

Evaluation of Different Genotypes at Codons 11, 72 and 248 of p53 Gene in Samples of Endometriosis

Hajar Dahim¹, Kahin Shahanipour^{2*}, Ahmad Shabanizadeh Darehdori³

1. MSc in Biochemistry, Department of Biochemistry, Falavarjan Branch, Islamic Azad University, Flavarjan, Iran.

2. PhD in Biochemistry, Department of Biochemistry, Falavarjan Branch, Islamic Azad University, Flavarjan, Iran.

3. PhD in Anatomy, Department of Anatomy, Rafsanjan University of Medical Sciences, Rafsanjan, Iran



Hajar Dahim, MSc works on biochemistry and teaches it to medical sciences students. Also, she works in Shahid Beheshti hospital laboratory, Isfahan.

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ABSTRACT

Introduction: Endometriosis is a prevalent gynecological disorder among women which is diagnosed by the growth of endometrial tissue outside of uterus and is mainly accompanied by severe pelvic pain and infertility. P53 also known as cellular tumor antigen P53 inside codons 11, 72 and 248 are contained with single nucleotide changes in which tends to be nearly rampant. This will probably be increasing the chances of endometriosis infection to some great extent.

Our aim was to evaluate the connection between endometriosis and polymorphism inside the codons 11, 72 and 248 of P53 gene.

Methods: In this study, single nucleotide changes in codons 11, 72 and 284 of TP53 gene among 44 persons infected with endometriosis and the same studying population for non-infected group have been examined. After primer design and amplification of polymorphic sequences by PCR, the polymorphisms of related codons have been evaluated by the digestion method (RFLP).

Results: In this study on the codon 72, there were seen differences in the distribution of genotype frequencies of normal polymorphic subjects and control subjects with endometriosis. At codons 11 and 248, there were observed no significant correlations between polymorphic and normal genotypes of endometriosis and non-endometriosis groups.

Conclusion: According to the results, we can say that probably polymorphism of codon 72 of p53 gene is one of the predisposing factors of endometriosis.

* Corresponding Author:

Kahin Shahani, PhD

Department of Biochemistry, Falavarjan Branch, Islamic Azad university, Flavarjan, Iran.

Tel: +98 (913)1267418

E-mail: shahani@iaufala.ac.ir

1. Introduction

Endometriosis is a prevalent gynecological and multi-factorial disease that is due to the interactions of variant genes and environmental factors. In this disease, endometrium is seen outside of uterus. Endometriosis is diagnosed first time by Baron Carl Von Rokitansky in 1860. There are more infected Asian and European women in numbers compared to what have been observed among African and American women.

The symptoms of disease are varied but it is usually associated with pelvic pain, painful menstruation, and reduced fertility. The most common symptom is referral pelvic pain [1-3].

Pathologic findings associated with this disease are fibrosis, peritonitis, adhesion, and ovarian cysts [4, 5].

Several reasons expressed as the reason of disease including menstrual reflux hypothesis (peritoneal macrophages cannot phagocyte ectopic endometrial), and being mullerianic (the cells with the potential of becoming endometrial from their path along their evolutionary course and logged organogenesis are located somewhere else). There are also some genetic defects which are heritable [6-8].

Endometriosis could occur for all women from the beginning of menstruation to menopause stage despite of their race, type and whether they have given birth to any child or not. However, this particular type of disease is associated with pregnant women. However, the contributing factors to endometriosis such as race, age, their child birth experience, uterine abnormalities, and diet could be affective to its cause [5].

The prevalence of this particular type of illness varies between 6 to 10 percent among infected women approximately. Furthermore, infertile women and women suffering from chronic pelvic pain are considered to be more under the risk [9].

Endometriosis cannot be only diagnosed with the given symptoms, but rather requires a set of results and examinations. Health background and physical examination help the physician to a better diagnosis for a large number of patients. The best diagnostic method of endometriosis is the direct observation of implanted tissue. Laparoscopy is known to be a golden method and the superior technique in diagnosing the disorder. The main aim of the treatment is to reduce the pain,

limit the disease progression, and lastly to maintain the fertility.

In general, the endometriosis treatment is divided into medical and surgical treatments. At both methods, infertility and pain can be cured [10]. First-degree relatives of women with endometriosis have an increased risk of receiving endometriosis by 7 percent more. Such figure is comparatively 7 times higher than women without this particular disorder [10]. Therefore, it is apparent that genetic changes play a crucial role in endometriosis. It has been shown that disruption in suppressor gene tumors might be relative to the root cause of endometriosis [11].

One of the most well-known suppressor gene tumors called P53 is located in the region P13-1, chromosome number 17. Codons' numbers 11, 72 and 248 of this particular gene contains approximately common polymorphisms. These polymorphisms depends on geographical and racial features and the occurrence of endometriosis has turned out to be reported differently in various world populations [4, 5]. The aim of this study was to evaluate these polymorphisms among women with endometriosis and comparing them with healthy women subsequently.

2. Materials and Methods

At this case-control study, the samples are all collected in form of paraffin blocks in pathology archives. As far as the research and statistical consulting are concerned, 44 endometrial samples were entitled as case group and the other 44 samples which are non-endometrial tissue were selected to be the instance. After the samples collection, DNA was extracted from kit. At the next step, the consecutive polymorphic of codons number 11, 72 and 284 belonged to gene 53TP by PCR and the implementation of designed specific primer pairs were augmented. The consecutive specific primer pairs to achieve consecutive polymorphic augmentation include as such : Codon 11 F: ACTTTCCTTGCAGCAGC and R: TTTCGGCTTCCCAGGTCTC

Codon 72 F: ATGATTGATGCTGTCCCC and R: GGAAGGGACAGAAGATGACAG, Codon 248F: TGCTTGCCACAGGTCTCCC and R: CAGGGTG-GCAAGTGGCTCC to augment the consecutive polymorphic for each codon. PCR conditions are as listed below:

PCR by using 300-100 ng of extracted DNA, 1 unit of each primer, 200mMMgcl2, 1.5 m/M each of dATP,

dCTP, dTTP, dGTP. First stage: A - Denaturation 94 °C to 30 seconds for codon 11, 95 °C to 30 seconds for codon 72, 94 °C to 30 seconds for the codon 248. B - Annealing temperature, 55 °C to 30 seconds for codon 11, 58 °C for codon 72 to 30 seconds and 30 seconds to codon 248. C - Extension, 72 °C to 30 seconds for codon 11, 72 °C to 45 seconds for codon 72, 72 °C to 30 seconds for codon 248. Then, PCR has been affected by the restriction enzymes and the related polymorphic have been evaluated through gel electrophoresis study. One unit of restriction enzyme, BstI at the temperature of 37° Celsius for a period of 30 minutes was used to evaluate the polymorphic of codon number 72. At genotype of normal homozygous (Arginine - Arginine) (GG), one piece of bp 279, at heterozygous (Arginine - Proline) (GC), three pieces of 279 bp and 60R and 119 and at polymorphic heterozygous (Proline - Proline) (CC), two pieces of 160 bp and 119 are produced. To evaluate polymorphic of codon number 11, one unit of restriction enzyme of IqTa for a period of 30 minutes at the temperature of 65°Celsius was used[4, 5].

At genotype of normal homozygous (Glutamine - Glutamine) (GG) one piece of 379 bp, at heterozygous (Glutamine - Lysine) (GC), three pieces of 379 bp and 239 and 140, and at polymorphic heterozygous (Lysine - Lysine) (CC), two pieces of 239 and 140 are produced. To evaluate the polymorphism of codon number of 248, one unit of restriction enzyme of IIIHap for

a period of 30 minutes at the temperature of 65°Celsius was used. At genotype of normal homozygous (Arginine - Arginine) (GG) one piece of 379 bp, at heterozygous (Arginine-Tryptophan) (CT) three pieces of 379 bp and 239 and 140, and at polymorphic heterozygous (Tryptophan - Tryptophan) (TT), two pieces of 239 and 140 are produced. The acquired information was statistically analyzed by SPSS software [4, 5].

To compare frequency distribution of three different genotypes of each codon at the samples of endometriosis, Chi-square-test was performed. It turned out that P value was perceived to be less than 0.05.

3. Results

According to table 1, genotype of Arg/Arg was 21 of 44 cases (47/72%) in endometriotic samples and 31 of 44 cases (70/45%) in nonendometriotic samples. Genotype of Arg/Pro was 22 of 44 cases (50%) in endometriotic samples and 13 of 44 cases (29/54%) in nonendometriotic samples. Genotype of Pro/Pro was 1 of 46 cases in endometriotic samples and there was not found cases in nonendometriotic samples. To compare frequency distribution of polymorphic and nonpolymorphic genotypes between two endometriotic and nonendometriotic group there was found significance differences (Pvalue <0/05) indicated polymorphic variation in codon 72 of endometriotic samples.

Table 1. Frequency of distribution of genotypes at the instance group and the endometriosis of codon 72. (P value ≤ 0.05)

Genotypes	Endometriosis, n=44(%)	Non- Endometriosis, n=44(%)
Arg/Arg G/G	21(47.72)	31(70.45)
Arg/Pro G/C	22(50)	13(29.54)
Pro/Pro C/C	1(2.27)	0

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Table 2. Frequency of distribution of genotypes at the instance group and the endometriosis of codon 11. (P value ≥ 0.05)

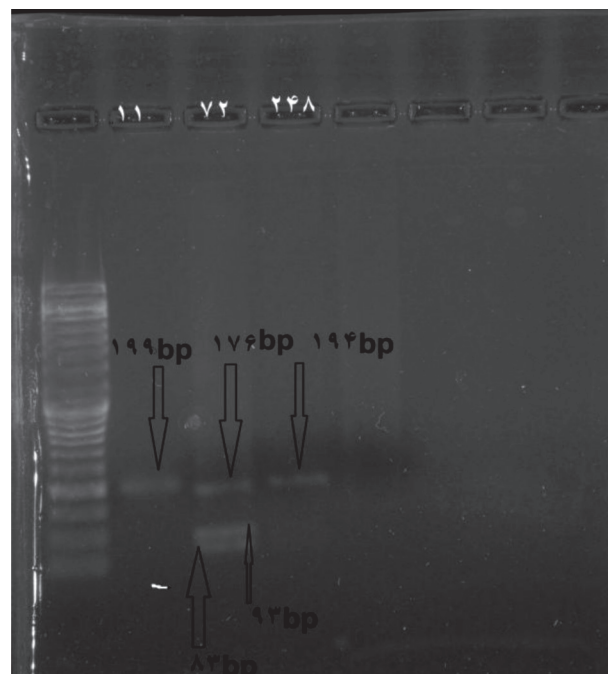
Genotypes	Endometriosis, n=44(%)	Non- Endometriosis, n=44(%)
Gln/Gln G/G	42(95.45)	44(100)
Gln /Lys G/C	2(4.55)	0
Lys/ Lys C/C	0	0

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Table 3. Frequency of distribution of genotypes at the instance group and the endometriosis of codon 248. (P value≥0.05)

Genotypes	Endometriosis, n=44(%)	Non- Endometriosis, n=44(%)
Arg/Arg C/C	43(97.72)	44(100)
Arg/Trp C/T	1(2.27)	0
Trp/ Trp T/T	0	0

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Figure 1. View details of polymorphisms codon 11, 72, and 248 after the effects of an enzyme.

To compare frequency distribution of polymorphic and non polymorphic genotype of codon 11 and 248 between two groups of endometriotic and non endometriotic samples, there was not found significant differences that indicated these codons not polymorphic variation in endometriotic samples.

4. Discussion

Endometriosis can be seen as one of the most common disorders in women who has similar characteristics of malignant tumors. Infertility is a major complication of the disease. Symptoms of this disorder including dyspareunia, pelvic and menstrual pain. Genetic changes

including significant events that occur during the creation of endometriosis. Single nucleotide change in the gene sequence in the lower of a percentage of the population is called polymorphism that is inherent. Certain genetic defects heritable may cause endometriosis. These changes in genes of tumor suppressor including p53 can cause the more affect of certain disorders and diseases. Defective gene to some 17, which is 53p can affect gene function and thus may cause promotional endometriosis offers[12]. In polymorphisms 72CG P53, replacement of Cytosine (C) instead of guanine (G) lead to amino acid Arg conversion to proline. So, this process is a polymorphism that is single nucleotide. Arginine (Arg) with CGC sequence and the pro-

line (Pro) with the sequence CCC. Given that genotypes may occur include: Arg / Arg (G / G) that is naturally homozygous, Arg / Pro (G / C) are heterozygous and Pro / Pro (C / C) that is polymorphic homozygous. In polymorphism C248TP53, replacement of nucleotides cytosine (C) by thymine (T) causes the conversion of the amino acid arginine, Arg to proline. Therefore, two alleles of arginine (Arg) with sequence (CGG) and another Tryptophan Trp with sequence (TGG) and three genotypes Arg / Arg (C / C) that normal homozygous, Arg / Trp (C / T) which is heterozygous, and Trp / Trp (T / T). The polymorphic homozygous found in polymorphism codon 11GP53C, cytosine substituted guanine and thus two alleles - glutamine (Gln) with sequence GAG) and a lysine (Lys) with sequence (CAG) and three genotypes Gln / Gln (G / G), which is normal homozygous, also Gln / Lys (G / C), which is heterozygous and Lys / Lys (C / C) that are polymorphic homozygous. This polymorphism and its genotypes indifferent geographic and ethnic populations may have different effects on the risk of the disease [12]. The Role of single nucleotide changes in codons listed in susceptibility to endometriosis in previous studies, results of the anti-conflicting provided. Therefore, single nucleotide changes in codon P 53 genes listed in different geographic and ethnic populations may have different effects on endometriosis. This polymorphism is associated with geographic location and race [12]. In a study on codon 72, Change has reported an association between alleles in a Chinese population proline and endometriosis. Two types of homozygote including the (Pro / Pro) and heterozygous for it (Arg / Pro) with the occurrence of endometriosis is associated while the Arg / Arg genotype is not a relationship with endometriosis. They have said that there is a defensive role of homozygous genotype Arg / Arg against the disease and if someone has this genotype who will have a more immunity against infection with endometriosis. According to researches, Homozygous genotype Arg / Arg of codon 72 have low risk of endometriosis while the heterozygous allele and homozygous of proline (Pro) has a higher chance of suffering from this disease [13]. This study was conducted in a Japanese population in Northern Italy and results have shown that the genotype Arg / Arg in comparison with the Arg / Pro and Pro / Pro is associated with an increased risk of endometriosis [14]. In this study, dissimilarity was seen in the frequency of codon 72 between normal controls and patients with endometriosis. Frequency of Allele Arg / Pro (G / C) in the patient group 50 % and in non-endometriosis was 29.54 %. Allele Pro / Pro (C / C) in the case group was 2.27%, compared

with zero percent in Group endometriosis and allele Arg / Arg group with 47.72 % was compared with 70.45 % in non-endometriosis. Therefore, the results of this study indicate that the most likely genotype of GC (Arg / Pro) and genotype of CC (Pro / Pro) which has a proline allele, can be considered as a potential risk for developing endometriosis. These findings match with the results provided by the change on a Chinese population as well as on an Italian population closely. According to the results, we can say that probably polymorphism of codon 72 of P 53 genes is a predisposing factor for endometriosis and it is considerable and useful indicator for predicting endometriosis and at least we can say people with this genotype are more susceptible to endometriosis. It can serve as a marker for identifying people with endometriosis. About codons 11 and 284, we found no significant correlations between polymorphic genotypes (polymorphic heterozygous and homozygous) and normal (normal homozygous) between groups of endometriosis and non-endometriosis. Therefore, by having this information, people with susceptible genotype can be identified, and preventive measures can be carried out before treatment.

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