists at the women clinic of Hajar Hospital, and uterine leiomyomas was confirmed in them by vaginal ultrasound. Inclusion criteria included: Diagnosis of uterine leiomyomas in the patient group and absence of leiomyomas in the control group by vaginal ultrasound and clinical diagnosis and being in the reproductive age range.

Exclusion criteria included: smoking, pregnancy, postmenopausal women (menopause), estrogen drug use, having estrogen receptor alpha-dependent cancers such as breast, ovary and endometrium cancers.

To comply with ethical considerations, necessary information on how to conduct study were given to the participants in the study before conducting the study and after obtaining written consent and completing questionnaire, 5 ml blood were taken from patients and healthy individuals and was mixed with EDTA 0.5M. Samples were kept at -20°C until completion of sampling and testing. DNA was extracted through the standard method of phenol-chloroform and the quality of the extracted DNA was evaluated based on OD 260/280, this ratio was between 1.7-1.9 in all samples. PCR-RFLP was performed to reproduce the desired sequence and ultimately determine the genotypes in situations IL-1β-511 C>T and IL-1β3954 C>T. Two sequences of 250 and 305bp were performed by thermocycler TC-412 (Made in Canada) using primers designed in previous studies [15] (Table 1) and according to the following conditions.

Qualitative PCR reaction was arranged to reproduce two regions IL-1β-511 and IL-1β3954 in volume 25 μl which included 0/3 μl of both forward and reverse primers (10 PM), MgCl2 (50 mM) 1.5 l, TaqDNA lbuffer (10X) 2.5 l, Mix dNTP (10mM) 0.5 l, 0.1 l Taq DNA Polymerase (5U/ l) and 1 l of DNA (about 100ng) which was distilled twice with distilled water to reach the final volume of 25 l. After optimization, the temperature conditions for IL-1β-511 included the following items: initial denaturation temperature 94°C for 6 minutes, then 32 cycles of 94°C for 1 min, 57°C for 1min, and finally, elongation at 72°C for 5 min and for IL-1β3954 included the initial denaturation temperature at 94°C for 5min, then 38 cycles including 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 45 seconds and eventually, elongation at 72°C for 10 minutes. After amplification of target sequences, IL-1β-511 polymorphism was digested by 1 unit of AvaI restriction enzyme (Fermentase-Canada) and IL-1β3954 gene by 1 unit of TaqI restriction enzyme (Fermentase-Canada) at 37°C for 16 hours (according to protocols recommended by the manufacturer). Value of 4 l of the PCR product digested by enzyme were brought on polyacrylamide gel 8% and electrophoresis was performed for 2 hours with 200 voltage and the obtained gel was stained with silver nitrate.

Results

PCR products were investigated after RFLP on polyacrylamide gel. In gene IL-1β-511 (Promoter), if C converts to T, the enzyme identification position will be lost and the fragment remains intact and if nucleotide is C, the fragment will be cut and two bands will be formed one in 190bp and the other in the region 115bp (Fig. 1). If the nucleotide is C in the region IL1β3954 (exon 5), the fragment will be cut and two bands will be formed one in 114bp and the other in the region 136bp. If C changes to T, cut position will be lost and the fragment remains intact (Fig. 2). Then, genotypic and allelic frequencies of -511 polymorphisms in the two groups were compared via \( \chi^2 \) test, and a statistically significant difference was observed between genotypic and allelic frequency of this polymorphism and the risk of uterine leiomyoma in patient and control groups (\( p=0.01 \) (Table 2). In the +3954 region, genotypic and allelic frequencies were studied in two groups. However, in this polymorphism, no significant relationship was observed between the two groups (Table 3).

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

<table>
<thead>
<tr>
<th>SNP</th>
<th>ID SNPs</th>
<th>Primer sequence</th>
<th>Restriction enzyme</th>
<th>Sizes of digested fragments (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β-511 C/T</td>
<td>rs 16944</td>
<td>5'-TGG CAT TGA TCT GGT TCA TC-3'</td>
<td>AwaI</td>
<td>305</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GTT TAG GAA TTC TCC CAC TT-3'</td>
<td></td>
<td>190+115</td>
</tr>
<tr>
<td>IL-1β3954 C/T</td>
<td>rs 11436334</td>
<td>5'-GTT GTC ATC AGA CTAT TGA CC-3'</td>
<td>TaqI</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-TTCAGT TCA TAT GGA CCA GA-3'</td>
<td></td>
<td>114+136</td>
</tr>
</tbody>
</table>
IL-1β Gene polymorphisms and uterine leiomyoma

Table 2. Genotype and allele frequencies of IL-1β -511 gene polymorphism

<table>
<thead>
<tr>
<th>IL-1β -511 Avai</th>
<th>Control (157) No (%)</th>
<th>Myoma (159) No (%)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>31(19.7)</td>
<td>28(18)</td>
<td>0.01</td>
</tr>
<tr>
<td>TC</td>
<td>117(74.5)</td>
<td>95(60)</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>9(5.8)</td>
<td>36(22)</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>179(57.3)</td>
<td>151(47)</td>
<td>0.01</td>
</tr>
<tr>
<td>C</td>
<td>135(42.9)</td>
<td>167(53)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Genotype and Allele Frequencies of IL-1β 3954 gene polymorphism

<table>
<thead>
<tr>
<th>IL-1β 3954 TaqI</th>
<th>Control (157) No (%)</th>
<th>Myoma (159) No(#)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>117(7)</td>
<td>13(8.2)</td>
<td>0.05</td>
</tr>
<tr>
<td>TC</td>
<td>59(37.6)</td>
<td>58(36.5)</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>87(55.4)</td>
<td>88(55.3)</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>81(26)</td>
<td>84(26.4)</td>
<td>0.05</td>
</tr>
<tr>
<td>C</td>
<td>233(74)</td>
<td>234(73.6)</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

The results showed that the polymorphism IL-1β-511 in the promoter region is associated with leiomyomas. However, the polymorphism located in region +3954 showed no statistically significant association with increased risk of disease. Evidence has shown that the analysis of SNPs (single nucleotide polymorphisms) can determine the diseases associated genes and improve our knowledge about the development of neoplasms. Generally, polymorphisms are not directly associated with specific diseases, but they are useful tools to study multifactorial diseases such as uterine leiomyomas [15]. Studies have shown that polymorphisms in different cytokines cause susceptibility to some diseases [16, 17]. Polymorphisms that occur in the regulatory or structural regions can change the expression level of genes associated with immunological reactions [18, 19]. It seems that predisposing genes interact with other genes and environmental factors and accelerate disease progression [20, 21]. Studies have shown that interleukin and other cytokines are involved in occurrence of various gynecological (female) neoplasms [22-24]. In this study, we observed that the polymorphism which is in the IL-1β-511 promoter is associated with uterine leiomyomas. While polymorphism located in the structural region (exon 5, position +3954) of IL-1β gene was not associated with leiomyoma, our results are inconsistent with the study conducted by Hsieh et al. This study has shown that IL-1β-511 polymorphism is associated with uterine leiomyomas in Asian women [14]. Another study conducted by Pietrowsi et al. on women of Middle Europe, it has been reported that there is a relationship between IL-1β-511 polymorphism and leiomyomas. However, +3954 polymorphism of this gene is not related. The results of this study are consistent with the results of our study [15]. These different results may be due to different genetic background of studied populations, or it is caused by heterogeneous associated with specific genes polymorphisms in different populations [25].

It seems that IL-1β polymorphism in some races and ethnicities that are exposed to some specific environmental factors is a predisposing factor for uterine leiomyomas but not in another population. A report has proven this genetic variation in several genes, including IL-1β gene between Asian and Caucasian populations [25]. On the other hand, linkage disequilibrium (LD) may be the cause of these differences. LD will be differently separated in various populations [26]. However, results obtained from this study showed that there is a relationship between IL-1β-511 polymorphism and risk of uterine leiomyomas in the female population of Chaharmahal and Bakhtiari, and it seems that this polymorphism increases the risk of leiomyomas in the studied women and may be involved in the disease. These SNPs can be a useful mark for predicting predisposition to leiomyomas and provide valuable insights into the pathogenesis of the disease and prepares information for further studies on polymorphisms of this gene. However,
the actual role of this polymorphism in leiomyoma should be clarified and the other role of gene polymorphisms should be investigated in larger studies. For a real understanding of what was said, it is recommended to conduct more extensive studies on larger numbers of people of a community and other ethnic groups from the viewpoint of genetic and environmental factors in order to discuss the role of IL-1β polymorphism in leiomyomas pathogenesis more firmly.

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Authors’ Contributions
All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest
No conflict.

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References
14. Hsieh YY, Chang CC, Tsai CH, et al. Interleukin (IL)-12 receptor beta1 coding 378G homozygote and allele, but not IL-1 (beta-511 promoter, 3953 exon 5, receptor antagonist), IL-2 114, IL-4-590 intron 3, IL-8 3e-UTR 2767, and IL-18 105, are associated with higher susceptibility to leiomyoma. Fertil Steril 2007; 87(4): 886-895.
