

## ORIGINAL ARTICLE

# Study of common nucleotide changes in ACE gene in women with Uterine Fibroids or Myoma

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### ABSTRACT

Better understanding of the genetic characteristics of myoma as the most common benign tumors of the significant gynecologic impact on preventive measures and treatment process it. Abnormal angiogenesis and vascular growth factors involved in the development of myoma. Gene ACE (Angiotensin-Converting Enzyme) is involved in the creation of several tumors. The present study examined the association between polymorphism I / D ACE gene odds ratio of the myoma in the female population of Iran. This case-control study has been done on 100 women with uterine leiomyomas as the case group and 100 healthy women as the control group between the years 2013-2016 were referred to Mohebe Yas hospital in Tehran. The Genomic DNA extracted from the blood cells and the genotyping was done by using of Single Specific Primer-Polymerase Chain Reaction (SSP-PCR). Statistical analysis is done by using SPSS software version 19 and a lower than 0.05 significant level was considered. There was a significant difference in frequency of D allele in patient and control group ( $p=0.03$ ). The obtained results showed that the presence of D allele would increase the myoma infection chance around 1.59 times. ( $P<0.02$ ). On the other hand, D/D genotype will increase the myoma infection chance around 2.8 times in compare with I/I genotype and I/D genotype ( $P<0.01$ ). In I/D polymorphism in ACE gene, D allele is considered as a risk allele in myoma infection. However, more studies with more samples in other population to confirm the results will be necessary.

**Keywords:** Uterine myoma, ACE gene, I/D polymorphism, SSP-PCR

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## INTRODUCTION

One of the most common tumors of gynecological disorder in women of reproductive age are uterine fibroids (leiomyomas or Myomata) that will arise in the 70 to 80% of women up to age 50, and have the signs in 25 to 30 [1,2,3]. Fibroids is one of the problems of public health and because of the complications like abnormal bleeding and pregnancy is one of the main reasons for surgery in women. Myomas are spherical benign tumors originated from uterus smooth muscles cells, and they have fibrous connective tissue that contains firm consistency and bulging [1, 3, 4]. Fibroids can be dispersed throughout the uterus, and they have three different types based on the location of them such as Submucosal, Subserosal, and Intramular [1].

Although a definitive cause of fibroids is unknown, but the creation of such risk factors include race (American - African) and can be cited obesity [3, 7]. Numerous hormones and cytokines are involved in the formation leiomyoma, including estrogen, progesterone, insulin-like growth factor, epidermal growth factor, fibroblast growth factor, etc [7, 8, and 9]. There is also the arteries and blood flow to part of the growth of tissue involved. So angiogenic factors also play a role in the creation of myoma [10]. It seems renin - angiotensin - aldosterone in the regulation of proliferation of endometrial cells affected. Some steroid hormones involved in the regulation of the renin system. Estrogen hormone causes to increase renin production and renin-angiotensin causes to improve the conversion Angiotensinogen to

Angiotensinogen I ACE causes angiogenesis I to Angiotensinogen II that the active form. Therefore, the use of ACE inhibitors with loss of Vascular Endothelial Growth Factor (growth factor I vascular endothelial) inhibits the growth of myoma and angiogenesis [11]. Angiotensin converting enzyme (ACE), are a component of the renin-angiotensin-aldosterone (12, 13) that up to present investigated its relationship with various diseases, including cancer [14]. ACE gene is located on the 17th chromosome in 17q23 location. This gene contains 26 exons and 25 introns in human and the length of this gene is around [15,16]. A polymorphism at intron 16 of this gene will cause the creation of three different genotypes such as insertion/insertion (I/I), insertion/deletion (I/D), and deletion/deletion (D/D) [11, 17]. I/D polymorphism of this gene will cause cardiovascular diseases due to the presence or absence of a 287bp piece in intron 16 [18]. Despite the importance and prevalence of fibroids and high health costs imposed on society in recent years, much research has been done about it. Regarding the relationship between ACE gene and forth between myoma and high prevalence of the disease, this study is the first study to assess the role of polymorphism I / D ACE gene is associated with myoma so as to make this connection as a marker of early detection to diagnosis and treatment of myoma so that in this way can be used to improve women's quality of life.

## MATERIAL AND METHODS

This case-control study was conducted from September 2013 to July 2014. In this study, 200 patients (100 patients and 100 healthy controls) than among women of the fertile age distribution of persons in compliance with the Comprehensive Women's Hospital in Tehran Jasmine lover were identified by laparoscopy, were selected as the study population. Before starting the work of all those moral principles written consent was received. The profile of these individuals was collected in the form of a questionnaire. The questionnaire included questions such as age, race, blood type, body mass index and was the myoma. To determine the genotype of every individual blood samples with a volume of 5 cc up in 500 microliter tubes containing ethylene diamine tetra-acetic acid anticoagulant material were stored. Samples maintaining cold chain and transferred to the laboratory biosafety and extraction time were kept at a temperature of c 4. After extracting DNA from peripheral blood with chloroform, the purity of DNA extracted by using optical absorption Nanodrop 260: 280 nm were examined. In this study, OD 1.6 to 1.9 was considered desirable. The polymorphism I / D ACE gene by PCR (SSP PCR) Sequence-specific Primers with two primers Forward and Reverse (KBC Co, Tehran, Iran) were used.

This method is based on amplification of target DNA using primers specific for each gene or set of genes that code for the respective antigen is performed. In this method, based on the difference between the three nucleotide primer that is designed for different alleles, a PCR reaction using a DNA template is done in a tube. In each tube, there are two pairs of primers specific primers for the target gene and the other one is for internal controls. PCR reactions were performed using specific primers with the following sequences.

**Table1: specific primers which were used**

PRIMERS	PRIMERS SEQUENCES	TM (°C)
FORWARD	5'CTGGAGACCACTCCCATCCTTTCT3'	64.4
REVERSE	5'GATGTGGCCATCACATTTCGTCAGAT3'	63

PCR reaction solution is prepared in 12µl as the final volume which is using following protocol: 6µl of Tag DNA Polymerase Master Mix Red (AMPLIQON), 0.9µl of primers mixture, and 3.8µl DNA with 30ng dilution and 1.3µl distilled water. PCR reactions on the genomic DNA samples were done by using of thermocycler machine under the following condition: For first denaturing, 5 minutes at 95°C followed by 30 cycles at 94°C for 40 seconds, 63°C in 40 seconds for annealing and 72°C for 40 seconds for extension phase and 72°C in 5 minutes as final extension. The PCR products were run on the 2% agarose gel for 45 minutes at a voltage of 80 and amplified bands were investigated by using documentation Gel under UV light. Comparing of allelic and genotypic frequency in 2 groups, control and patient, and also Hardy-Weinberg equilibrium for genotypes frequency was investigated by using Chi-square analysis. Statistical analysis is done by using SPSS software version 19 and a lower than 0.05 significant level was considered.

## RESULTS

In this study, 100 patients with myoma with age ranging from 20-48 years with an average of 31.5 years and 100 healthy women aged 48-20 years with a mean age of 34 years were studied. After gel

electrophoresis samples of genotype I / I single-band 490, genotype I / D-band 490 and 190, genotype D / D single-band 190, respectively.

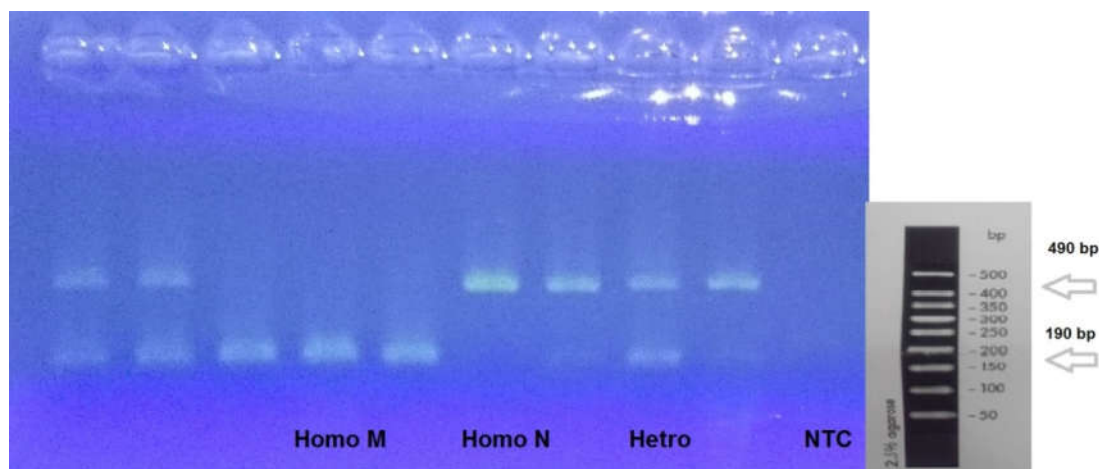


Figure 1: Image of ACE gene electrophoresis bands created with bp490 size as a normal band (Homo N) and Mark bp190 band mutant band (Homo M) and the combination of both bands in the heterozygous state (Hetro). Or negative control NTC (No Template Control) contains all the ingredients except the reaction of DNA samples or RNA (where instead Template used RNase free water) negative control in the PCR is the accuracy.

The results showed significant differences in the frequency of genotype D / D in the study group (35%) than controls (23%), respectively (p=0.016). Overall, the total population (200) genotype I / D or about 52.5% of heterozygous individuals with frequency 105 Frequency, then genotype D / D or mutant genotype frequency with a frequency of 58 to 29% ranks after normal I / I in 37 cases 18.5% of the patients. To investigate the allele frequency of allele I 78 200 cases (39%) in the group with uterine fibroids and 122 (61%) in the control group there. In connection with the D allele frequency of about 101 people (50.5%) in the group with 99 cases (49.5%) in the control groups (p=0.02). The results obtained indicate a significant correlation between the presence of genotype D / D and the risk of uterine myoma (P =0.01655). The genotype D / D risk of uterine myoma compared with the control group to increase 809/2.

**Table 2: Association of ACE gene genotypes with myoma infection**

P*	OR** (CI*** 95%)	The number of Patient (%)	Healthy	Genotype
	1(reference)	13(13)	24(24)	<b>I/I</b>
0/130	1/811(0/83-3/93)	52(52)	53(53)	<b>I/D</b>
0/016	2/809(1/194-6/611)	35(35)	23(23)	<b>D/D</b>
	1(reference)	78(39)	101(50/5)	<b>I</b>
0/020	1/596(1/073-2/373)	122(61)	99(49/5)	<b>D</b>

\* Statistical analysis: Chi-square and P <0.05 was significant  
 \*\* OR = odd ratio, \*\*\* CI = confidence interval, \*\*\*\* Reference Category (Odds ratio: 1.00)

Hardy-Weinberg equilibrium test was done for two groups using Chi-square test. Calculate the amount of Chi showed that patients were in Hardy-Weinberg equilibrium because if the disease-causing alleles in the population with the disease, the patient population is not established in Hardy-Weinberg equilibrium (p=0.035, chi2=0.863). Hardy-Weinberg equilibrium populations in different genetic models at 95% for recessive genetic model Recessive) was statistically significant, meaning that two copies of the defective allele were necessary for the occurrence of disease in populations.

**DISCUSSION AND CONCLUSION**

Fibroids are the most common gynecological tumors, which are causing many problems indication for hysterectomy. Angiogenic growth factors improved in myoma not have abnormal angiogenesis [10]. ACE is one of the genes involved in angiogenesis; tumor growth requires angiogenesis and tissue [11]. ACE is

associated with tumors [14]. The many studies done on the relationship between polymorphisms of this gene suggest the gene known gynecologic conditions such as endometriosis and risk of breast cancer and ovarian laziness [20, 21, and 22].

Among gene polymorphisms of ACE I / D is that the different ethnic groups in Iran (17). The results show a significant correlation between genotype D / D and the risk of myoma (P =0.0165). Based on observations of allele D allele is a risk and there is a significant difference between the experimental and control groups (P =0.02073). This allele in homozygous genotype D / D with 8/2 the risk of disease increases. It can be genotype D / D as a risk factor for prediction of uterine myoma considered in Iranian population.

Freitas and his colleagues have shown that the presence of endometrial cancer associated allele I on growth and age (P =0.029). They observed that women with normal blood pressure are up to 3/6 times more likely to have an allele I at risk fall endometrial cancer (P =0.037, OD =3.6) (20) . While Namazi and his colleagues showed that the ACE gene polymorphism and breast cancer, there is no communication (P =0.14) [21]. Jung and his colleague's study demonstrated that the ACE gene could be important as a marker to identify primary ovarian failure have a role (P =0.04) [22]. Studies Hung and his colleagues showed that genotype D / D to 0.41 the risk of cancer of the cervix (cervical) increases [23].

In a study by HSIEH *et al* also showed that polymorphism D / D Taiwanese women in the development of uterine myoma is a significant difference (P <0.0001) (24). However, in a study conducted by Gültekin and his colleagues statistically significant difference between genotype D / D were observed in healthy subjects (p =0.339,  $\chi^2 =2 .162$ ) [25]. Gomaa and his colleagues also showed that the ACE gene polymorphism and risk of uterine myoma in Egyptian women there is no significant relationship (P =0.4978) [26].

This polymorphism may be due to differences in the expression of multiple processes and enzymatic reactions, differences in the classification of diseases, diversity, and environmental sustainability and so on can be used in the design of screening tests, clinical diagnosis, treatment and presentation of the inheritance pattern are HGSHA.

Clinical patients with genotype D / D and I / D compared to if there is a statistically significant relationship of this relationship can be used as a marker predicting. If you find a statistical association, predictive criteria readily available to physicians to diagnose disease will be the risk factor.

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