Klinik Çalışma

Plasminogen Activator İnhibitor Type-1 (PAI-1) Gene 4G/5G and p53 Codon72 Polymorphisms and Genetic Susceptibility to Endometriosis PAI-1 4G/5G, p53 codon 72, Endometriosis

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Abstract

Aim: In this study it was aimed to determine whether plasminogen activator inhibitor type-1 gene 4G/5G and p53 codon 72 polymorphisms are genetic markers of endometriosis development in Turkish endometriosis patients.

Method: Genomic DNA was extracted from 60 women (30 with endometriosis and 30 controls) in the study. DNA was amplified with 4G and 5G specific primers for detection of plasminogen activator inhibitor type-1 gene 4G/5G polymorphism and PCR-RFLP technique was used to analyze p53 codon 72 polymorphism. Products were assessed with ultraviolet transilluminator by being exposed to agarose gel electrophoresis.

Results: According to the plasminogen activator inhibitor type-1 gene 4G/5G polymorphism the 4G allele frequency was indicated as 37% and 5G allele was as 63% in patients, whereas this was 53-47% in the control group. According to p53 codon 72 polymorphism the proline allele frequency was indicated as 43.3% and arginine allele was as 56.7% in patients, whereas this was 40-60% in the control group.

Conclusion: As a result of our study we may assert that plasminogen activator inhibitor type-1 gene 4G/5G and p53 codon polymorphisms cannot be considered as genetic markers to develop endometriosis in Turkish population. However the significance of our result remains to be further investigated in different and even larger groups being combined with other genetic polymorphisms considered as risk factors for endometriosis.

Key words: Endometriosis, PAI-1 4G/5G, p53 codon 72 polymorphism

Introduction

Endometriosis is a gynecological disease where tissue similar to the lining of the uterus (the endometrial stroma and glands, which should only be located inside the uterus) is found elsewhere in the body (1-3). It affects as many as 10-15% of premenopausal women.

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The basic etiology and pathogenesis of endometriosis remain unknown (4). It is a multifactorial and polygenic entity in which the fibrinolytic system may be involved. The persistence of a fibrin matrix in peritoneal pockets, as a result of hypo fibrinolysis, could allow menstrually deposited endometrial fragments to initiate endometriosis (5). Plasminogen activator inhibitor type-1 (PAI-1) is one of the fibrinolytic system inhibitor. It was determined that PAI-1 levels are related to a polymorphism created by the guanosine insertion/deletion variation (4G or 5G) gene which codes PAI-1. Plasma PAI-1 levels of the 4G allele carriers are found to be higher compared with 5G allele carriers (6,7). Thus, PAI-1 gene 4G allele should be a genetic marker for developing endometriosis. Alterations in tumor suppressor genes may also be related to development of endometriois. p53, a representative tumor suppressor, is involved in

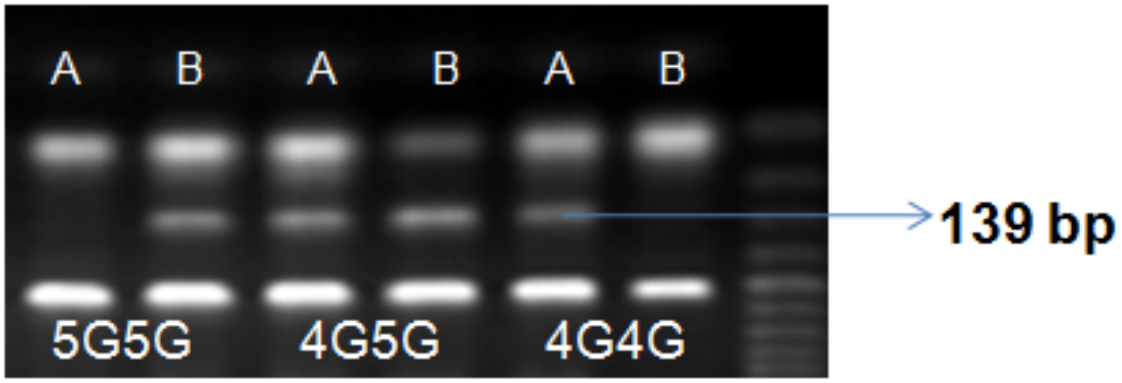

Figure 1. Gel image of PAI-1 gene 4G/5G polymorphism (A) PCR products of 4G primer (B) PCR products of 5G primer

cell proliferation and progression of various tumor types (8,9). p53 codon 72 shows a single nucleotide substitution resulting in the presence of either arginine or proline in amino acid sequence. This change affects biochemical and functional properties of p53: the proline variant is a stronger transcriptional activator, whereas the arginine variant is a stronger apoptosis (10-12). The association between endometriosis and p53 codon 72 remains unclear. In some studies it was observed that proline allele is related to a higher risk in endometriosis (8-13,14) but on the other hand some studies contradicted this result, suggesting that p53 codon 72 polymorphism does not confer genetic susceptibility to endometriosis (9-12). Based on these findings, this study was aimed at determining whether or not PAI-1 gene 4G/5G and p53 codon 72 polymorphisms are genetic markers of endometriosis development in Turkish endometriosis patients.

Material and Methods

Patients:

30 endometriosis patients (31±,1.26 years), applied to the department of Obstetrics and Gynecology in Private Muş Şifa Hospital in Muş, Turkey, with symptoms such as like pelvic pain, infertility and etc., diagnosed as endometriosis by pathology after a surgery in other hospitals, and 30 pregnant individuals (28.5±0.73 years) as a control group, have no infertility story and no mass in their ovaries, were included in this research. Informed consent in accordance with the study protocol approved by the ethics committee of Medical Faculty, Eskisehir Osmangazi University, Eskisehir, Turkey was obtained from each patient.

DNA isolation:

DNA was extracted from venous blood according to kit procedure (Vivantis, Malesia) and stored at -20^{0}C. PAI-1 gene 4G/5G polymorphism genotype determination DNA was amplified by polymerase chain reaction (PCR) in a thermal cycler (Amplitronyx 4, USA). Allele-specific primers were used in the PCR. These primers were as follows: for 5G allele, 5'-GTC TGG ACA CGT GGG GG-3' and for 4G allele, 5'-GTC TGG ACA CGT GGG GA-3'. Each in combination with a common downstream primer 5'-TGC AGC CAG CCA CGT GAT TGT CTA G-3' gives rise to a 139 bp DNA fragment. A control upstream primer, 5'-AAG CTT TTA CCA TGG TAA CCC CTG GT-3', was used as a positive control in the PCR. 1 µl of DNA sample was amplified for 35 cycles with denaturation at 94^{0}C for 60 s, annealing at 54^{0}C for 30 s, and extension at 72^{0}C for 40 s using a 25 µl PCR mixture contained a 10 pmol allele-specific primer, a 10 pmol common downstream primer, a 10 pmol control upstream primer, 10X PCR buffer, 2 mM dNTPs, and 5U Taq polymerase. The PCR products were separated by electrophoresis on 2% agarose gel containing 4 µl ethidium bromide and were visualized using a UV transilluminator (Nyxtechnik, USA) and photographed with a CCD camera (Cleaver, UK) (Figure 1).

p53 codon 72 polymorphism genotype determination:

DNA was amplified by polymerase chain reaction (PCR) in a thermal cycler (Amplitronyx 4, USA). Allele-specific primers were used in the PCR. These primers were as follows: forward, 5'-TTG CCG TCC CAA GCA ATG GAT GA-3' and reverse, 5'-TCT GGG AAG GGA CAG AAG ATG AC-3'. 5 µl of DNA sample was amplified for 35 cycles with denaturation at 95^{0}C for 30 s, annealing at 59^{0}C for 30 s, and extension at 72^{0}C for 35 s using a 50 µl PCR mixture contained a 10 pmol each primer, 10X PCR buffer, 2 mM

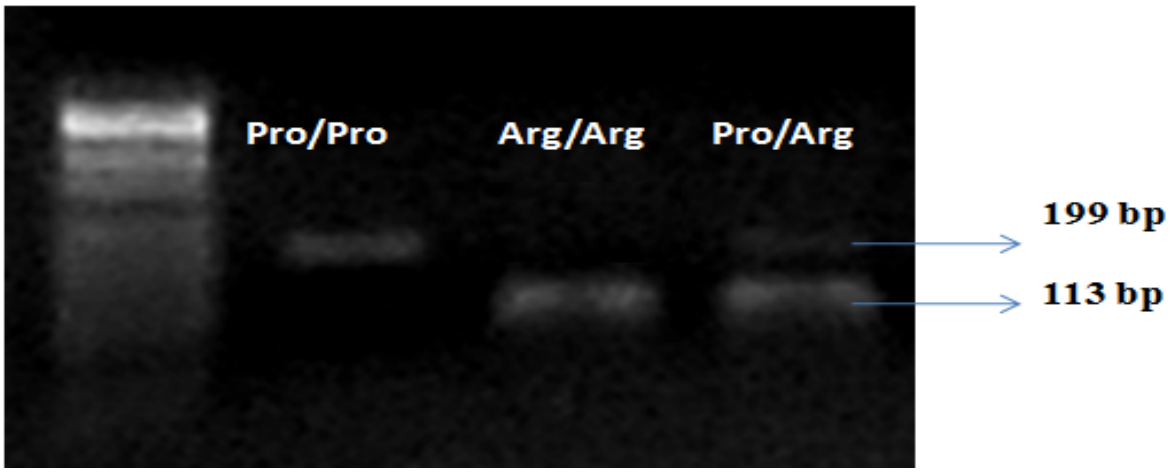

Figure 2. Gel image of p53 codon 72 polymorphism BstU1 products

dNTPs, and 5U Taq polymerase. The PCR products were separated by electrophoresis on 2% agarose gel containing 4 µl ethidium bromide and were visualized using a UV transilluminator (Nyxtechnik, USA) and photographed with a CCD camera (Cleaver, UK). After confirmation of an amplified fragment of the expected size (199 bp), the PCR products were digested with 1 unit of restriction enzyme BstFN1 (Vivantis, Malesia) at 60^0C for 1 hour. Digested PCR products were separated by electrophoresis on 2% agarose gel containing 4 µl ethidium bromide and were visualized using a UV transilluminator (Nyxtechnik, USA) and photographed with a CCD camera (Cleaver, UK) (Figure 2).

Statistical analysis:

Data were analyzed using the Statistical Package for Social Sciences (SPSS ver.15). The values were expressed as means ± SE. Alleles and genotype frequencies between patients and control subjects were compared by Pearson $\chi 2$-test. A Pvalue less than 0.05 was considered statistically significant.

Table 1. Genotype distribution of PAI-1 gene 4G/5G polymorphism

PAI-1 gene 4G/5G polymorphism genotypes	Patient (n=30)	Control (n=30)	p value
4G/4G	3	10	
4G/5G	16	12	p<0.05
5G/5G	11	8	

Table 2. Allele frequencies of PAI-1 gene 4G/5G polymorphism

PAI-1 gene 4G/5G polymorphism alleles	Patient (n=30)	Control (n=30)	p value
4G Allele	22 (%36.6)	32 (%53.3)	p<0.05
5G Allele	38 (%63.4)	28 (%46.7)	

Table 3. Genotype distribution of p53 codon 72 polymorphism

p53 codon 72 polymorphism genotypes	Patient (n=30)	Control (n=30)	p value
Proline/Proline	4	8	
Proline/Arginine	18	8	p<0.05
Arginine/Arginine	8	14	

Results

According to genotype distribution of PAI-1 gene 4G/5G polymorphism 4G4G genotype is found statisticaly high (p<0.05) in controls than patients (Table 1). There was also a significant different between allele frequencies. The 5G allele frequency was found statistically higher in patients than controls (Table 2). According to genotype distribution of p53 codon 72 polymorphism Pro/Arg genotype is found statisticaly higher (p<0.05) in patients than controls (Table 3). But there was no a significant

different between allele frequencies in patients and controls (Table 4).

Discussion

Hypofibrinolysis is associated with the 4G allele of the PAI-1 gene 4G/5G polymorphism in endometriosis development (5). According to our results 5G allele frequency was found statistically high in patients than controls. Also 4G4G genotype was statistically higher in controls than patints. So we can assert that PAI-1 gene 4G/5G polymorphism is not a genetic marker in Turkish endometriosis patients. Consistent with our results Ramon et. al and Gentilini et al reported that PAI-1 gene 4G/5G polymorphism is not associated with endometriosis in Spain and Italian population respectively (15,16).

Table 4. Allele frequencies of p53 codon 72 polymorphism

p53 codon 72 polymorphism alleles	Patient (n=30)	Control (n=30)	p value
Proline Allele	26 (%43.3)	24 (%40)	p>0.05
Arginine Allele	34 (%56.7)	36(%60)	

Nevertheless, as distinct from our findings, Bedaiwy et al.. have found a significant association between PAI-1 gene 4G/5G polymorphism 4G allele and endometriosis in Canadian population (5).

The genetic distribution of p53 codon 72 polymophism genotypes also showed a significant difference between patients and controls. Pro/Arg genotype was significantly higher in patient group than control group. In recent studies it has been reported that proline homozygosity and heterozygosity is related to a higher risk in endometriosis (8,13,14). But when we look at proline homozygosity between patients and controls it is found higher in control group. Allele frequencies also did not show any difference between groups. So we should not consider that p53 gene codon 72 polymorphism could not be a marker to predict endometriosis in Turkish population. Similar to our results, in Italian and Japanese populations investigators also suggested no association between p53 gene codon 72 polymorphism and endometriosis (9-12). In contrast with these results proline homozygosity and heterozygosity have been found associated with endometriosis in Chinese and Brazilian populations (8,13,14). As a conclusion we did not found any association between PAI-1 gene 4G/5G and p53 codon 72 polymorphism and endometriosis development in Turkish population. However, genetic polymorphism studies in larger populations will provide more significative results. As a result, it could not be suggested that results of this study can be generalized to the whole Turkish population, rather it renders an opinion. Also when we consider gene pools, life style, and gene–environment interactions, the risk shall not be supposed as identical in every population with respect to genotypes (17). In addition, genetic polymorphism studies with larger numbers of the population provides more meaningful results. The significance of our result remains to be further investigated in different and even larger populations being combined with other genetic polymorphisms considered as risk factors for endometriosis.

Plazminojen Aktivatör İnhbitör tip-1 (PAI-1) Geni 4G/5G ve p53 Kodon 72 Polimorfizmleri ve Endometriyozise Genetik Yatkınlık PAI-1 4G/5G, p53 Kodon 72, Endometriyozis

Özet

Amaç: Bu çalışmada plazminojen activator inhibitor tip-1 geni 4G/5G ve p53 kodon 72 polimorfizmlerinin endometriyozisli Türk hastalarda endometriyozis gelişiminde genetik belirteçler olup olmadıklarının belirlenmesi amaçlanmıştır.

Yöntem: Genomik DNA 60 kadından (30 endometriyozisli kadın ve 30 kontrol) izole edilmiştir. DNA, plazminojen activator inhibitor tip-1 geni 4G/5G polimorfizmi 4G ve 5G spesifik primerler ile amplifiye edilerek, p53 kodon 72 polimorfizmi için PCR-RFLP yöntemi uygulanarak analiz edilmiştir. Ürünler agaroz jel elektroforezinde ultraviyole transillüminatör ile değerlendirilmiştir.

Bulgular: Plazminojen activator inhibitor tip-1 geni 4G/5G polimorfizmi değerlendirildiğinde hasta grubunda 4G alel frekansı %37, 5G alel frekansı %63 olarak, kontrol grubunda ise sırasıyla %53-47 olarak belirlenmiştir. p53 kodon 72 polimorfizmi açısından ise hasta grubunda prolin alel frekansı %43.3, arginin alel frekansı %56.7 olarak, kontrol grubunda ise sırasıyla %40-60 olarak tespit edilmiştir.

Sonuç: Çalışmamızın sonucunda plazminojen activator inhibitor tip-1 geni 4G/5G ve p53 kodon 72 polimorfizmlerinin Türk popülasyonunda endometriyozis gelişiminde birer genetik belirteç olarak düşünülemeyeceğini söyleyebiliriz. Ancak sonuçlarımızın daha anlamlı olabilmesi açısından daha geniş çalışma grubu ile, endometriyozis için risk faktörü olarak düşünülen diğer genetik polimorfizmler ile kombine edilerek çalışılması gerektiğini düşünmekteyiz.

Anahtar kelimeler: Endometriyozis, PAI-1 4G/5G, p53 kodon 72, polimorfizm

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