Combination effect of cytochrome P450 1A1 gene polymorphisms on uterine leiomyoma: A case-control study

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ABSTRACT

Uterine leiomyoma (UL) is an estrogen-dependent neoplasm of the uterus, and estrogen metabolizing enzymes affect its progression. This study aimed to evaluate the association between two single-nucleotide polymorphisms of cytochrome P450 1A1 (*CYP1A1*) gene and UL risk. The study consisted of 105 patients with UL and 112 healthy women as controls. Ile462Val (A/G) and Asp449Asp (T/C) polymorphisms of *CYP1A1* gene were analyzed by DNA sequencing and polymerase chain reaction-restriction fragment length polymorphism methods, respectively. The findings indicated no association between Ile462Val (A/G) and Asp449Asp (T/C) polymorphisms of *CYP1A1* gene and UL ($p < 0.05$). However, the combination effect of TT/AG genotypes of the Asp449Asp (T/C) and Ile462Val (A/G) polymorphisms was associated with 4.3-fold higher risk of UL. In addition, haplotype analysis revealed that TG haplotype of the Asp449Asp (T/C) and Ile462Val (A/G) polymorphisms could increase the UL risk nearly 4.9-fold. Asp449Asp (T/C) and Ile462Val (A/G) polymorphisms of *CYP1A1* gene were not associated with UL susceptibility; however, the combination of the TT/AG genotypes and TG haplotype could increase the UL risk.

KEY WORDS: Cytochrome P450 1A1; haplotype; gene; polymorphism; uterine leiomyoma
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INTRODUCTION

Uterine leiomyoma (UL) as a common benign neoplasm of the uterus involves almost 30-40% of reproductive age women. There are large amounts of extracellular matrix components in this tumor, including collagen, fibronectin, and proteoglycans [1]. Age, race, caffeine, number of pregnancies, endogenous hormone levels (estrogen and progesterone), enzymes, obesity, and genetic factors are associated with UL risk [1,2]. During pregnancy, UL may result in a number of problems such as hemorrhage in the first trimester, placenta displacement, premature labor, and miscarriage [3,4]. Although the exact cause of the disease is unknown, the effects of hormonal, genetic, and growth factors on UL progression have been established [5].

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The growth and development of UL are dependent on estrogen. Estrogens are chiefly generated in ovaries and testes. Estrogen influences the growth, differentiation, and function of many target tissues. The hormone is distributed throughout the inner and outer surface of cells, but maintained with high affinity and specificity in target cells by an intranuclear binding protein, called the estrogen receptor (ER) [6]. The physiological effects of estrogens are mediated by ER; both subtypes, ERα and ERβ are found in the uterus [7].

Several enzymes are known to be involved in the biosynthesis and metabolic activation of steroid hormones [8,9]. Cytochrome P450 17a (CYP17) and cytochrome P450 19 (CYP19) participate in the biosynthesis of estrogen, however, two other enzymes, cytochrome P450 1A1 (CYP1A1) and cytochrome P450 1B1 (CYP1B1) are the basic enzymes which metabolize the estrogen resulting in the production of 2-hydroxy-4-hydroxy estrogen metabolite [10]. The *CYP1A1* gene which is mapped on chromosome 15q22–q24, spans 5,987 base pairs and encodes a 512-amino acid protein [11]. In

the first stage of estrogen metabolism, hydroxylation is carried out by CYP1A1 [12]. CYP1A1 is influential in the estrogen to 2-hydroxy catechol metabolites and 2-hydroxylation of estradiol for O-methylation to 2-methoxy conversion [13]. *CYP1A1* polymorphisms have been investigated in relation to hormonally-related diseases such as endometriosis, breast cancer, and endometrial adenocarcinoma [14-16]. So far, 19 polymorphisms of *CYP1A1* gene have been detected [2]. Several studies have considered the association between *CYP1A1* single-nucleotide polymorphisms Asp449Asp (T/C) and Ile462Val (A/G) and UL risk; however, the results are contradictory [2,17-23].

Haplotypes are the series of variants on a chromosome inherited from one parent without substantial recombination. In addition, the term "haplotype" shows closely linked genetic loci. Linkage disequilibrium (LD) is the non-random association of alleles at various loci and shows the degree to which alleles are associated. Observing higher haplotype distribution among affected individuals is a useful method for association studies [24]. The current study aimed to evaluate the association between Asp449Asp (T/C) and Ile462Val (A/G) polymorphisms of *CYP1A1* gene and UL susceptibility in southeast Iran. Moreover, the relation between the Asp449Asp (T/C) and Ile462Val (A/G) haplotypes was evaluated with regard to UL susceptibility.

MATERIALS AND METHODS

Study population

This case-control study was performed on 105 patients with UL (at the stage before menopause having leiomyoma) referred to Ali Ibn Abitaleb Hospital, Zahedan, southeast Iran. Their disease was diagnosed by medical examination and confirmed by ultrasound. In total, 112 healthy females in their premenopausal stage were selected as the control group among females referring for a routine annual check-up and performing the Pap smear test. None of the patients and control group women had a history of blood transfusions. Convenience sampling was employed, and the women were matched for age and ethnicity (Fars or Balouch). The exclusion criteria included systemic diseases and history of malignancy.

Ethics

All participants were Iranian and delivered their informed consents before participating in the study. The study protocol was accepted by the Ethics Committee of Zahedan University of Medical Sciences.

Genomic DNA extraction and genotyping

Genomic DNA was extracted from 2 mL peripheral blood leukocytes using the salting out phenol-chloroform method and stored at – 20°C until analysis. The analysis of Ile462Val (A/G) and Asp449Asp (T/C) polymorphisms of *CYP1A1* gene was performed by the polymerase chain reaction (PCR)-restriction fragment length polymorphism method. The fragment containing the Ile462Val (A/G) was amplified using the forward and reverse primers 5′-CTGTCTCCCTCTGGTTACAGGAAG-3′ and 5′-TTCCACCCGTTGCAGCAGGATAGCC-3′, respectively. The fragment containing the Asp449Asp (T/C) or *MspI* polymorphism region was amplified using the forward and reverse primers of 5′-CAGTGAAGAGGTGTAGCCGCT-3′ and 5′-TAGGAGTCTTGTCTCATGCCT-3′, respectively. The total volume of the PCR mixture was 25 μL containing 200 ng genomic DNA, 25 pM of each primer, 0.1 mM dNTP, 1.5 mM $MgCl_2$, 2.5 μL PCR buffer × 10, and 1 U of Taq polymerase (Fermentas, Lithuania). Amplification was carried out in a Bio-Rad thermal cycler using a thermal profile of initial denaturation at 95°C for 1 minute, followed by 30 cycles at 95°C for 30 seconds, annealing at 66°C for the Ile462Val (A/G) polymorphism and 60°C for the Asp449Asp (T/C) polymorphism for 45 seconds, and primer extension at 72°C for 60 seconds. The 204-bp PCR product of the Asp449Asp (T/C) polymorphism was digested by MspI restriction enzyme (Fermentas, Lithuania) and incubated at 37°C overnight. Post-digestion PCR products were identified by electrophoresis on 2.5% agarose gel. *MspI* cleavage site for T allele of the Asp449Asp (T/C) polymorphism produced 207- and 136-bp fragments. The PCR products of the Ile462Val (A/G) polymorphism were sequenced in a forward direction with the same primers as applied in the PCR, using ABI BigDye Terminator chemistry and ABI PRISM 3730/3730 × l instrument (Applied Biosystems, Foster City, CA, USA). The DNA sequencing results are displayed in Figure 1.

Statistical analysis

Statistical analyses were performed using SPSS software (Version 20; SPSS Inc., Chicago, IL, USA). The rates of the genotypes and alleles were compared using the χ^2-test and/or Fisher's exact test. Quantitative variables were compared using the independent samples *t*-test. The independent effect of each risk polymorphism and haplotypes on UL was assessed via logistic regression analysis. Haplotype frequency and LD were analyzed using Cube X software [25]. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Clinical and demographic characteristics of the patients with leiomyoma and the control group are provided in Table 1. The mean age was not significantly different between the

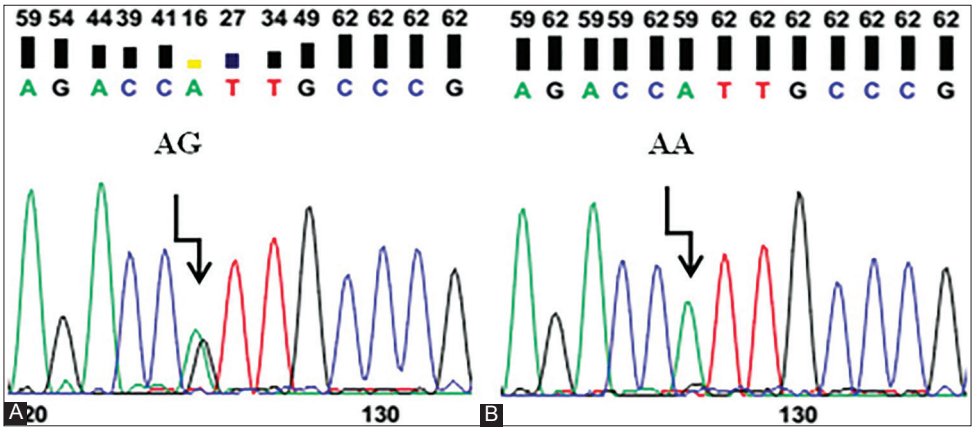

FIGURE 1. DNA sequencing results of Ile462Val (A/G) polymorphism in *CYP1A1* gene, (A) AG heterozygous genotype and (B) AA homozygous genotype

TABLE 1. Clinical and demographic characteristics of the women with UL and controls

Parameter	UL women (n=105)	Control (n=112)	*p* value
Age (years)	38±9.8	36.7±5.7	0.23
Marrieds; n (%)	100 (95.2)	109 (97.3)	0.49
BMI (kg/m^2)	25.1±5.5	25.4±4.7	0.7
Age at menarche (years)	13.5±1.5	13.1±1.6	0.07
Duration of menses(days)	6.1±1.6	5.8±1.5	0.07
Menstrual cycle (days)	28.3±3.3	28.7±2.1	0.3
Bleeding; n (%)	60 (57)	4 (4)	<0.001
Pain; n (%)	30 (29)	6 (5)	<0.001

BMI: Body mass index; UL: Uterine leiomyoma. $p<0.05$ was regarded as statistically significant

two groups ($p > 0.05$). There were no significant differences regarding the other parameters such as menstruation intervals (day), body mass index, menarche age (year), and menstruation intervals (day) between the women with UL and controls ($p > 0.05$). The frequency of abnormal bleeding and pain was significantly higher in the women with UL.

The frequencies of alleles and genotypes of Ile462Val (A/G) and Asp449Asp (T/C) polymorphisms of *CYP1A1* gene are presented in Table 2. The Ile462Val (A/G) polymorphism conformed to the Hardy–Weinberg equilibrium in the UL patients; however, this polymorphism did not conform to Hardy–Weinberg equilibrium in the control group. Moreover, the Asp449Asp (T/C) polymorphism conformed to Hardy–Weinberg equilibrium in both groups. The frequencies of the alleles and genotypes of the Ile462Val (A/G) polymorphism were not significantly different between the women with UL and controls. In addition, there were no differences regarding the alleles and genotypes of the Asp449Asp (T/C) polymorphism between the two groups ($p > 0.05$).

Although, there was no correlation between Asp449Asp (T/C) and Ile462Val (A/G) polymorphisms of *CYP1A1* gene and the UL susceptibility, the combination effect of the TT/AG genotypes of the Asp449Asp (T/C) and Ile462Val (A/G) polymorphisms was associated with 4.3-fold higher risk of UL (odds ratio [OR], 4.3 [95% confidence interval (CI), 1.1 to 16.1], $P = 0.03$) (Table 3).

In addition, the haplotype analysis indicated that the TG haplotype of the Asp449Asp (T/C) and Ile462Val (A/G) polymorphisms could increase the UL risk almost 4.9-fold (OR, 4.9 [95% CI, 1.4 to 17.3], $P = 0.009$) (Table 4).

DISCUSSION

CYP1A1 enzyme is an important phase-I enzyme involved in the metabolism of estrogens and environmental carcinogens such as alkaloids or heterocyclic aromatic amines. Evidence shows that the polymorphisms of *CYP1A1* gene might influence the efficiency of estrogens and environmental carcinogens' metabolism. Ile462Val (A/G) polymorphism of *CYP1A1* gene causes substitution of Isoleucine to Valine. Consequently, the effectiveness of the CYP1A1 enzyme increases and higher amounts of carcinogenic active molecules might be involved in UL development [2].

UL is a multifactorial disease with unknown etiology, and factors such as race, sex hormones, genetic factors, growth factors, cytokines, obesity, family history, lifestyle, and environment are possibly involved in the pathogenesis of the disease. In addition, there are higher levels of ERα and ERβ in leiomyoma cells compared to normal uterus tissue.

It is suggested that genetic polymorphisms in the enzymes of estrogen metabolism such as CYP1A1 and CYP1B1 (the main enzymes of monooxygenase family of cytochrome P450) may affect the pathogenesis of leiomyoma [20]. In this study, no association was observed between Asp449Asp (T/C) (rs4646903) and Ile462Val (A/G) (rs1048943) polymorphisms of *CYP1A1* gene and UL in southeast Iranian women. Several studies have also indicated no association between these polymorphisms and UL, confirming the results of our study.

In 2008, Ye et al. reported that Ile462Val (A/G) polymorphism of *CYP1A1* gene was associated with the increased risk of UL in Chinese women, while reporting no association

TABLE 2. Allelic and genotypic frequencies of Ile462Val (A/G) and Asp449Asp (T/C) polymorphisms of *CYP1A1* gene in women with UL and healthy controls

CYP1A1	UL women (n=105)	Control (n=112)	*p* value	Non adjusted OR (95% CI)	*p* value	Adjusted OR (95% CI)
Ile462Val (A/G) (rs1048943)						
AA, n (%)	70 (67)	80 (71.5)		1		1
AG, n (%)	35 (33)	32 (28.5)	0.5	1.3 (0.7-2.2)	0.5	1.2 (0.7-2.2)
GG, n (%)	0 (0)	0 (0)	-	-	-	-
Allele						
A, n (%)	175 (83)	192 (86)		1		
G, n (%)	35 (17)	32 (14)	0.5	1.2 (0.7-2)		
Asp449Asp (T/C) (rs4646903)						
TT, n (%)	71 (67.5)	73 (65)		1		1
TC, n (%)	29 (27.5)	34 (30)	0.7	0.9 (0.5-1.6)	0.6	0.8 (0.5-1.5)
CC, n (%)	5 (5)	5 (5)	1	1 (0.5-1.9)	1	1 (0.5-1.9)
TC+CC, n (%)	34 (32.5)	39 (35)	0.7	0.9 (0.5-1.6)	0.6	0.8 (0.5-1.5)
Allele						
T, n (%)	171 (81)	180 (80)		1		
C, n (%)	39 (19)	44 (20)	0.9	0.9 (0.6-1.5)		

$p<0.05$ was regarded as statistically significant. OR: Odds ratio; CI: Confidence interval; UL: Uterine leiomyoma

TABLE 3. Combination analysis of Asp449Asp (T/C) and Ile462Val (A/G) genotypes of *CYP1A1* gene in women with UL and healthy controls

Asp449Asp (T/C)/Ile462Val (A/G)	UL women (n=105)	Control (n=112)	*p* value	OR (95% CI)
TT/AA	60 (57.1)	70 (62.5)		1
TT/AG	11 (10.5)	3 (2.7)	0.03	4.3 (1.1-16.1)
TC/AA	8 (7.6)	3 (2.7)	0.10	1.8 (0.9-3.5)
TC/AG	21 (20)	31 (27.7)	0.48	0.9 (0.7-1.2)
CC/AA	5 (4.8)	4 (3.6)	0.59	1.1 (0.8-1.5)
CC/AG	0 (0)	1 (0.9)	1	-

$p<0.05$ was regarded as statistically significant. OR: Odds ratio; CI: Confidence interval; UL: Uterine leiomyoma

TABLE 4. Haplotype analysis of Asp449Asp (T/C) and Ile462Val (A/G) polymorphisms of *CYP1A1* gene in women with UL and healthy controls

Asp449Asp (T/C)	Ile462Val (A/G)	UL women	Control	*p* value	OR (95% CI)
T	A	0.7545	0.7892		1
T	G	0.0598	0.0144	0.009	4.9 (1.4-17.3)
C	A	0.0931	0.0545	0.14	1.9 (0.9-3.9)
C	G	0.0926	0.1419	0.3	0.7 (0.4-1.3)

$p<0.05$ was regarded as statistically significant. OR: Odds ratio; CI: Confidence interval; UL: Uterine leiomyoma

between Asp449Asp and Leu432Val of *CYP1B1* gene and Asp449Asp (T/C) polymorphism of *CYP1A1* gene and the risk of UL [18]. In another multicenter case-control study performed by Shen et al. in Han Chinese women, Ile462Val (A/G) and Gly45Asp loci of *CYP1A1* gene were reported as risk factors for UL development [22]. Barao et al. did not find any correlation between Ile462Val (A/G) and Asp449Asp (T/C) polymorphisms of *CYP1A1* gene and UL in Brazilian women [19]. Contradictory to the current results, Herr et al. found C allele of *CYP1A1* polymorphism as a risk factor for UL susceptibility in German women [17]. El-Shennawy et al. concluded that the carriage of AG genotype of Ile462Val (A/G) polymorphism of *CYP1A1* gene and CC genotype of Leu432Val polymorphism of *CYP1B1* gene were associated with UL in Egyptian women [20].

Similarly, there was no relation between Ile462Val (A/G) polymorphism of *CYP1A1* gene and UL in the study of Taghizade Mortezaee et al. carried out in West Iran [26]. Wang et al. performed a meta-analysis on nine case-control studies including 2157 women with UL and 2197 healthy controls and suggested that Ile462Val (A/G) polymorphism of *CYP1A1* gene is significantly associated with UL risk. These differences may be due to various genetic backgrounds or heterogeneity effect of specific gene polymorphisms in different populations. In addition, LD may be a cause for these differences [2].

Although no association was observed between Asp449Asp (T/C) and Ile462Val (A/G) polymorphisms of *CYP1A1* gene and UL, the combination effect of the TT/AG genotypes and TG haplotype of the Asp449Asp (T/C) and Ile462Val (A/G) polymorphisms was associated with the UL risk. To the best of our knowledge, there is no published report on the combination or haplotype effects of *CYP1A1* polymorphisms and UL.

Ile462Val (A/G) polymorphism of *CYP1A1* gene conformed to Hardy-Weinberg equilibrium in the UL patients; however, this polymorphism did not conform to Hardy–Weinberg equilibrium in the control group. Moreover, Asp449Asp (T/C) polymorphism of *CYP1A1* gene

conformed to Hardy-Weinberg equilibrium in both groups. Hardy-Weinberg disequilibrium could be due to population structure in the southeast of Iran (different ethnic groups) and small sample size. Furthermore, the findings would have been more valuable if the study was performed on both myomatous and intact tissues.

In conclusion, the results showed no association between Ile462Val (A/G) and Asp449Asp (T/C) polymorphisms of *CYP1A1* gene and UL ($p < 0.05$). However, the combination effect of the TT/AG genotypes and TG haplotype of the Asp449Asp (T/C) and Ile462Val (A/G) polymorphisms could increase the UL risk.

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DECLARATION OF INTERESTS

The authors declare no conflict of interests.

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