International Journal of Medical Laboratory 2016;3(3):185-190.

International Journal of Medical Laboratory
Shahid Sadoughi University of Medical Sciences

Original Article

Frequency of Polymorphism Alu Insertion in Progesterone Receptor Gene in Endometriosis

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Article history
Received 10 Apr 2016
Accepted 29 Aug 2016
Available online 29 Oct 2016

Key words
Endometriosis
Polymerase chain reaction
Progesterone receptor gene

A B S T R A C T

Background and Aims: This research aimed to study a possible link between endometriosis and polymorphism of the progesterone receptor gene.

Materials and Methods: The control group consisted of 86 women without endometriosis and the case group comprised 86 patients with a diagnosis of endometriosis by laparoscopy. Genotypes for Alu insertion polymorphisms (A1/A1, A1/A2 and A2/A2) were described by polymerase chain reaction and determined on a 2% agarousegel.

Results: The genotype frequencies of A1/A2 and A2/A2 were not significantly higher in patients than in the control group without endometriosis. On the other hand differences in the Alu insertion polymorphism frequencies were not significant.

Conclusion: According to our investigations, we conclude that there is not a significant correlation between Alu insertion polymorphism and endometriosis.

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Introduction

Endometriosis is a common gynecological disease, which is defined as a development of endometrial tissue which is outside the uterine cavity [1]. It causes some problems such as dysmenorrheal, pelvic pain, dyspareunia, and infertility [2, 3]. Endometriosis has a significant negative influence on women life, and it has a long-term major health issue [4]. Endometriosis could affect up to 10% of women in reproductive age [5], in postmenopausal [6], women with infertility [7, 8] and with a history of endometriosis or ovarian cancer [9]. Progesterone is a potent antagonist of estrogen. Endometriosis and ovarian cancer could be stimulated by estrogens and inhabited by progesterone [10]. Endometriosis occurs by genetic and environmental factors,which determine disease phenotype. Some genes that may be related to endometriosis onset and progression were studied previously [11]. Progesterone receptor (PR) polymorphism gene (PROGINS) reduced response to progesterone. Some studies found an association between this polymorphism gene and endometriosis [12, 13]. The human PR gene, located on chromosome 11q22–23, comprises eight exons and seven introns. One PR polymorphic variant, consisted of a 320 bp PV/HS-1 Alu insertion in intron G between exon 7 and 8 and two point mutations in exons 4 and 5 and was named PROGINS [14, 15].

Previous study found a relation between a mutated progesterone receptor allele and ovarian cancer, which has more transcriptional activity compared to the wild-type receptor. Greater transcriptional activity increases stability and higher expression of the mutant protein [16, 17].

Proteins encodes two isoforms of the receptor included PR-A and PR-B. Isoform protein A has some anti proliferative effects on the endometrium and isoform B activated in the absence of isoform A receptor, which leads to addition proliferation in the epithelium. The genetic changes in the function of these isoforms could cause the unwanted proliferation of the endometrial tissue at every place, which results in endometriosis [17-20].

Materials and Methods

Eighty six peripheral blood samples were collected from women with diagnosed endometriosis, using laparoscopy referred to Yazd Reproductive Sciences Institute, and 86 normal controls. The Ethics Committee of Shahid Sadoughi University of Medical Sciences approved this research.

DNA extraction

Genomic DNA was extracted from lymphocytes of the peripheral blood using salting out method.

Polymerase Chain Reaction (PCR)

PCR was carried out in a final volume of 25 µl to detect the polymorphism in the progesterone receptor. The steps for cycling were an initial denaturation step at 94ºC for 5 min., and 35 cycles for denaturation at 94ºC for 1 min., followed by a final extension at 72ºC for 7 min. The sequences of primers used to amplify

the region including progesterone receptor gene polymorphism in intron G of the PR gene are: 5' –GGC AGA AAG CAA AAT AAA AAG A-3'(primer 5'), and 5'-AAA GTA TTT TCT TGC TAA ATG TC-3'(primer3') [21].

The amplification products were subjected to electrophoresis on 2% agarose gels in 1X Tris-borate–Ethylenediaminetetraacetic acid (TBE). They are stabled and visualized with ethidiume bromide (5 µg/ml) and video documentation system (VDS). The PCR product showed A1 allele, 149 bp without Alu insertion that refers to the wild-type allele, and A2 allele, 455 bp indicates the insertion of 306 bp in intro G of the receptor gene. Therefore each woman suffered endometriosis and subjected to analysis of two alleles, indicates being homozygous of the wild type (A1/A1) and/or being polymorphic (A2/A2), or heterozygous (A1/A2) (Fig.1).

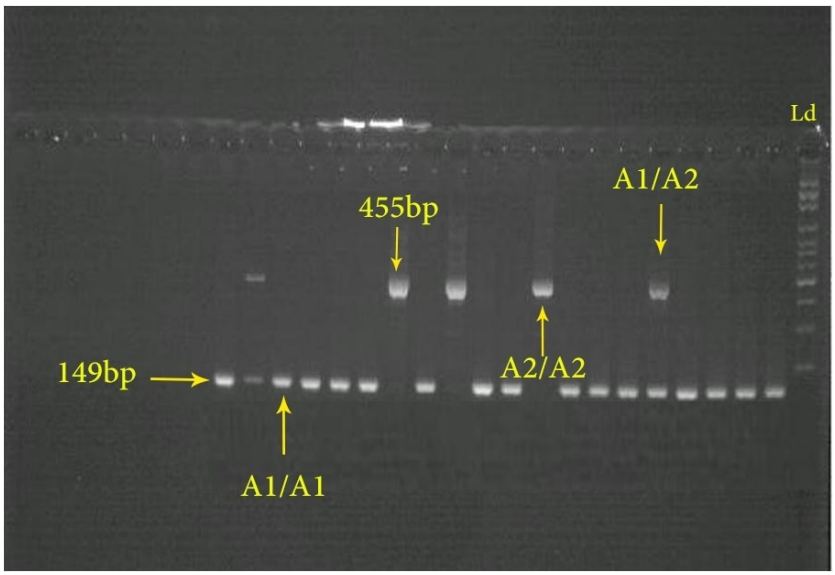

Fig.1. Electrophoresis of the products of the PCR. The 149 bp band Shows A1allele, and 455 bp with Alu insertion of 306 bp shows A2 allele.

Statistical Analysis

The genotypes of PROGINS were compared by the chi-square test using SPSS soft ware (version 17). Significant results achieved in P-value were less than 0.05.

Results

The results showed 73 cases (84.9%) were A1/A1 homozygous (73/86), but only 9 (10.5%) A1/A2 heterozygote and 4 (4.7%) A2/A2 were homozygous. 75 cases (87.2%) were A1/A1 homozygous, 10 (11.6%) A1/A2 heterozygote, and only 1 (1.2%) A2/A2 was homozygous in the control group. The frequency of genotypes theses were different between cases and controls, but these differences were not significant (p= 0.391). The results are presented in table 1.

Table 1. Frequency of genotypes A1/A1, A1/A2 and A2/A2 of PROGINS polymorphism in cases and controls

	A1/A1		A1/A2		A2/A2		A1/A2+A2/A2		P-value
	N	%	N	%	N	%	N	%	
Endometriosis (N=86)	73	84.9	9	10.5	4	4.7	13	15.2	0.391
Control (N=86)	75	87.2	10	11.6	1	1.2	11	12.8	

Chi-Square test showed no significant differences between cases and controls in genotypes frequency.

Discussion

Several Studies have focused on the increased incidence of endometriosis in infertility and some disorders, which encourage them try to understand the quick diagnosis and prevention of endometriosis [22-24]. One of the accurate diagnostic procedure is laparoscopy or laparotomy, but this procedure is invasive [25, 26]. If this study could find a significant relation between biochemical or molecular marker and endometriosis, it could be very important and effective for diagnosis [27, 28]. Previous studies have shown an alteration in PROGINS leads to the reduction of the expression of CYP1A1, which changes in dioxin metabolism and influences the risk of endometriosis onset [29]. Present study does not find a significant correlation between frequencies of PROGINS polymorphism and endometriosis. However, in Brazil population, Costa et al. have shown a significant correlation between PROGINS polymorphism and endometriosis [21]. Lattuada et al also found that the PROGINS polymorphism of the progesterone receptor might be associated with endometriosis [30] in another study in Italian women, which was found by Wieser et al. [31], and Carvalho et al. [32]. However, Govindan et al stated that, in Asian Indian women, Alu insertion could be considered as a risk factor for breast cancer but not for endometriosis [33]. The studies done by Treloar et al. [34], van Kaam et al. [35], and Gimenes et al. [36] did not find a correlation between PROGINS polymorphism and endometriosis. The results obtained in the Australian and Dutch in different research focus was not the same.

Conclusion

The present Study does not show any significant correlation between PROGINS 306 insertion polymorphism and endometriosis. However there are some evidences that show the relation between PR and endometriosis. It encourages working in different focus on progesterone receptor.

Conflict of Interest

The authors confirm that this article content has no conflict of interest

Acknowledgment

There is no Acknowledgment to declare.

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