

**Original Article:****Association between Vascular Endothelial Growth Factor (VEGF) -1154G/A Polymorphism and Endometriosis in North West of Iran****Gholamreza Niaei<sup>1</sup>, Somayeh Heidaryan<sup>2</sup>, Alireza Niaei<sup>3</sup>, Nasim Alsadat Didevar<sup>4</sup>**<sup>1</sup>Department of Biology, Faculty of Sciences, University of Guilan, Rasht, Iran<sup>2</sup>Cellular and Molecular Sciences, Department of Biology, Faculty of Guilan Pardis, Department of Biology, University of Guilan, Rasht, Iran.<sup>3</sup>Department of Medicine, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran<sup>4</sup>Department of biology, East Azerbaijan Science and Research Branch, Islamic Azad University, Tabriz, Iran\*Corresponding Author: email address: [Gniaei@iaushab.ac.ir](mailto:Gniaei@iaushab.ac.ir) (Gh. Niaei)**ABSTRACT**

Endometriosis is considered as a multifactorial disease that affects up to 10% of all women of fertile age. Vascular endothelial growth factor (VEGF) is one of the most important activators of angiogenesis. VEGF is known to be a key molecule in the pathogenesis of endometriosis. A great number of studies have referred to genetic polymorphisms as a factor that contributes to the development of endometriosis. The present study was aimed to find out the frequency of the VEGF -1154G/A polymorphism and its relationship with endometriosis risk in Iranian women with endometriosis. This study involved 175 patients with endometriosis and 131 healthy controls. Following extraction of genomic DNA from patients and controls, genotyping of the -1154G/A polymorphism of the VEGF gene were performed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis. Multivariate analysis was used to examine the association between the risk of endometriosis and VEGF -1154G/A polymorphism. There was statistically a significant difference in the frequency of the -1154G/A polymorphism between patients and the controls. The percentage distribution of the three -1154G/A genotypes was (GG 3.4%, GA %46.8%, AA 49.7%) in endometriosis patients.

**Conclusion:** Data supports the hypothesis that angiogenesis is of pivotal importance in the development of endometriosis. Our study indicates that there is significant association between the VEGF gene -1154G/A polymorphism and the risk of endometriosis in North West of Iran.

**Keywords:** Endometriosis; vascular endothelial growth factor; genetic polymorphisms

**INTRODUCTION**

Endometriosis is a gynecological disease defined as the presence of endometrial-like glands and/or stroma outside the uterus. There is no known cause for endometriosis. Some experts have come up with a few possibilities, but nothing has been conclusively proven [1-3]. Many genetic studies were carried out to inspect the contribution of encoded genes whereas various studies exposed the association between different genotypes and the disease vulnerability [3-5]. The most common areas for endometriosis to implant are on the ovaries, the outside wall of

the uterus, fallopian tubes, pelvic cavity, and reproductive organ ligaments. In endometriosis, neovascularization is essential for the implantation of endometrial cells in ectopic sites [1-5]. Vascular endothelial growth factor (VEGF), known as vascular permeability factor, is a heparin-binding glycoprotein, playing a critical role in angiogenesis. VEGF is strongly expressed by epidermal keratinocytes in wound healing and psoriasis, conditions that are characterized by increased microvascular permeability and angiogenesis [4-7]. Some investigators have suggested higher peritoneal

concentrations of VEGF in women with advanced stage endometriosis, and others have shown increased VEGF mRNA and protein expression in the eutopic endometrium from subjects with endometriosis [5-8].

The VEGF gene is located at 6p21.343, comprises a 14-kb coding region with 8 exons and 7 introns, which is responsible for forming several proteins [6-8]. Several association studies on single nucleotide polymorphisms (SNPs) were described in this gene. Moreover, association has been reported between endometriosis and the VEGF 5'-untranslated region (UTR) [+405G>C (rs2010963)] or 3'-UTR [+936C>T (rs3025039)] polymorphisms, where polymorphisms in the promoter region [loci -2578C>A (rs699947) and -460T>C (rs833061)] might alter VEGF expression [6-10]. VEGF is also regulated at the level of mRNA stability. The T allele (-460T>C polymorphism) may be associated with decreased VEGF promoter activity. The C allele of the +405G>C polymorphism has been associated with lower VEGF levels as well [7, 9]. The present study was designed to explore the association between the VEGF -1154G/A (rs1570360) gene polymorphism and the risk of advanced endometriosis in Iranian Azeri population. We investigated the frequency of -1154G/A polymorphism in patients with and without advanced endometriosis.

## MATERIALS AND METHODS

### Subjects

From August 2014 to June 2015, all individuals involved in this comparative case and control study gave written informed consents for the genetic analysis. This study was approved by the ethic committee of Tabriz University of medical sciences as a research project.

All Controls were of Iranian and selected from women who self-reported as never being diagnosed with any pelvic findings of endometriosis, inflammatory disease or uterine fibroid, and were therefore considered to be at low risk of having endometriosis. The diagnosis of endometriosis was confirmed by laparoscopy and classified by histological criteria according to the Revised American Society for Reproductive Medicine. Only patients with stages 1 and 2

endometriosis have been included in this study. Patients who received endocrine therapy prior to surgery were excluded from this study. The characteristics of the samples are shown in Table 1.

**Table 1.** Characteristics of the samples

Characteristics	Women with Endometriosis (n=175)	Controls (n=131)
Maternal age (years) (range, mean)	18–44 (28.8)	19–41 (30)
Body mass index (range, mean)	18.4–29.3 (23.5)	19.0–27.6 (24.0)
Length of menstrual cycle (days) (range, mean)	25–30 (27.5)	27–33 (28.7)
Family history of miscarriage	51	15

### Genomic DNA analysis

Peripheral blood was drawn from each patient and collected in an EDTA containing tube. DNA concentrations were quantified with a spectrophotometer [11]. Purified genomic DNA was maintained at a temperature of -20°C until the appearance of PCR reactions. Genotyping of the VEGF gene polymorphisms were performed by PCR and restriction fragment length polymorphism (RFLP) analysis, as previously described by Liu Qing, et al (2009) for the -1154G/A (rs1570360) polymorphism, with minor modifications [10]. PCR products were digested with restriction enzyme MnlI (New England Biolabs) for -1154G/A. The digested products were analyzed on 3% agarose gels stained with ethidium bromide. The sequence of used primers is presented in Table 2.

**Table 2.** PCR conditions for identifying VEGF -1154G/A restriction fragment length polymorphism

Polymorphisms	Primers	PCR product length	Restriction enzyme
-1154G/A	5'-TCCTGCTCCCTCT CGCCAATG-3' (F) 5'-GGCGGGGACAGGC GAGCATC-3' (R)	206 bp	MnlI

### Statistical analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS), version 9.0. Alleles and genotypes frequencies were compared between groups using the Fisher's exact test. A *p* value of < 0.05 was considered significant.

### RESULTS

The age of the patients ranged from 22 to 37 years. Chi-square ( $\chi^2$ ) test was used to evaluate each allele and genotype frequency of -1154G/A polymorphism among the cases and controls. There was no significant difference in terms of distribution of age between the cases and controls (*P* = 0.02). Endometriosis symptoms were also reported by undiagnosed women. We have evaluated the genotypes data of 175 endometriosis patients and 131 healthy individuals by PCR-RFLP. The distribution of the -1154G/A genotype, and the genotype specific ORs for patients with endometriosis and controls are shown in Table 3. The percentage distribution of the three -1154G/A genotypes GG, GA and AA in endometriosis patients was 3.4, 46.8 and 49.7%, respectively, whereas, in the control group these frequencies were 10.7, 51.1, and 38.2%, respectively. There was a significant trend between G/A genotype and higher stage of endometriosis (*P* < 0.01).

**Table 3.** Genotype and allele distributions of VEGF -1154 G/A in Iranian Azeri women with endometriosis (n 175) and controls (n 131) and their association with the risk of developing endometriosis

VEGF -1154G/A Genotypes	Control n (%)	Endometriosis n (%)	OR (95%CI)	P-value
G/G	14 (10.7)	6 (3.4)	Ref	-
G/A	67 (51.1)	82 (46.8)	3.3 (1.2-9.0)	0.01
A/A	50 (38.2)	87 (49.7)	1.6 (1.0-2.5)	0.04
<b>Alleles</b>				
G	95 (36.3)	94 (26.9)	Ref.	-
A	167 (63.7)	256 (73.1)	1.5 (1.1-2.2)	0.01

### DISCUSSION

Genetic studies in endometriosis have been reviewed by several groups, showing that published studies are often conflicting due to small sample size in many studies. Previous studies have shown that the increased production of VEGF in females with endometriosis and increased VEGF levels have been demonstrated in the peritoneal tissue and blood plasma and peritoneal fluid of females with endometriosis [3-6]. In order to determine a genetic predisposition to endometriosis, some studies were developed to investigate polymorphisms of the VEGF gene in women with endometriosis. These studies were conducted in China [12-13], Korea [14, 15], India (16), Japan [17], Turkey [18], Italy [19], and Spain [20]. Lee and Ho reported that VEGF in endometriosis significantly inhibits sperm motility, acrosome reaction and sperm-oocyte interaction, which may result in endometriosis associated infertility [21].

The VEGF gene is located at 6p21.3, at least 30 SNPs were described in this gene. The -1154 G/A polymorphism of the VEGF gene was assessed by Liu et al. [13] and by Lamp et al. [22]. They studies have shown -1154A allele may be protective against the development of endometriosis in North Chinese women [13]. Studies by the same group of researchers showed that VEGF -1154G/A polymorphism was associated with susceptibility to endometriosis and adenomyosis, and the GG genotype was associated with high risk for the disease [23]. Different alleles of the -2578 C/A SNP were associated with reduced risk for endometriosis. The A allele of this polymorphism was reported to be protective in relation to the development of endometriosis [13]; This polymorphism was not associated with endometriosis in a study by Zhao et al [24]. The study by Zhao et al which assessed VEGF +936 C/T and -2578 A/C SNPs, was the only study that did not associate these SNPs with endometriosis [24]. It has therefore become an important goal to illuminate the pathogenesis of infertility and explore the newly identified genes related to endometriosis. Based on the above research, it was assumed that VEGF polymorphisms might indeed play a certain role in endometriosis. The present study applied this approach to the role of -1154G/A VEGF

polymorphism in endometriosis development. To the best of the researchers' knowledge, this is the first study on Iranian women to examine the association of VEGF -1154G/A polymorphism with endometriosis. Data, support the hypothesis that angiogenesis is of pivotal importance in the development of endometriosis. Since genetic polymorphisms are different among different ethnic groups, the different results in different populations may be due to different genetic background. Therefore, further studies are needed to clarify whether the association between genetic polymorphisms and pregnancy outcome can be modified by VEGF polymorphism.

In conclusion, this pilot study which was carried out in Iran focused on 175 women with endometriosis. A strong association between VEGF -1154G/A polymorphism and endometriosis was confirmed. The findings of the present study indicate that there is significant association between the VEGF gene -1154G/A polymorphism and the risk of endometriosis.

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*"The authors declare no conflict of interest"*

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