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The –2549 insertion/deletion polymorphism of VEGF gene associated with uterine leiomyoma susceptibility in women from Southeastern Iran

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ABSTRACT

Objectives: Vascular endothelial growth factor (VEGF) is an important angiogenic factor that regulates angiogenesis and mediates sex steroid-induced cell growth. The present study investigated the association of VEGF gene-2578C/A (rs699947) and –2549 insertion/deletion polymorphisms in the promoter region of VEGF-A gene and uterine leiomyoma susceptibility in Southeast of Iran.

Material and methods: One hundred and fifty five women with uterine leiomyoma and 157 age, BMI, and ethnicity matched healthy women were enrolled in this study. VEGF gene –2578C/A polymorphism genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, and the –2549 insertion/deletion polymorphism was analyzed by PCR method.

Results: The frequency of alleles and genotypes of VEGF-2578C/A polymorphism was not different between women with uterine leiomyoma and the controls; however, a significant association was revealed between II genotype of –2549 insertion/deletion (I/D) polymorphism of VEGF gene and uterine leiomyoma.

Conclusions: The findings showed that VEGF gene –2549 insertion/deletion polymorphism was associated with uterine leiomyoma.

Key words: VEGF, uterine leiomyoma, polymorphism

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INTRODUCTION

Uterine leiomyomas (ULs) are benign and monoclonal tumors of the smooth muscle cells in the myometrium. It is estimated that the prevalence of these tumors is 77% of all reproductive-age women in the United States [1]. ULs are asymptomatic in 50–40% of women over 35 years old; however, it could be associated with symptoms such as menorrhagia, pain, infertility, and recurrent pregnancy loss [2]. UL may affect the pregnancy in one third of women and enlarge its size in the first and third trimesters of pregnancy due to altered levels of hormones especially estrogen [3]. Different risk factors may affect UL development including: obesity, ethnicity, family history, diet rich in meat, smoking, age increasing, use of oral contraceptive pills, and biological biomarkers [4].

Although the exact etiology of UL is unknown, there are several factors influencing the growth of UL [5]. They

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include not only ovarian steroids, angiogenesis, and growth factors, but also the apoptosis related factors [6]. It is well known that ULs respond to the ovarian hormones such as estrogen and progesterone, and their epidemiology parallels the ontogeny and life-cycle changes in genital hormones [7].

Several studies have shown that different growth factors such as epidermal growth factor (EGF), insulin-like growth factors I and II (IGF-I/IGF-II), VEGF, transforming growth factor-beta (TGF-β), platelet-derived growth factor (PDGF), and basic fibroblast growth factor (bFGF), which are the mediators of cell proliferation, increase the extracellular matrix and hypertrophy involved in UL [8, 9]. VEGF is an angiogenic peptide that plays an important role in the growth of numerous tumors [10, 11]. Amongst seven different types of VEGF (VEGF-A, -B, -C, -D, -E, -F, and placental growth factor), VEGF-A and B are mostly angiogenic [12, 13].

VEGF binds to VEGF receptors (VEGFR-1 and VEGFR-2) on endothelial cells, triggering a Tyrosine Kinase pathway leading to angiogenesis. VEGF expression is potentiated by a variety of cytokine and hormones [13]. The human VEGF-A gene is located on chromosome 6 (6p12.1) and contains a 14-kb coding region with eight exons and seven introns [14, 15]. There are numerous VEGF polymorphisms, some of which are associated with altered VEGF expression [16]. A number of genetic studies have revealed the association between UL development and genetic polymorphisms; for example, VEGF [17], estrogen receptors [18, 19], XRCC1 and p53 [20], CYP1A1 [21], Catechol-O-methyl transferase [22] and MDM2 [23] genes.

Although there is one report about the relation between VEGF gene 5′-UTR –460 polymorphism and UL, there is no published report about the association between-2578C/A (rs699947) and –2549 insertion/deletion polymorphisms of VEGF gene and UL susceptibility.

The current study aimed to evaluate the association between –2578C/A (rs699947) and –2549 insertion/deletion polymorphisms in the promoter region of VEGF gene and UL susceptibility.

MATERIAL AND METHODS

This study was performed on 155 women with UL (aged 38.5 ± 9.7 years) and 157 healthy women (aged 38.4 ± 7.9 years) as controls without any systematic disease, homogenous with the patient group (age, race, and BMI). The study protocol was approved by the Ethics Committee of Zahedan University of Medical Sciences, and all the patients granted their informed consent.

Samples containing 2 mL of peripheral blood were taken from all women for genetic analysis. They were poured in tubes containing EDTA and stored at –20°C.

Genotype analysis

Genomic DNA was extracted from EDTA treated whole blood through salting out method. VEGF polymorphisms genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism method (PCR-RFLP). The fragment containing VEGF-2578 C/A polymorphism was amplified, using forward; 5'-GGATGGGGCTGACTAGGTAAGC-3' and reverse; 5'-AGCCCCCTTTTCCTCCAAC-3' primers. The target fragment containing –2549 insertion/deletion polymorphism was amplified using forward; 5′-CCTGGAGCGTTTTGGTTAAA-3′ and reverse; 5′-ATATAGGAAGCAGCTTGGAA-3′ primers.

The PCR conditions were set as follows: one cycle at 95°C for 5 min, 30 cycles at 95°C for 20 s, annealing respectively at 58°C and 62°C for 30 s and 2 min (VEGF C-2578A and–2549 insertion/deletion), and extension at 72°C for 45 s, and a final extension cycle at 72°C for 5 min. The 325bp-PCR product of –2578C/A polymorphism was digested with 2-U *BglII* restriction enzyme (Fermentas, Vilnius, Lithuania) at 37°C overnight. Digested products were electrophoresed on 2% agarose gel containing ethidium bromide. The C allele of C-2578A had no *BglII* cleavage site, whereas in presence of A allele 325 bp fragment cleaved to 202 and 123 bp fragments.

The PCR product of –2549 insertion/deletion polymorphism was loaded into 3% agarose gel containing ethidium bromide for electrophoresis. I and D alleles of VEGF I/D polymorphism produced 234bp and 216 bp bands, respectively.

Statistical analysis

Statistical analyses were performed using SPSS V-20. Clinical and demographic characteristics of UL women and control groups were compared by the independent student's t-test or Fisher's exact test whenever appropriate. The alleles and genotypes' frequencies between the groups (patients and controls) were compared applying Fisher's exact test. To determine the effect of polymorphisms on the risk of UL Logistic Regression analysis was used. p-value < 0.05 was considered statistically significant.

RESULTS

Clinical and demographic characteristics of UL women and control group are given in Table 1. The maternal age and menarche age were not statistically different between two groups. The frequency of bleeding and pain were significantly higher in the UL group compared to the controls.

Alleles and genotypes frequency of VEGF-2578C/A polymorphism is presented in Table 2. VEGF-2578C/A polymorphism was in Hardy-Weinberg equilibrium in both groups ($p > 0.05$). There was no association between genotypes of the VEGF-2578C/A polymorphism and UL susceptibility. In

Table 1. Clinical and demographic characteristics of UL women and control group

	UL women (n = 155)	Controls (n = 157)	p-value
Maternal age (years)	38.5 ± 9.7	38.4 ± 7.9	NS
Marriage status, n (%)	146 (94)	151 (96)	NS
BMI [kg/m^2]	25.8 ± 5.3	25.3 ± 4.6	NS
Age of menarche (years)	13.5 ± 1.6	13.2 ± 1.5	NS
Duration of menses (days)	6.1 ± 1.6	5.9 ± 1.6	NS
Menstrual cycle (days)	28.2 ± 3.6	28.5 ± 2.9	NS
Bleeding, n (%)	94 (61)	6 (3.8)	< 0.0001
Pain, n (%)	43 (28)	10 (6.4)	< 0.0001

NS — not significant; UL — uterine leiomyoma

Table 2. Comparison of genotypic and allelic frequency of VEGF –2578C/A polymorphism in UL women and control group

VEGF –2578C/A	UL women (n = 155)	Controls (n = 157)	p-value	OR (95% CI)*
CC, n (%)	51 (33)	49 (31)		
CA, n (%)	76 (49)	85 (54)	0.6	0.85 (0.5–1.35)
AA, n (%)	28 (18)	23 (15)	0.7	1.1 (0.8–1.5)
Allele				
C, n (%)	178 (57)	183 (58)		
A, n (%)	132 (43)	131 (42)	0.9	

*Adjusted for age, ethnicity and primiparity
CI — confidence interval; OR — odds ratio; UL — uterine leiomyoma; VEGF — vascular endothelial growth factor

addition, the frequency of VEGF-2578C/A alleles was not different between two groups.

Alleles and genotypes' frequency of VEGF –2549 insertion/deletion polymorphism is presented in Table 3. The frequency of II genotype of VEGF –2549 insertion/deletion polymorphism was significantly higher than DD genotype in UL women compared to controls (25 vs. 12 %), and the risk of UL was 1.5 fold higher in presence of II genotype ([OR, 1.5 [95% CI, 1–2.1]; p = 0.03).

Moreover, the risk of UL was 2.4 fold higher in women with II genotype compared to DD + ID genotypes (recessive model) ([OR, 2.4 [95% CI, 1.3–4.3]; p = 0.004). The frequency values of I and D alleles were not different between two groups.

DISCUSSION

In the present study, two polymorphisms of VEGF gene in UL women and controls were evaluated. The findings showed no association between VEGF-2578C/A (rs699947) polymorphism and UL; however, a significant association was revealed between II genotype of –2549 insertion/deletion (I/D) polymorphism of VEGF gene and UL in Southeast Iranian women.

Table 3. Comparison of genotypic and allelic frequency of VEGF I/D polymorphism in UL women and control group

VEGF I/D polymorphism	UL women (n = 155)	Controls (n = 157)	p-value	OR (95% CI)*
DD	44 (28.5)	46 (29)		1
ID	72 (46.5)	92 (59)	0.45	0.8 (0.5–1.4)
II	39 (25)	19 (12)	0.03	1.5 (1–2.1)
DD+ID	116 (75)	138 (88)		1
II	39 (25)	19 (12)	0.004	2.4 (1.3–4.3)
Alleles				
D	160 (52)	184 (59)		
I	150 (48)	130 (41)	0.09	1.3 (1–1.8)

*Adjusted for age, ethnicity and primiparity
CI — confidence interval; OR — odds ratio; UL — uterine leiomyoma; VEGF — vascular endothelial growth factor

UL is the most common tumor in women; however, its molecular etiology is still unknown. Many hormones and cytokines are associated with leiomyoma formation, including progesterone, estrogen, insulin-like growth factor, fibroblast growth factor, epidermal growth factor and VEGF. VEGF, as an endothelial cell specific regulator, is a key factor influencing tumor related angiogenesis. Several studies showed that over expression of VEGF might be related to numerous tumors, including endometrial cancer [24], breast cancer [25], hepatocellular carcinoma, and UL [26].

Considering the importance of VEGF in angiogenesis, the association of VEGF polymorphisms and various tumors has been investigated. Nevertheless, there is only one report on the association of VEGF-460 polymorphism in 5'-untranslated region of VEGF gene and UL. Accordingly, the present study evaluated the association between VEGF –2578 and –2549 insertion/deletion polymorphisms and UL susceptibility.

In 2008, Hsieh et al. genotyped –460 polymorphism at 5'-untranslated region of VEGF gene in 159 women with leiomyoma and 131 nonleiomyoma women in Taiwan, indicating that VEGF –460T allele and –460TT genotype were associated with higher UL susceptibility.

There are several studies which have investigated the relation between VEGF polymorphisms and different tumors and cancers. Liu et al. found that –2578A allele is a protective factor against the development of endometriosis in Chinese women. There was an association between VEGF +936 C/T polymorphism, but not VEGF –460 C/T, +405 G/C polymorphisms and endometriosis in Tunisian population in the study of Henidi et al. [27]. Perini et al. showed an association between VEGF –1154G > A polymorphism and the risk of developing endometriosis in Brazil [28].

Liu et al. found higher risk of endometriosis and adenomyosis in individuals with GG genotype of VEGF –1154G/A

polymorphism, however, showing no relation between 460C/T polymorphism and these diseases [29]. The study of Kang et al. the –2578A or –1154A allele of VEGF gene significantly decreased the risk of adenomyosis and was considered potentially protective factor for adenomyosis development [30].

In a meta-analysis performed on 14 studies (3313 endometriosis cases and 3393 healthy controls), Li et al. suggested that rs3025039 (+936 C/T) polymorphism of VEGF gene increases endometriosis risk, but VEGF-2578C/A (rs699947) and –1154G/A (rs1570360) polymorphisms might be protective factors for endometriosis [31].

The reason for these discrepancies remains unknown. It was suggested that these differences in the genotypes and alleles' distribution might be due to different sample sizes and different selection criteria adopted for patients and controls in particular clinical manifestations, ethnicities, and environmental risk factors. Since ethnicity is a crucial factor in genetic studies, these differences could be more related to ethnic and clinical heterogeneity between populations.

In conclusion, the results showed no association between VEGF-2578C/A (rs699947) polymorphism and UL. However, there was a significant association between –2549 insertion/deletion (I/D) polymorphism in the promoter region of the VEGF gene and UL. To the best of our knowledge, the present work is the first study regarding the effect of –2549 insertion/deletion (I/D) polymorphism of VEGF gene on UL susceptibility, and further studies are required to evaluate the association between VEGF polymorphisms and UL in different populations.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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