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Vascular endothelial growth factor genetic polymorphisms at +405C/G and -460 C/T and the association with advanced stages of endometriosis

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Summary

Purpose of investigation: Endometriosis is a common gynecologic disease, with a genetic component, a series of studies involving different vascular endothelial growth factor (VEGF) genetic variants in endometriosis pathogenesis. In this study, the authors aimed to describe the presence of VEGF genetic polymorphisms in relationship with endometriosis, to evaluate their relationship with VEGF serum levels, and to investigate its impact on endometriosis susceptibility. *Materials and Methods:* The authors studied the frequency of alleles, genotypes, and haplotypes of VEGF +405 C/G and -460 C/T genetic polymorphisms in patients with endometriosis, by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). *Results:* This study did not find any significant relationship between the presence of mutant allele and endometriosis. Regarding VEGF serum levels and the presence of a different allele, there was also no statistically significant difference. *Conclusion:* This research shows that VEGF +405 C/G and -460 C/T genetic polymorphisms are not associated with advanced endometriosis, and VEGF serum levels did not correlate significantly in relationship with the presence of a different allele and/or genotypes, thus suggesting no involvement for this polymorphism in the pathogenesis of endometriosis.

Key words: Endometriosis; Genetic polymorphism; Infertility; Angiogenesis.

Introduction

Endometriosis is a benign gynecologic disease, characterized by the presence and growth of endometrial tissue outside the uterus, often associated with chronic problems, such as dysmenorrhea, dyspareunia, pelvic pain, and infertility [1]. It affects around 10% of women in their reproductive years, but among infertile women, its prevalence can reach up to 40% [2].

The diagnosis of endometriosis relays on the histologic confirmation of endometrial glands and stroma outside of the uterus and it is usually based on invasive procedures such as laparoscopic surgery [3]. To date, there are no reliable non-invasive tests for endometriosis, thus, a non-invasive diagnostic tool for diagnosis of endometriosis is required.

Although many investigations have been carried out, the pathogenesis of the disease remains unclear, with Sampson's retrograde menstruation being considered as the predominant theory involved [4]. On the other hand, it is considered that women with first-degree relatives that have endometriosis have a five- to seven-fold greater risk to develop the disease. Moreover, different reports have indicated that women with a family history of endometriosis tend to have more advanced stages of endometriosis, associated with severe pelvic pain, and other related symptoms [5, 6]. Thus, it seems that endometriosis has a genetic component and through the years a search for candidate genes on which to base an endometriosis-specific test has been challenging.

For endometriosis to develop and progress, the endometriotic lesions require an adequate blood supply to survive, thus angiogenesis is very important during this process [7]. The development of new blood supplies is a complex process, which is regulated by a signal sequence of different angiogenic factors. Endometriosis-related angiogenesis is influenced by different inducers and growth factors, from which the significant one is vascular endothelial growth factor (VEGF). It is one of the most important angiogenic factors involved in endometriosis and it determines an increase in vascular permeability, it induces endothelial cell proliferation, migration, differentiation, and capillary formation [8].

Previous studies on VEGF serum and peritoneal fluid levels in women with endometriosis have shown conflicting results, with some of them indicating increases, or decreases, while several others have reported no change [9-14]. A very recent study has found that plasma levels of VEGF were elevated in endometriosis patients compared with controls [15].

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In the recent years, different studies focusing on diverse genetic polymorphisms in women with endometriosis have indicated that VEGF is one of the most important candidate genes in association with endometriosis, and that VEGF genetic polymorphisms might be associated with an increased risk of endometriosis [8, 16-20]. On the other hand, a very recent meta-analysis aiming for a better understanding of the effects of VEGF +405G>C genetic polymorphism on the risk of endometriosis has concluded that VEGF +405G>C genetic polymorphism is not significant associated with the risk of endometriosis [21]. Following the same idea, a meta-analysis [8] concluded that VEGF + 936T/C polymorphism is capable of causing endometriosis susceptibility and there was no significant association between the -460C/T and + 405G/C polymorphisms and risk of endometriosis. Thus, the findings from the published articles are somewhat conflicting, and at the same time are not conclusive.

In the present study, the authors aimed to describe the frequency of alleles, genotypes, and haplotypes of VEGF +405 C/G and -460 C/T genetic polymorphisms, to evaluate their relationship with VEGF serum levels, and to investigate their impact on endometriosis susceptibility.

Materials and Methods

The author conducted a case-control study in which they included 160 patients divided into two groups, as follows: group 1 (endometriosis group) – 80 women with regular menses, with no history of pelvic infections, autoimmune and neoplastic diseases, and undergoing laparoscopy or laparotomy for suspected endometriosis. Histopathological examination established the endometriosis diagnosis for all included patients. The severity of endometriosis was staged according to the revised American Society for Reproductive Medicine (rASRM) classification; all included patients were staged III or IV according to rASRM. Group 2 (control group) – 80 healthy non-pregnant women aged between 18-45-years-old, without clinical and paraclinical evidence of endometriosis, undergoing laparoscopy for unexplained infertility or tubal ligation. Exclusion criteria: patients with previous pelvic surgeries, history of cancer, suspected malignancy, adenomyosis or leiomyoma, pre-surgical suspicion of evidence of premature ovarian failure, or the use of ovarian suppressive drug, such as oral contraceptives, GnRH agonists, progestins or danazol in the preceding six months were excluded from the study. None of the patients had taken anti-inflammatory medications or had been diagnosed with an inflammatory or infectious condition for at least six months before the study.

The study design was approved by the Local Ethics Committee, and signed informed consent was received from each woman before sample collection. The study was conducted under the tenets of Helsinki Declaration. Ten ml of venous blood was collected from each patient *à jeun*, which was centrifuged and the serum obtained was stored at -70ºC for future determinations. Five patients were excluded from the endometriosis group, because not enough blood was harvested at inclusion in the study.

The authors used multiplex cytokine kits in order to measure VEGF serum levels. Measurements were performed in accordance with the manufacturer's specifications. The sensitivity of the test was specified by the manufacturer. The average sensitivity of the test was < 0.5 pg/mL, with an inter-assay variation coefficient of 7.8%.

Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis for the −460 C/T polymorphism and for the +405 C/G polymorphism. The PCR primers were forward 5′-TGTGCGT-GTGGGGTTGAGCG-3′ and reverse 5′-TACGTGCG-GACAGGGCCTGA-3′ for the −460 C/T and forward 5′-TTGCTT-GCCATTCCCCACTTGA-3′ and reverse 5′-CC-GAAGCGA-GAACAGCCCAGAA-3′ for the +405 C/G polymorphism. The PCR products were digested with a restriction enzyme and migrated on agarose gel by electrophoresis and stained with ethidium bromide in order to obtain UV fluorescence [22].

Data collection was performed in Excel. Statistical analysis was done using SPSS version 23 software and CDC Epi info version 7. All statistical tests assumed a 0.05 significance level. Categorical data distribution was compared with Chi-square test (uncorrected and with the Mantel-Haenszel correction) and with the Fisher-exact test. Normal distribution was verified with Shapiro-Wilk test and because the probability obtained was above 0.05 quantitative variables were compared with the Kruskal-Wallis test or the Mann-Whitney U test.

Results

Genotyping by RFLP of the VEGF *+405 C/G* and *-460 CT* polymorphisms was successfully achieved for all subjects. All observed genotypes frequencies in both endometriosis and control groups were in Hardy-Weinberg equilibrium, the Chi square parameter was less than 3.84 for all distributions (Table 1).

In the case of +405 C/G polymorphism, the distribution of genotypes and of allele frequencies in the endometriosis group was similar to control group and no significant odds-ratio was calculated.

As for the relationship between VEGF serum levels and genotypes, because the data were not normally distributed, the authors performed a Kruskal-Wallis test, which showed no statistical significance in the VEGF serum levels between genotypes ($p = 0.932$) (Table 2). Regarding VEGF serum levels and the presence of a different allele, the non-parametric Mann-Whitney test for abnormal distributed data showed no statistically significant difference ($p = 0.709$) (Table 3).

For the second studied polymorphism, -460 C/T, the distribution of genotypes and of allele frequencies in the endometriosis group compared to controls, did not show any significant presence of the mutant allele in relationship with the endometriosis. Table 4 presents the statistical data, revealing an estimated risk of developing endometriosis with a confidence interval overlapping 1.

Investigating the relationship between VEGF serum levels and genotypes, or alleles, the Kruskal-Wallis test showed no significant difference for VEGF serum levels in relationship with -460C/T genotype ($p = 0.996$), and no sta-

Table 1. — *The Hardy-Weinberg equilibrium for the observed genotypes frequencies.*

	Endometriosis group			Control group		
Genotype	**Observed (O)**	**Expected (E)**	**(O-E)^2/E**	**Observed (O)**	**Expected (E)**	**(O-E)^2/E**
-405 CC	16	12.02	0.66	16	12.02	0.20
-405 CG	30	37.98	0.84	30	37.98	0.10
-405 GG	34	30.02	0.26	34	30.02	0.01
		Chi square	**1.76**		**Chi square**	**0.31**
	Observed (O)	**Expected (E)**	**(O-E)^2/E**	**Observed (O)**	**Expected (E)**	**(O-E)^2/E**
-460 CC	12	11.26	0.03	20	18.06	0.02
-460 CT	36	37.50	0.03	36	39.90	0.13
-460 TT	32	31.26	0.01	24	22.06	0.20
		Chi square	**0.06**		**Chi square**	**0.36**

Table 2. — *Distribution of genotypes and allele frequencies of the VEGF +405 C/G polymorphism in the studied groups.*

	Genotype				Allele		
Group (n)	**-405 CC**	**-405 CG**	**-405 GG**	**Total**	**C allele frequency**	**G allele frequency**	**Total**
E (80)	16 (20,0%)	30 (37,50%)	34 (42,50%)	80 (100,0%)	62 (38,75%)	98 (61,25%)	160 (100,0%)
C (80)	16 (20,0%)	30 (37,50%)	34 (42,50%)	80 (100,0%)	62 (38,75%)	98 (61,25%)	160 (100,0%)
Total	32 (20,0%)	60 (37,50%)	68 (42,50%)	160 (00,0%)	124 (38,75%)	196 (61,25%)	320 (100,0%)

	Odds ratio			Statistics		
	Estimate	**Lower**	**Upper**	**Uncorrected Chi-square**	**Mantel-Haenszel**	**Fisher Exact**
CC + CG vs GG	1.000	0.412	2.427	1.000	1.000	1.000
CC vs CG + GG	1.000	0.334	2.991	1.000	1.000	1.000
CC vs CG	1.000	0.297	3.365	1.000	1.000	1.000
CC vs GG	1.000	0.308	3.282	1.000	1.000	1.000
CG vs GG	1.000	0.375	2.670	1.000	1.000	1.000
C vs G	1.000	0.529	1.890	1.000	1.000	1.000

E: Endometriosis group; C: control group.

tistical significance in the VEGF serum levels in relation with the presence of a different allele, C or T ($p = 0.931$), (Table 5).

Discussions

Endometriosis is a complex gynecological condition characterized by the presence of endometrial tissue outside the uterus. The pathogenesis and the molecular mechanisms that are involved in the development and progression of endometriosis have troubled investigators through many years, being thoroughly investigated, but still many gaps are impairing the development of efficient therapies [23].

Different theories on the endometriosis pathogenesis involve growth factors and pro-inflammatory or anti-inflammatory cytokines associated with dysregulation in cell multiplication and angiogenesis. Different studies have stated that immunological abnormalities are associated with the presence and development of endometriosis. In addition, it is considered that genic polymorphisms could affect the serum levels of cytokines by influencing transcriptional regulation. The role of single-nucleotide polymorphisms (SNPs) in some immunological disorders has been previously reported [24-26], and some exact genetic polymorphisms have been identified in relationship with endometriosis. In the myriad of studied endometriosis associated genetic polymorphisms, a large body of evidence suggests that VEGF is a promising candidate gene for mediating the genetic influence on the risk of endometriosis [20, 27-30]. Furthermore, VEGF genetic polymorphisms have been indicated to have a key effect on VEGF gene expression regulation which will determine al-

Table 3. — *VEGF serum levels in relationship with +405 C/G genetic polymorphism.*

	Endometriosis group	Control group		Global
+405 C/G	Mean (SD)	Mean (SD)	**Mann-Whitney U test**	Mean (SD)
CC	1.811 (0.060)	1.904 (0.116)	0.065**	1.858 (0.101)
CG	1.805 (0.070)	1.904 (0.154)	0.074**	1.855 (0.128)
GG	1.776 (0.060)	1.951 (0.152)	0.000*	1.864 (0.144)
Alleles				
C	1.808 (0.063)	1.904 (0.132)	0.002*	1.856 (0.113)
G	1.785 (0.063)	1.937 (0.151)	0.000*	1.861 (0.138)

*SD: standard deviation; *significant test; ** tendency to obtain test significance*

Table 4. — *Distribution of genotypes and allele frequencies of the VEGF -460 C/T polymorphism in the studied groups.*

	Genotype				Allele		
Group (n)	**-460 CC**	**-460 CT**	**-460 TT**	**Total**	**C allele frequency**	**T allele frequency**	**Total**
E (80)	12 (15,0%)	36 (45,0%)	32 (40,0%)	80 (100,0%)	60 (37,50%)	100 (62,50%)	160 (100,0%)
C (80)	20 (25,0%)	36 (45,0%)	24 (30,0%)	80 (100,0%)	76 (47,50%)	84 (52,50%)	160 (100,0%)
Total	32 (20,0%)	72 (45,0%)	56 (35,0%)	160 (100,0%)	136 (42,50%)	184 (57,50%)	320 (100,0%)

	Odds ratio			Statistics		
	Estimate	**Lower**	**Upper**	**Uncorrected Chi-square**	**Mantel-Haenszel**	**Fisher Exact**
CC + CT vs TT	0.643	0.255	1.623	0.348	0.351	0.482
CC vs CT + TT	0.529	0.172	1.631	0.264	0.267	0.402
CC vs CT	0.600	0.180	2.001	0.404	0.409	0.549
CC vs TT	0.450	0.128	1.585	0.210	0.215	0.347
CT vs TT	1.200	0.414	3.476	0.737	0.739	0.791
C vs T	0.663	0.353	1.246	0.201	0.202	0.263

E: endometriosis group; C: control group.

Table 5. — *VEGF serum levels in relationship with -460C/T genetic polymorphism.*

	Endometriosis group	Control group		Global
- 460C/T	Mean (SD)	Mean (SD)	Mann-Whitney test	Mean (SD)
CC	1,794 (0,064)	1,924 (0,145)	0.031*	1,859 (0,129)
CT	1,783 (0,067)	1,897 (0,119)	0.001*	1,854 (0,115)
TT	1,786 (0,065)	1,948 (0,155)	0.090**	1,867 (0,143)
Alleles				
C	1.785 (0.064)	1.926 (0,152)	0.000*	1.858 (0.129)
T	1.800 (0.064)	1.924 (0.144)	0.000*	1.859 (0.129)

*SD: standard deviation; *significant test; ** tendency to obtain test significance*

tered levels of VEGF, and may contribute to the pathogenesis of endometriosis [8, 31].

In the current study the authors investigated a possible association between two VEGF SNPs, +405 C/G and -460 C/T, and advanced endometriosis, and if VEGF serum levels are modified in relationship with the presence of SNPs.

In a previous study, the present authors have shown that VEGF serum levels are significantly lower in endometriosis patients compared to healthy controls [13]. The results the current study showed regarding VEGF +405C/G and -460C/T genetic polymorphisms did not find any association between advanced endometriosis and the presence of the studied SNPs. In addition, there was no statistical significance between VEGF serum levels in relationship with the presence of a different allele and genotypes, thus showing that VEGF +405C/G and -460C/T genetic polymorphisms are probably not involved in the susceptibility to endometriosis.

VEGF, a major angiogenic factor, is considered to play an important role in the development of endometriosis. Several studies performed in past years, have focused particularly on VEGF genetic polymorphism involvement in endometriosis due to the fact that VEGF polymorphisms may affect the process of angiogenesis, and it is considered that angiogenesis represents a critical step in the establishment and pathogenesis of endometriosis. Most of these studies have analyzed a series of VEGF SNPs like, +405 C/G, -2578C/A, -460T/C, -1154G/A, or +936C/T. The main problem is that, the results from those published observations were controversial or ambiguous. Thus, a very recent study regarding the association of three VEGF (−460 C/T, +405 G/C, and +936 C/T) polymorphisms with the risk of endometriosis in the Tunisian population has shown that patients with stages III-IV endometriosis had higher VEGF + 936T allele frequency than controls, but the distribution of genotypes and allele frequencies of the VEGF −460 C/T and +405 G/C polymorphisms did not differ significantly between endometriosis patients and controls [32]. At the same time, Kim *et al.* demonstrated that the VEGF +405G/C genetic polymorphism may be associated with the risk of advanced stage endometriosis in a Korean population [22], and Bhanoori *et al.* suggested that the VEGF -460T/+405C haplotype was significantly less common in women with endometriosis than in controls [33]. Furthermore, very recent genetic studies have provided evidence that VEGF +405G>C genetic polymorphism is not significantly associated with the risk of endometriosis [21], and that there is a positive association between VEGF -1154G/A and the risk of developing endometriosis with no significant differences in allele or genotype distributions of the -2578C/A, -460T/C, +405G/C, and +936C/T polymorphisms between endometriosis cases and controls. The present results are in line with previous studies, as the present authors have provided more evidence that genetic polymorphism in VEGF +405C/G and -460C/T are not involved in advanced cases of endometriosis.

The present study has some obvious limitations such as the lack of differentiation between patients with ovarian endometrioma (OE) and patients with deep infiltrating endometriosis (DIE); all included patients were diagnosed with OE with or without associated DIE, and not only DIE patients. Another possible limitation could be the fact that all included patients were of Caucasian origin; one could say that a genetic association, although valid for a specific ethnic population, may not be relevant to individuals of another ethnicity. Lastly, sample size is an important limitation, although the authors had included a fair number of cases and controls, large-scale case-control studies are needed for a more precise estimation regarding a single SNP involvement in the pathogenesis of one disease.

In conclusion, the present research shows that VEGF +405 C/G and -460 C/T genetic polymorphism are not associated with advanced endometriosis. Moreover, VEGF serum levels did not correlate significantly in relationship with the presence of a different allele and/or genotypes, thus suggesting no involvement for this polymorphism in the pathogenesis of endometriosis. Thus this study, confirms with the previous research which has showed that these genetic variants have no implication in the susceptibility to endometriosis. On the other hand, taking in consideration the low number of investigated polymorphisms, small sample size, the single ethnicity, and the case-control design of the study, larger-scaled and well-designed studies are needed to elucidate a more precise role for VEGF genetic polymorphism in endometriosis susceptibility, development, and progression.

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References

[1] Burney R.O., Giudice L.C.: "Pathogenesis and pathophysiology of endometriosis". *Fertil. Steril*., 2012, *98*, 511.
[2] Meuleman C., Vandenabeele B., Fieuws S.: "High prevalence of endometriosis in infertile women with normal ovulation and normospermic partners". *Fertil. Steril*., 2009, *92*, 68.
[3] Othman Eel D., Hornung D., Salem H.T., Khalifa E.A., El-Metwally T.H., Al-Hendy A.: "Serum cytokines as biomarkers for nonsurgical prediction of endometriosis". *Eur. J. Obstet. Gynecol. Reprod. Biol.,* 2008, *137*, 240.
[4] Sampson JA.: "Peritoneal endometriosis due to menstrual dissemination of endometrial tissue into the peritoneal cavity". *Am. J. Obstet. Gynecol.*, 1927, *14*, 422.
[5] Gebel H.M., Braun D.P., Tambur A., Frame D., Rana N., Dmowski D.P.: "Spontaneous apoptosis of endometrial tissue is impaired in women with endometriosis". *Fertil. Steril.,* 1998, *9*, 1042.
[6] Bischoff F.Z., Simpson J.L.: "Heritability and molecular genetic studies of endometriosis". *Hum. Reprod. Update.,* 2000, *6*, 37.
[7] Perini J.A., Cardoso J.V., Berardo P.T., Vianna-Jorge R., Nasciutti L.E., Bellodi-Privato M., *et al.*: "Role of vascular endothelial growth factor polymorphisms (-2578C>A, -460 T>C, -1154G>A, +405G>C and +936C>T) in endometriosis: a case-control study with Brazilians". *BMC Womens Health,* 2014, *14*, 117.
[8] Xu S., Wu W., Sun H., Lu J., Yuan B., Xia Y., *et al.*: "Association of the vascular endothelial growth factor gene polymorphisms (-460C/T, +405G/C and +936T/C) with endometriosis: a meta-analy-

sis". *Ann. Hum. Genet.,* 2012, *76*, 464.
[9] Wang H., Gorpudolo N., Li Y., Feng D., Wang Z., Zhang Y.: "Elevated vascular endothelia growth factor-A in the serum and peritoneal fluid of patients with endometriosis". *J. Huazhong. Univ. Sci. Technolog. Med. Sci*., 2009, *29*, 637.
[10] García-Manero M., Alcazar J.L., Toledo G.: "Vascular endothelial growth factor (VEGF) and ovarian endometriosis: correlation between VEGF serum levels, VEGF cellular expression, and pelvic pain". *Fertil. Steril.,* 2007, *88*, 513.
[11] Dziunycz P., Milewski Ł., Radomski D., Barcz E., Kamiński P., Roszkowski P.I., *et al.*: "Elevated ghrelin levels in the peritoneal fluid of patients with endometriosis: associations with vascular endothelial growth factor (VEGF) and inflammatory cytokines". *Fertil. Steril*., 2009, *92*, 1844.
[12] Gagné D., Pagé M., Robitaille G., Hugo P., Gosselin D.: Levels of vascular endothelial growth factor (VEGF) in serum of patients with endometriosis. *Hum Reprod.,* 2003, 18, 1674-1680.
[13] Malutan A., Drugan T., Georgescu C., Ciortea R., Bucuri C., Bobric A., *et al.*: "Vascular Endothelial Growth Factor Serum Levels in Women with Advanced Endometriosis". *Acta. Endo. (Buc).*, 2016, *12*, 7.
[14] Pupo-Nogueira A., de Oliveira R.M., Petta C.A., Podgaec S., Dias J.A. Jr., Abrao M.S.: "Vascular endothelial growth factor concentrations in the serum and peritoneal fluid of women with endometriosis". *Int. J. Gynaecol. Obstet.,* 2007, *99*, 33.
[15] Vodolazkaia A., Yesilyurt B.T., Kyama C.M., Bokor A., Schols D., Huskens D., *et al.*: "Vascular endothelial growth factor pathway in endometriosis: genetic variants and plasma biomarkers". *Fertil. Steril.,* 2016, *105*, 988.
[16] Gentilini D., Somigliana E., Vigano P., Vignali M., Busacca. M, Di Blasio A.M.: "The vascular endothelial growth factor +405G>C polymorphism in endometriosis". *Hum. Reprod.*, 2008, *23*, 211.
[17] Emamifar B., Salehi Z., Mehrafza M., Mashayekhi F.: "The vascular endothelial growth factor (VEGF) polymorphisms and the risk of endometriosis in northern Iran". *Gynecol. Endocrinol*., 2012, *28*, 447.
[18] Liang S., Huang Y., Fan Y.: "Vascular endothelial growth factor gene polymorphisms and endometriosis risk: a meta-analysis". *Arch. Gynecol. Obstet.*, 2012, *286*, 139.
[19] Rotman C., Fischel L., Cortez G., Greiss H., Rana N., Rinehart J., et al.: "A search to identify genetic risk factors for endometriosis". *Am. J. Reprod. Immunol.*, 2013, *69*, 92.
[20] Tan S., Li Y., Li S.: "Methodological remarks concerning the recent metanalysis on vascular endothelial growth factor polymorphism and endometriosis risk". *Arch. Gynecol. Obstet.,* 2013, *287*, 167.
[21] Fang F., Gong L., Wang X., Zhang L.: "The association between vascular endothelial growth factor (VEGF) +405G>C genetic polymorphism and endometriosis". *Exp. Biol. Med. (Maywood)*, 2015, *240*, 1177-1182.
[22] Kim S.H., Choi Y.M., Choung S.H., Jun J.K., Kim J.G., Moon S.Y.: "Vascular endothelial growth factor gene +405 C/G polymorphism is associated with susceptibility to advanced stage endometriosis". *Hum. Reprod.,* 2005, *20*, 2904.
[23] Trovó de Marqui AB.: "Genetic polymorphisms and endometriosis: contribution of genes that regulate vascular function and tissue remodeling". *Rev. Assoc. Med. Bras.*, 2012, 58, 620-632.
[24] Amirzargar A.A., Bagheri M., Ghavamzadeh A., Alimoghadam K., Khosravi F., Rezaei. N, *et al.*: "Cytokine gene polymorphism in Iranian patients with chronic myelogenous leukaemia". *Int. J. Immunogenet.,* 2005, *32*, 167.
[25] Amirzargar A.A., Rezaei N., Jabbari H., Danesh A.A., Khosravi F., Hajabdolbaghi M., *et al.*: "Cytokine single nucleotide polymorphisms in Iranian patients with pulmonary tuberculosis". *Eur. Cytokine. Netw*., 2006, *17*, 84.
[26] Rezaei N., Amirzargar A.A., Shakiba Y., Mahmoudi M., Moradi B., Aghamohammadi A.: "Proinflammatory cytokine gene single nucleotide polymorphisms in common variable immunodeficiency". *Clin. Exp. Immunol.,* 2009, *155*, 21.
[27] Saliminejad K., Memariani T., Ardekani A.M., Kamali K., Edalatkhah H., Pahlevanzadeh Z.: "Association study of the TNF-alpha -1031T/C and VEGF +450G/C polymorphisms with susceptibility to endometriosis". *Gynecol. Endocrinol.*, 2013, *29*, 9747.
[28] Toktam M., Kioomars S.N., Kourosh K., Adel S., Behrokh M.M., Mohhamad Mehdi A. *et al.*: "Association of vascular endothelial growth factor (VEGF) +405 g>c polymorphism with endometriosis in an Iranian population". *J. Reprod. Infertil*., 2010, *11*, 33.
[29] Ikuhashi Y., Yoshida S., Kennedy S., Zondervan K., Takemura N., Deguchi M., et al.: "Vascular endothelial growth factor +936 C/T polymorphism is associated with an increased risk of endometriosis in a Japanese population". *Acta. Obstet. Gynecol. Scand*., 2007, *86*, 1352.
[30] Attar R., Agachan B., Kuran S.B., Toptas B., Eraltan I.Y., Attar E., et al.: Genetic variants of vascular endothelial growth factor and risk for the development of endometriosis. *In Vivo*, 2010, 24, 297-301.
[31] Cosin R., Gilabert-Estelles J., Ramon L.A., Espana F., Gilabert J., Romeu A., et al.: "Vascular endothelial growth factor polymorphisms (-460C/T, +405G/C, and 936C/T) and endometriosis: their influence on vascular endothelial growth factor expression". *Fertil. Steril*., 2009, 92, 1214–1220.
[32] Henidi B., Kaabachi W., Naouali A., Kaabachi S., Zhioua A., Haj Sassi F., *et al.*: Vascular endothelial growth factor (-460 C/T, +405 G/C, and +936 C/T) polymorphisms and endometriosis risk in Tunisian population. *Syst. Biol. Reprod. Med.,* 2015, *61*, 238.
[33] Bhanoori M., Arvind Babu K., Pavankumar Reddy N.G., Lakshmi Rao K., Zondervan K., Deenadayal M. et al.: "The vascular endothelial growth factor (VEGF) +405G>C 5'-untranslated region polymorphism and increased risk of endometriosis in South Indian women: a case control study". *Hum. Reprod.* 2005, *20*, 1844.

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