Journal of Tissues and Materials 2018 1(1): 25-31

Journal homepage: www.jourtm.com

Association Between Single Nucleotide Polymorphism rs113488022 of BRAF and Endometriosis in Iranian Population

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Received: 01 June 2018, Accepted: 14 July 2018

Abstract

Endometriosis is a benign gynecologic disorder that has malignant-like characteristics such as invasion, and it can cause malignancy and cancer in advanced stages. Genetic, epigenetic and environmental factors play significant roles in the establishment and maintenance of endometriosis. Studies have demonstrated that proto-oncogenes may be an important regulator of cell proliferation in endometriotic tissue. B-Raf is a proto-oncogene, which participates in the MAP kinase/ERK signaling pathway and plays an important role in different types of cancers and disorders. The aim of the present study was to evaluate the association of BRAF gene single nucleotide polymorphism (SNP), rs113488022, with the risk of endometriosis in Iranian women, in order to identify a potential biomarker for the early non-invasive detection of endometriosis and endometriosis-related cancers.

In a population based case-control study conducted in Tehran, in-person interviews were completed for 65 women aged 15– 49 years and an equal number of controls frequency-matched to cases by age. All cases were newly diagnosed with endometriosis and all controls were with no laparoscopic evidence of disease. The genomic DNA was extracted from peripheral blood leucocytes and subsequently the rs113488022 SNP of the BRAF gene was genotyped using amplification refractory mutation system- polymerase chain reaction (ARMS-PCR). Statistical analysis was performed using SPSS. In comparison of case and control groups, no significant differences were found in genotype and allele frequencies. Moreover, the results showed that there

doi: 10.22034/jtm.2018.69395

was no significant association between the presence of risk allele (A) and increased risk of endometriosis (Odds ratio=0.781, 95% CI: 0.480 -1.272, P=0.321).

In conclusion, there was no evidence of an association between the rs113488022 SNP of the BRAF gene, and overall risk of endometriosis in Iranian population. Larger studies with different ethnics are needed to validate our findings.

Keywords: Endometriosis, B-Raf proto-oncogene, single nucleotide polymorphism, Iranian women

1. Introduction

Endometriosis is an estrogen-dependent chronic gynecological disease and is known as one of the main causes of infertility in women (1). The main symptoms include dysmenorrhea, rectal pain on defecation, pelvic masses, chronic pelvic pain. Endometriosis may be described as a typical endometrial tissue growing outside of uterus cavity, especially in fallopian tubes, ovaries or rectovaginal septum (2,3). Endometriosis is a benign disease that it has malignant-like characteristics such as invasion and metastasis (3). A number of studies have revealed that invasion, angiogenesis, apoptosis and cell adhesion in ectopic endometrium of women with endometriosis have differences compared with normal endometrium of women without endometriosis (4-6).

Several genetic studies have found evidence of association between genetic polymorphisms and endometriosis. At least 19 single-nucleotide polymorphisms (SNPs) associated with endometriosis have been identified by genome-wide association studies to date (5, 9).

Many genes have been suggested as candidate genes in the pathogenesis of endometriosis, including genes involved in inflammation, cell cycle regulation, growth factors, hormone receptors and adhesion molecules (1). However, a portion of these data have inconsistent results due to the lack of replication in independent populations.

As regards women with endometriosis are more susceptible to gynecologic malignancies such as ovarian and endometrial cancer (3, 10), it seems that investigating mutations in proto-oncogenes that are frequently mutated in endometrial and ovarian cancers provide new insights into possible pathways leading to endometriosis, and also can be useful for early diagnosis endometriosis-related cancers.

Previous findings have suggested that oncogenes such as BRAF may be used as potential biomarkers for the diagnosis of endometriosis and understanding the pathogenesis of the disease (11,12). The BRAF gene is involved in the MAPK/ERK signaling cascade as well as plays an important role in causing normal cells to cancerous. The MAPK/ERK signaling pathway regulates important cell functions including cellular growth, differentiation, proliferation, and apoptosis (13). BRAF mutations are found in various types of cancer. The mutation of valine 600 to glutamic acid (V600E) in exon 15 is the most common mutation (14, 15).

As regards endometriosis is associated with increased risk of women's cancers and BRAF is used as a biomarker for a prognosis and target therapy in cancers, we conducted the current study to investigate whether rs113488022 of *BRAF* gene is associated

with the risk of endometriosis in the Iranian population in order to facilitate the early detection and diagnosis of endometriosis and endometriosis-related malignancy.

2. Materials and Method

2.1. Data collection

The current case-control study was conducted on 65 women (15–49 years old) with endometriosis who were undergoing total hysterectomy selected among visitors of Mirza Kuchak Khan (MohebYas) Women's General Hospital. The control group including 65 healthy with a normal pregnancy history and had no previous medical record of chronic pelvic pain, dysmenorrhea, or dyspareunia. The exclusion criteria for the present study were: A history of leiomyoma, fibroid, pelvic inflammatory diseases, unsuccessful pregnancies such as abortion or ectopic pregnancy and endometrial and ovarian cancer. Also, patients who had any other types of cancer were not eligible for inclusion in the study. Participants had to complete the same self-reported medical history form and the same study questionnaire. Moreover, two groups were homogenized based on age, ethnicity and physical conditions. The study protocol was approved by the Ethics Committee of Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Informed consents were obtained from all participants.

2.2. Blood sample collection

Whole blood samples from the patients with endometriosis were collected before treatment. The peripheral blood samples of both cases and controls were collected in ethylene diaminetetra acetic acid (EDTA)-containing tubes, then centrifuged at (3000 rpm, 10 min, +4°C) and finally, the buffy coat layer was separated and transferred to a new tube for isolation of nucleated cells. All tubes stored at -20 °C until DNA extraction and analysis.

2.3. DNA extraction from peripheral blood leucocytes

DNA was isolated from peripheral blood leukocytes by using the Proteinase K method. After DNA extraction, the DNA pellet was washed with 70% ethanol, the final DNA pellets were air dried, and then dissolved in 30µl DEPC-water (diethylpyrocarbonate) and stored at −20 °C. Also, DNA concentration and purity was evaluated with spectrophotometry (NanoDrop 2000 machine, Thermo Fisher Scientific, USA) and electrophoresis on agarose gel.

2.4. BRAF Genotyping by ARMS-PCR

Genotyping of the samples for rs113488022 was performed by the Amplification Refractory Mutation System (ARMS)-PCR technique using designed primers that the primer sequences are shown in (Table 1). This is a single assay that identifies the BRAF c.1799T>A trans version in exon 15 of the BRAF proto-oncogene resulting in an amino acid change from valine to glutamic acid in codon 600 of BRAF protein (p. V600E) (16). The reaction mixture was carried out in the total volume of 20µL using ready to use Ampliqon 2X-PCR master mix. The amplification was done using PCR program with initial heating for 5 min at 95˚C, followed by 35 cycles of denaturing at 94°C for 50 sec, annealing for 40 sec at 60˚C, and chain extension at 72˚C 50 sec with a final elongation for 5 min at 72˚C. In order to ensure accuracy of the results, all clinical samples were tested in duplicate. The PCR products were loaded on 2% agarose gel supplemented with Gel Red in reference to a molecular weight marker (100 bp DNA ladder) for DNA product visualization under UV light.

2.5. Statistical analysis

Allelic and genotypic associations were evaluated by the Chi-square test using the SPSS statistical software package, version 16 (SPSS Inc., Chicago, IL, USA).

3. Results

Our subjects were 130 women aged 15–49 years old (average 31.6 ± 7.3 and 31.8 ± 7.5 for patient and control groups, respectively, P = 0.91). ARMS-PCR results demonstrated that heterozygote genotype "AT" has the highest frequency (83.1 %) in case group (Table 2). On the other hand, in control group the highest genotype frequency belonged to AT (86.2 %). The results revealed that there were no significant differences between case and control groups in terms of genotype frequency (p =0.116).

In this study, allele "T" had the highest frequency (50.8 %) in case group, meanwhile; allele "A" had the highest frequency (56.9 %) in control (Figure 1). The results also showed that the allele frequency had no significant differences between case and control groups (p =0.214).

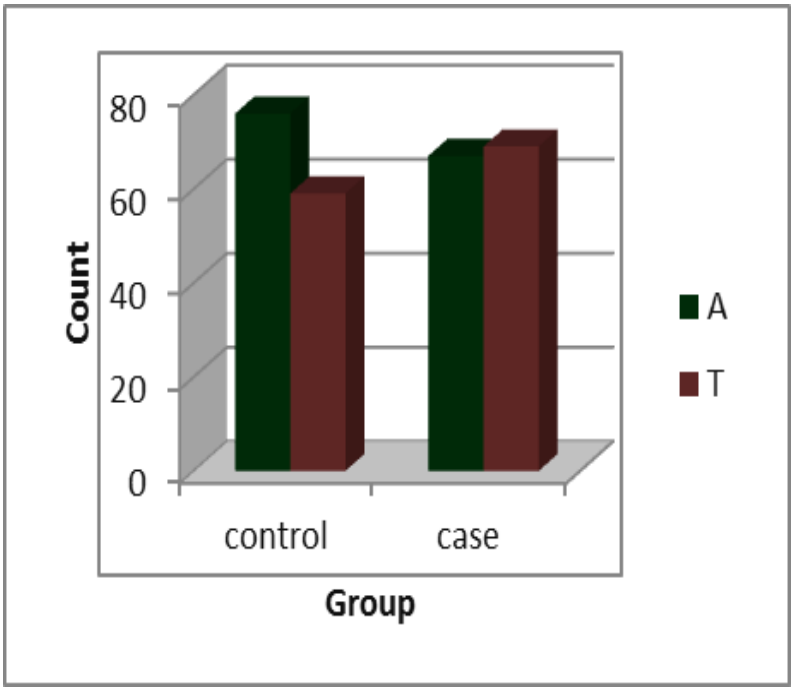

Figure 1. allele frequency in control and case groups

Table 1. Designed primers

Primers	Tm (°C)	Sequence (5' → 3')	Product length (bp)
Common	61.6	TCACCTCATCCTATCACATTTCAAG	
Normal	57.7	AGGTGATTTTGGTCTAGCTACTGT	320
Mutant	59.5	GACCCACTCCATCGAGATTACT	153

The findings showed that heterozygous AT genotype had no effect on increased risk of endometriosis compared to wild type genotype (homozygote TT) (odds ratio: 0.648, 95% CI:0.200-2.106, p =0.468). Also the results obtained in this study indicated that the presence of mutant allele "A" compared with wide allele "T" had no role in increased susceptibility to endometriosis in Iranian population (Odds ratio: 0.781, 95% CI:0.480 -1.272, p =0.321) (Table3).

Table 2. The frequency of different genotypes in case and control groups

			Genotype			Total
			AA	AT	TT	
Group	Control	Count % within group	8 12.3%	56 86.2%	1 1.5%	65 100.0%
	Case	Count % within group	5 7.7%	54 83.1%	6 9.2%	65 100.0%
Total		Count % within group	13 10.0%	110 84.6%	7 5.4%	130 100.0%

Table 3. Risk estimate based on genotype and allele frequencies between case and control groups

		Case	Control	Total	P-value	OR(95%C.I)
Genotypes	AA	5 (7.7%)	8(12.3%)	13	0.043	0.104(0.010 - 1.141)
	AT	54 (83.1%)	56 (86.2%)	110	0.468	0.648(0.200 - 2.106)
	TT	6 (9.2%)	1 (1.5%)	7		1.00(Reference)
Alleles	A	64 (49.9%)	72(56.9%)	136	0.321	0.781(0.480-1.272)
	T	66 (50.8%)	58(43.1%)	124		1.00(Reference)

OR, odd ratio; C.I, confidence interval

4. Discussion

Recent advancement in genetic and new avenues of research resulted in the identification genetic risk factors in endometriosis and possible mechanisms of action. In the present study, we genotyped 65 women with endometriosis and 65 healthy controls for the presence of SNP rs113488022 (Val600Glu). To our knowledge, this is the first report about the risk of endometriosis and this SNP of BRAF gene in Iranian women. The BRAF gene is located on chromosome 7 (7q34) (13). Mutations in BRAF play important roles in the development of malignant tumors (17). Furthermore, studies have shown that the most common BRAF mutation is c.1799T>A in exon 15 (p.Val600Glu) and this mutation

have been identified in some types of cancer such as ovarian cancer(18, 19).

Overexpression of oncogenic BRAF promotes the activation of the RAS- BRAF-MAPK signaling pathway which in turn can result in different types of disorders and cancers (20, 21). As regards the endometriosis increases the risk of female cancers such as ovarian cancer (3,22) analysis of SNPs in BRAF gene in women with endometriosis can influence on ovarian and endometrial cancers diagnosis and prognosis as a molecular biomarker. In this regard, some studies have confirmed the relationship between BRAF mRNA/protein expression and endometriosis.

(23). We found no evidence of an association between Susceptibility to Endometriosis and rs113488022. This is consistent with results of studies conducted by Xiao Lv et al. (2018) and Vestergaard AL et al. (2011) that no mutation in BRAF exon 15 was identified in eutopic endometrium samples of patients with endometriosis. Meanwhile, analysis of BRAF expression showed a relationship between endometriosis and high levels of mRNA and protein expression (12, 24). In addition, Previous studies reported BRAF V600E mutation in ovarian carcinoma cell lines ES-2, and endometrial cancer (19). Accordingly, these data suggest that BRAF gene may play a role in endometriosis and the Progression to endometriosis associated cancers.

This study has some limitations that should be considered when interpreting the results. First, it was carried out in only one geographical area (Tehran), although some of subjects came from other cities. Second, our limited sample size was not sufficiently powered to detect association between BRAF SNP rs113488022 and risk of endometriosis.

4. Conclusion

Diagnosis of endometriosis is often delayed due to lack of non-invasive biomarkers for diagnosis of endometriosis. The evaluation of peripheral blood DNA as a noninvasive method for identification of biomarkers can be useful to early detection and diagnosis of endometriosis and also for the prognosis of the risk of endometriosis-related cancers. This study revealed no association between rs113488022 and endometriosis among Iranian women. However, further extensive researches with a large number of samples from different populations and ethnicities are needed to validate the results obtained in this study.

Acknowledgment

We gratefully acknowledge Parse Lab for preparing facilities and equipment for this thesis.

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