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Association of PTEN gene polymorphisms, protein levels and endometrial cancer: A hospital-based case-control study

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Abstract

Endometrial cancer is a type of cancer that develops in the inner lining of the uterus, called the endometrium. After breast, lung, and colon cancer, endometrial cancer is the most prevalent malignancy of the female genital system in industrialized nations like North America and Europe.

Phosphatase and Tensin homolog (PTEN), deleted on chromosome 10, is a tumor suppressor gene located on chromosome 10q23.31, which encodes a 403 amino acid protein with both lipid and protein phosphatase activities. One of the most common genetic abnormalities identified in human malignancies are somatic mutations in the PTEN gene. The tumor suppressor activity of the PTEN enzyme is reduced or completely lost as a result of PTEN gene mutations.

Considering this information, it is thought that PTEN gene polymorphisms may be associated with the pathogenesis of the disease. The purpose of our study is to determine the relationship between functional PTEN polymorphisms IVS4 (–/+) and -9 C/G and endometrial cancer.

DNAs belonging to 63 endometrial cancer patients and 63 control individuals were genotyped by PCR-RFLP method for 2 genetic variants of PTEN IVS4 (–/+) and -9 C/G gene. The expression level of PTEN protein was measured by the Elisa method from serum samples. Our results were evaluated with appropriate statistical methods.

As a result of our study, no statistically significant difference was found in the risk of endometrial cancer and genotype frequencies of –9 C/G and IVS4 (-/+) variants ($p > 0.05$). It was detected that the serum level of PTEN lessened significantly in patients with endometrial cancer in comparison to the control ($p < 0.001$).

As a consequence, there was no evidence that the PTEN gene variations -9 C/G and IVS4 (-/+), whose efficacy we examined in our investigation, increased the incidence of endometrial cancer in the Turkish population. However, endometrial cancer patients were shown to have lower serum levels of the tumor suppressor protein PTEN.

Key words: *Endometrial cancer, PTEN, polymorphism, IVS4 and -9C/G.*

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PTEN gen polimorfizmleri, protein seviyeleri ve endometrium kanseri ilişkisi: Hastane bazlı vaka-kontrol çalışması

Özet

Endometrium kanseri endometrium olarak isimlendirilen rahim iç zarında ortaya çıkan kanser türüdür. Endometrium kanseri Kuzey Amerika ve Avrupa bölgelerde kadınlarda meme, akciğer ve kolon kanserinden sonra 4. sıklıktadır ve kadın genital sisteminde en sık görülen kanserdir.

Kromozom 10 (PTEN) üzerinde silinen fosfataz ve Tensin homologu, hem lipit hem de protein fosfataz aktivitelerine sahip olan bir 403-amino asit proteinini kodlayan, kromozom 10q23.31'de bulunan bir tümör baskılayıcı genidir. PTEN genindeki somatik mutasyonlar, insan kanserlerinde bulunan en yaygın genetik değişiklikler arasındadır. PTEN genindeki mutasyonlar PTEN enziminin tümör süpresör fonksiyonunu azaltır veya ortadan kaldırır. Verilen bilgiler eşliğinde Bu çalışmada endometrium kanserinde arttığı bildirilen PTEN ekspresyonunun ve olası azalan PTEN

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protein ekspresyon seviyesi nedeninin fonksiyonel PTEN polimorfizmleri (IVS4 (rs no: 3830675) ve -9 C/G) olup olmadığını ve endometrium kanseri ile PTEN gen polimorfizmleri arasındaki ilişkinin hastalık patogenezine etkisinin belirlenmesi amaçlanmıştır.

63 endometrium kanser hastası ve 63 kontrol bireye ait DNA'lar PTEN IVS4 (–/+) ve -9 C/G, genine ait 2 genetik varyant için PCR-RFLP yöntemi ile genotiplendirildi. PTEN protein seviyesi serum örneklerinden Eliza yöntemi ile belirlendi. Sonuçlarımız uygun istatistik yöntemler ile değerlendirildi.

Çalışmamız sonucunda –9 C/G ve IVS4(–/+) varyantlarına ait genotip frekansları ile endometrium kanseri riski arasında istatistiksel olarak anlamlı fark tespit edilememiştir ($p>0.05$). PTEN serum seviyeleri kontrole göre endometrium kanserli hastalarda anlamlı oranda düşük olduğu belirlenmiştir ($p<0.001$).

Sonuç olarak araştırmamızda etkinliğini araştırdığımız PTEN genine ait –9 C/G ve IVS4(–/+) varyantların, Türk toplumunda endometrium kanser riski ile ilişkili bulunmadı. Ancak bir tümör süpresör olan PTEN proteinin serum seviyelerinin endometrium kanserli hastalarda azaldığı belirlendi.

Anahtar kelimeler: *Endometrium kanseri, PTEN, polimorfizm, IVS4(–/+), -9 C/G.*

1. Introduction

The endometrium is a structure that covers the uterus and the inner layer of the uterus, is prepared for a possible pregnancy every month by shedding a layer in each menstrual period, and in case of pregnancy, the baby clings. Cancers originating from here are also called endometrial cancer. Worldwide, approximately 142,000 women show endometrial cancer each year, and roughly 42,000 die from this cancer. It ranks fourth among cancers seen in women [1].

About 75% of all endometrial carcinomas are of the endometrioid type. Various endometrioid carcinoma variants, including secretory and villoglandular, with squamous differentiation with ciliated cells have been identified. Based on their molecular characteristics, Type 1 and Type 2 endometrial malignancies are separated. Long-term estrogen exposure is the cause of type 1 endometrial cancer, which spreads more slowly. This variety is typically diagnosed sooner, has a better prognosis, and is linked to obesity [2].

About 10-20% of sporadic endometrial carcinomas, termed type 2, are not estrogen-dependent and most can be associated with atrophic endometrium. Type 2 usually occurs at a later age, approximately 5-10 years after type 1 tumors, estrogen and progesterone receptor expression is usually negative. Most endometrial cancers, especially type 1, are hormone-dependent. The most important risk factor associated with endometrial cancer is exposure to excessive amounts of the hormone estrogen [3].

Previous studies have shown that in endometrial carcinomas, PTEN inactivation occurs as a result of a mutation in chromosome 10q23, which acts as a tumor suppressor, by coding a protein with tyrosine kinase effect, resulting in loss of expression [4]. PTEN mutations are observed in 83% of endometrial cancers [5].

PTEN is a well-known tumor suppressor gene with germline mutations that is one of the most often mutated genes in human cancers and causes cancer susceptibility syndromes. It was first discovered in 1997. The protein, which has a tensin-like domain and a phosphatase catalytic domain, is expressed in all body tissues [4, 6].

The PTEN gene, which is found on chromosome 10q23, produces a 403 amino acid, 48 kDa dominant protein that has sequence homology with tensin, oxylin, and exon 9 of the tyrosine phosphatase superfamily. The catalytic N-terminal phosphatase domain, which is necessary for membrane binding, and the C2 domain, which is present in the human PTEN crystal structure, are two strongly connected domains [7].

The PI3K/AKT cascade, which regulates cell growth, proliferation, survival, and metabolism, is negatively regulated by PTEN. This pathway's regulation is essential for oncogenic transformation [6]. PTEN is a crucial negative regulator of the signaling pathway made up of class I phosphoinositide 3 kinase (PI3K), AKT, and the mechanical target of rapamycin (mTOR) and is essential for regulating some crucial cellular functions, including cell growth, proliferation, survival, and metabolism. Lipid phosphatase PTEN prevents PI3K signal activation by turning PIP3 which PI3K produces back to PIP2. Loss of PTEN results in non-regulation of PIP3 levels, which promotes hyperactivation of the pathway and, as seen in experiments with PTEN-deficient tumor cell lines, immortalized fibroblasts, and tumors developing in PTEN-deficient mice causes cellular transformation and carcinogenesis [7].

Beyond its lipid phosphatase function, PTEN also promotes protein phosphatase activity, which results in actions unrelated to PI3K-AKT signaling. A study of PTEN mutations that lose lipid phosphatase but retain protein phosphatase activity in cell lines has shown that PTEN has multiple functions in regulating numerous novel protein substrates and cell adhesions via focal adhesion kinase 1 (FADK1) and non-receptor proto-oncogene tyrosine-protein kinase SRC10 [8].

PTEN has a critical role in managing the tumor microenvironment, and the initiation, development, and spread of illness by interacting with cancer cells, the stromal compartment, and the immune system at various levels. This is in addition to its ability to limit tumor growth. Loss of PTEN causes the tumor microenvironment to change by increasing tumor angiogenesis, macrophage recruitment, and malignant transformation in breast stromal fibroblasts [6].

PTEN deletions and mutations that impair or inactivate PTEN function typically arise during tumor formation. Glioblastomas, melanomas, and endometrial, prostate, colon, and bladder cancers are frequently reported to have genetically inactivated PTEN, while breast and lung malignancies have been discovered to have decreased PTEN expression [4, 6].

PTEN deletion, mutation, or change is present in a large number of sporadic cancers. Approximately 80% of people with Cowden syndrome have germline mutations that result in loss of PTEN function or low PTEN levels. A database of 1,904 PTEN mutations for 30 different tumor types is kept at the Sanger Institute. While there are greater frequency mutations in particular amino acids, known as mutation hotspots, PTEN mutations, minor insertions, and deletions seem to occur in sporadic cancers in this database. However, these hotspots do not only contain mutations in one form of cancer. For instance, although there have been more than 250 distinct PTEN mutations found in endometrial cancers, 19% of the 632 published alterations are related to the amino acid Arg130 in the phosphatase catalytic region. Mutations in Arg130 occur in other tumor types (4% of central nervous system tumors) but are most common in endometrial and ovarian tumors (19%) [9].

Endometrial hyperplasia, which is regarded to be a precursor lesion for endometrial cancer, has PTEN mutations as well. Short insertion or frameshift mutations, which are indicative of microsatellite instability, are present in the majority of endometrial cancers. Particularly, endometrial carcinomas linked to familial nonpolyposis colon cancer syndrome exhibit PTEN frameshift mutations (HNPCC). Additionally, DNA mismatch repair gene polymorphisms influence the incidence of endometrial cancers, indicating that alterations in PTEN that cause endometrial tumors may come from DNA repair processes [9].

In view of the available data, the goal of our investigation was to examine the association between endometrial cancer, PTEN IVS4 (-/+), and -9 C/G gene polymorphisms, PTEN protein serum level, and these variables.

2. Materials and methods

2.1. Subjects

The subject group consists of 63 cases with endometrium cancer and 63 control individualities of Turkish origin signed from the Department of Gynecology, Eskisehir Osmangazi University Faculty of Medicine in Eskisehir, Turkey.

2.2. Ethics Committee Approval

Informed concurrence by our protocol, and approval by the Ethics Committee of Eskisehir Osmangazi University Faculty of Medicine (approval number 2018/86), was attained from all cases and control individualities. We conducted the study by the ethical principles of the 1975 declaration of Helsinki.

2.3. DNA Isolation and Purity Determination

Genomic DNA isolation was performed from the collected blood samples using the PureLinkTM Genomic DNA Mini Kit (Invitrogen Corporation, Carlsbad, California, USA). The amount and purity of DNA samples were measured with NanoDrop (Multiskan™ GO Microplate Spectrophotometer uDrop, Thermo Scientific™). The DNA was diluted with elution solution to a stock concentration of 50 ng/µL and stored at -20 °C until use.

2.4. Polymerase Chain Reaction

PCR- RFLP system was used in the genotyping phase of our study. Applicable primer sequences were identified for PTEN IVS4(-/) and-9 C/ G gene variants, and gene regions were amplified by primers and PCR mix. A twenty-five (25) µl admixture was prepared for PCR amplification. PCR amplification was made ready with DNA, master mix (OneTaq ® Quick- cargo ® 2X Master Mix with Standard Buffer, New England Biolabs, Ipswich, MA, USA), forward-revers primers (Table1), and water (ddH2O). PCR reactions were performed in Bio-Rad Thermal Cycler (T100 ™, Foster City, CA, USA). After amplification, shearing was done with restriction endonucleases, and the cut products were subordinated to agarose gel electrophoresis to determine the genotype frequency with the gel attestation system. The sequences of PCR- RFLP primers, restriction enzymes, and annealing temperatures of manuals are presented in Table 1.

Table 1. Forward and reverse primer pairs designed for PCR, annealing temperatures and restriction enzymes

Gene	Forward (5'-3') Primer Sequence	Reverse (5'-3') Primer Sequence	Annealing temperature	Restriction enzyme
PTEN -9 C/G	GCAGCCGTTCGGAGGATT	GCCGCAGAAATGGATACAGG	57.3 ºC	*DraII*
PTEN IVS4 (-/+)	GGGGGTGATAACAGTATCTA	CTTTATGCAATACTTTTTCCTA	54 ºC	*AflII*

2.5. Elisa

The blood taken from the patient and control groups was placed in vacuum tubes with 5 ml gel. Then it was centrifuged for 15 min at 2500 g for serum. Supernatants were carefully collected. Serum PTEN levels were measured with the YL Biont Human Elisa Kit (Shanghai YL Biotech Co., Ltd) following the manufacturer's instructions.

Serum samples, standards, and reactants were prepared by the kit protocol. Biotin-stained secondary antibodies were annexed to the prepared samples and standards and incubated at 37ºC for 60 minutes. The plate was washed 5 times. Chromogens A and B were then added. After 10 min of incubation at 37ºC, the stop solution was added. Samples were read at 450 nm in a Thermo Scientific Multiskan GO spectrophotometer (ThermoFisher Scientific, Vantaa, Finland) device within 10 minutes. The results were calculated according to the standard curve plot.

2.6. Statistical analysis

Continuous data are presented as Mean ± Standard Deviation. Categorical values are shown as percentages (%). Shapiro Wilk test was used to determine the suitability of the data for normal distribution. Mann-Whitney U test was used compared to the groups that did not fit the normal distribution, and Pearson Chi-Square and Monte Carlo Pearson Chi-Square analyzes were used in the analysis of the cross tables.

Allele frequencies and genotype distributions, Hardy-Weinberg equilibrium (HWE) values were determined with the FINNETI program (https://ihg.gsf.de/cgi-bin/hw/hwa1.pl). P values less than 0.05 were accepted as substantial in all analyses.

3. Results

A total of 126 individuals, 63 patients with endometrial cancer and 63 control individuals, were included in our study. PCR-RFLP method was used for genotyping.

The amplification product for the PTEN -9 C/G region is 371 bp. In the presence of variation, *DraII* is cut into 266 and 105 bp fragments as a result of restriction enzyme cleavage. The uncut product has the CC genotype (371 bp), while the 371-266-105bp product CG has the 266-105bp product GG genotype. In our study, all individuals had the ancestral CC genotype and the PCR-RFLP image for the PTEN-9 C/G region is presented in **Figure 1**.

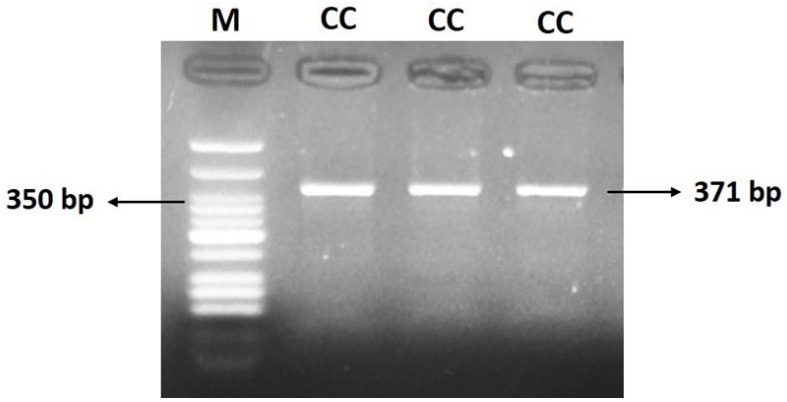

Figure 1. PCR product cut by the *DraII* restriction for the PTEN –9 C/G polymorphism. M: Marker 50bp, CC: 371bp

The amplification product for the PTEN IVS4 (-/+) region is 403 bp. In the presence of variation, a 5bp insertion occurs as a result of AflII restriction enzyme cleavage, and fragments of 335 and 73 bp are formed. Uncut product (ancestral genotype, no insertion,-/-) 403 bp, in the presence of insertion (5bp insertion) heterozygous genotype (-/+) 403-335-73 bp product, homozygous genotype (+/+) 335-73 bp constitutes a product. (+/+) genotype was not determined in our study. The PCR-RFLP image for the PTEN IVS4 (-/+) region is shown in Figure 2.

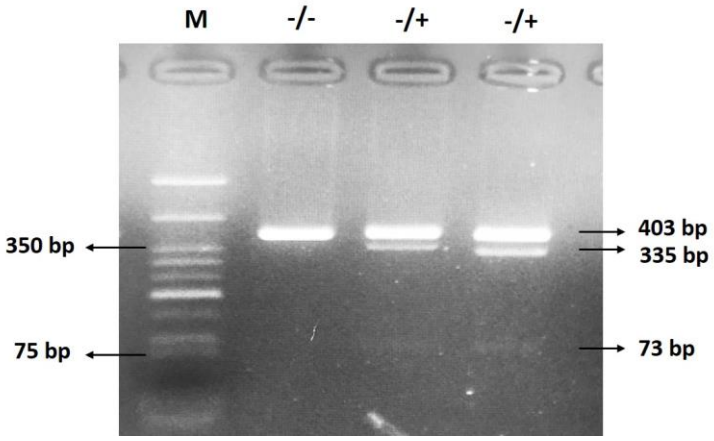

Figure 2. PCR product cut by *AflII* restriction for PTEN IVS4(–/+) polymorphism. M: Marker 50 bp, -/-: 403 bp, -/+: 403, 335, 73 bp

The genotype frequencies for PTEN IVS4 (-/+) and -9 C/G polymorphisms in endometrial cancer patients and controls are given in Table 3, respectively.

As a result of our study, when the PTEN gene IVS4 (-/+) genotypes of the control group and endometrial cancer were compared, no statistically significant difference was determined in point of disease risk and genotype frequency ($p>0.05$) (Table 2).

Table 2. Genotype and allele frequencies of PTEN IVS4 (-/+) polymorphisms in the endometrium cancer and control

Gene IVS4 (-/+)	**Allele**		Statistic P	OR (95 % CI)	**Genotype**			Statistic P	HWE
	- n(%)	+ n(%)			-/-	-/+	+/+		
Control	87 (69.0)	39 (31.0)	0.679	0.892 (0.520-1.532)	24	39	0	0.586	<0,001
Endometrium cancer	90 (71.4)	36 (28.6)			27	36	0		0.001
OR (95 % CI) (Risk allele +)									
Heterozygous -/- vs -/+		Homozygous -/- vs +/+		Dominant -/- vs -/+ + +/+		Recessive -/- + -/+ vs +/+		Armitage's trend test	
0.821 (0.402-1.673) p=0.586		0.891 (0.017-46.621) p=1.000		0.821 (0.402-1.673) p=0.586		1.000 (0.020-51.179) p=1.000		Common OR: 0.821 p=0.586	
OR: Odds ratio, CI: Confidence interval, HWE: Hardy-Weinberg equilibrium									

For the -9C/G variant of the PTEN gene, all of the patient and control individuals were determined to have the CC genotype, which is the ancestral genotype. Since there is no genotype difference, statistical evaluation was not made for this region.

The mean serum PTEN levels of endometrial cancer and control groups are presented in Table 4. The mean serum PTEN level of the endometrial cancer group was 5.322±1.62, and the mean serum PTEN level of the control group was 6.822 ± 2.72. PTEN serum levels were found to be significantly lower in patients with endometrial cancer in comparison to controls ($p<0.001$) (Table 3).

Table 3. Serum PTEN levels in the study population

Group	Control **(M ± SD) (n=63)**	Endometrium cancer **(M ± SD) (n=63)**	Statistic
PTEN (ng/ml)	6,822 ± 2,72	5,322 ± 1,62	p<0,001
Total **(n=126)**	6,072 ± 2,35		
M ± SD : Mean ± Standard Deviation			

4. Conclusions and discussion

Endometrial cancer is the most common gynecological cancer and the sixth most common cancer among women worldwide. The 5-year survival of patients with stage I endometrial carcinoma is 75-88%, whereas it is 50% for stage III disease and 15% for stage IV disease. For this reason, early detection can increase survival rates [10].

Determination of the basic molecular mechanisms related to cancer biogenesis and cancer, its prevention, early diagnosis, and the development of drugs and treatments for effective treatment are of great importance for the prevention and treatment of cancer, one of the most important diseases of our time. A study aimed at evaluating the efficacy of cancer gene mutations from endometrial biopsies to predict the development of malignant lesions used fifty cancer genes targeting a next-generation sequencing panel to search for mutations in matched non-cancerous and malignant samples. As a result of all biopsies taken from cancer tissues, mutations were detected in one or more of the APC, CTNNB1, FBXW7, HNF1A, KRAS, MTOR, NRAS, PIK3CA, PTEN, RB1, and TP53 genes. In addition, 50% of biopsies from matched non-cancerous tissues were found to exhibit mutations in the PTEN, KRAS, or PIK3CA genes. These findings indicate that the detection of pathogenic mutations in oncogenes or tumor suppressor genes in benign situations is associated with the risk of developing the malignant disease. Given the advantage of identifying mutations several months or years before the onset of malignancy, this information is invaluable as it warrants closer monitoring of patients who show such molecular changes in non-cancerous uterine mass [10].

Therefore, in our study, we planned to determine the relationship between PTEN genetic variants and protein levels, which have an active role in cancer, and endometrial cancer. Identification of these variants, which are seen to be studied very limitedly, will make an important scientific contribution to the field of prevention, early diagnosis, and treatment of endometrial cancer.

In this part of our study, the relationship between PTEN IVS4 (-/+) and -9 C/G gene variants, which have substantial functions in the pathogenesis of endometrial cancer, and PTEN protein levels and the disease will be discussed.

PTEN takes charge of cell cycle regulation by preventing cells from growing and dividing too quickly [11]. The tumor suppressor gene PTEN is a gene that is frequently mutated in endometrioid-type endometrial cancers (mutation rate is 30-50%). PTEN can exert its tumor suppressor function by inhibiting very rapidly growing and dividing cells as a negative regulator in the PI3K signaling pathway and can act as a regulator in cell cycle regulation, promoting apoptosis and cell adhesion, migration, diffusion, and differentiation. Loss of PTEN activity has been described in several human malignant cancers. [11-13].

PTEN inactivation occurs as a result of a mutation and results in loss of expression [14]. It has been stated that mice with homozygous deletion of the PTEN gene die in the early stages of embryogenesis, while those with heterozygous deletion show normal development but pose a risk for many cancers, including endometrial carcinoma [15-17].

Sun et al. (2014), in a meta-analysis study investigating whether the PTEN IVS4 polymorphism is associated with cancer risk, including seven case-control studies, individuals with the PTEN IVS4 (-/-) genotype compared to the (+/+) genotype have an increased risk of cancer and digestive system cancer shown to be significantly related. Allele analysis also detected that the (-) allele was substantially associated with increased cancer risk and gastrointestinal (tract) cancer compared to the (+) allele. In our study, it was determined that the (-/-) genotype and (-) allele were higher in endometrial cancer patients in comparison to the control, but this difference was not statistically important. In studies where the number of samples is increased, this difference may become significant as stated in the meta-analysis [18].

The PTEN IVS4 polymorphism (rs3830675), formed by ATCTT insertion 109 bp downstream of exon 4, is one of the common PTEN polymorphisms. Recently, there are some studies investigating the association of PTEN IVS4 polymorphism with different cancer risks. Recently, there are some studies investigating the association of PTEN IVS4 polymorphism with different cancer risks. The PTEN IVS4 polymorphism has been associated with several types of cancer. Wang et al. (2015) showed in their meta-analysis studies that the PTEN IVS4 (-/-) genotype was substantially associated with cancer risk, especially for tract cancer, compared to the (+/+) genotype. Moreover, the (-) allele of the PTEN IVS4 polymorphism was significantly associated with cancer risk, especially for digestive cancer, compared to the (+) allele. No significant association was observed between PTEN IVS4 (+/–) genotype and cancer risk [19].

Another study aimed at investigating the relationship between the PTEN IVS4 polymorphism and gastric cancer (GC) risk included 93 patients with GC and 113 healthy controls, and a hospital-based case-control study was made. As in our study, individuals were selected from the Turkish population and the PTEN IVS4 (rs no: 3830675) polymorphism was determined using the PCR-RFLP analysis used in our study. The PTEN IVS4 (-/-) genotype exhibited a significantly higher risk for GC compared to controls. Analyzes of clinicopathological parameters showed that PTEN IVS4 genotypes were not associated with any variant of patients with GC. As a result, it was determined that PTEN IVS4 polymorphism may contribute to the development of GC in the Turkish population [20].

In our study, individuals with PTEN IVS4 (+/+) genotypes were not determined in the Turkish population, and no significant difference was found for (-/-) and (+/-) genotypes when control and endometrial cancer individuals were compared. For the -9 C/G variant of the PTEN gene, all individuals were found to have the ancestral CC genotype, and

this region was defined as a conserved region. In our study, by the literature findings, significantly lower PTEN serum levels were determined in patients with endometrial cancer in comparison to the control.

As a result, PTEN serum levels were determined to be substantially lower in patients with endometrial cancer compared to controls, and it can be used as an important biomarker for endometrial cancer. PTEN IVS4 (-/+) and -9 C/G variations are not associated with endometrial cancer risk in individuals originating from Turkey. However, studies in larger populations related to the PTEN gene are needed.

Further studies, including a comparison of PTEN IVS4 polymorphism with plasma and tissue expressions of PTEN in larger study size groups, will enable further evaluation of PTEN IVS4 polymorphism in endometrial cancer patients.

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References

1. Amant, F., *Moerm an P, Neven P,[et al.]. Endometrial cancer.* Lancet, 2005. **366**(9484): p. 491-505.
2. Cibula, D., *Onkogynekologie*. 2009: Grada Publishing as.
3. Ambros, R.A., et al., *Endometrial intraepithelial carcinoma: a distinctive lesion specifically associated with tumors displaying serous differentiation.* Human pathology, 1995. **26**(11): p. 1260-1267.
4. Ortega-Molina, A. and M. Serrano, *PTEN in cancer, metabolism, and aging.* Trends in Endocrinology & Metabolism, 2013. **24**(4): p. 184-189.
5. Gougelet, A., et al., *Estrogen receptor α and β subtype expression and transactivation capacity are differentially affected by receptor-, hsp90-and immunophilin-ligands in human breast cancer cells.* The Journal of steroid biochemistry and molecular biology, 2005. **94**(1-3): p. 71-81.
6. Luongo, F., et al., *PTEN tumor-suppressor: the dam of stemness in cancer.* Cancers, 2019. **11**(8): p. 1076.
7. Álvarez-Garcia, V., et al. *Mechanisms of PTEN loss in cancer: It's all about diversity*. in *Seminars in Cancer Biology*. 2019. Elsevier.
8. Jamaspishvili, T., et al., *Clinical implications of PTEN loss in prostate cancer.* Nature Reviews Urology, 2018. **15**(4): p. 222-234.
9. Hollander, M.C., G.M. Blumenthal, and P.A. Dennis, *PTEN loss in the continuum of common cancers, rare syndromes and mouse models.* Nature Reviews Cancer, 2011. **11**(4): p. 289-301.
10. Lupini, L., et al., *Molecular biomarkers predicting early development of endometrial carcinoma: A pilot study.* European Journal of Cancer Care, 2019. **28**(6): p. e13137.
11. Reddy, P., et al., *Oocyte-specific deletion of Pten causes premature activation of the primordial follicle pool.* Science, 2008. **319**(5863): p. 611-613.
12. Djordjevic, B., et al., *Clinical assessment of PTEN loss in endometrial carcinoma: immunohistochemistry outperforms gene sequencing.* Modern pathology, 2012. **25**(5): p. 699-708.
13. Ozturk, O., et al., *HER2 Ile655Val and PTEN IVS4 polymorphisms in patients with breast cancer.* Molecular biology reports, 2013. **40**(2): p. 1813-1818.
14. Bansal, N., V. Yendluri, and R.M. Wenham, *The molecular biology of endometrial cancers and the implications for pathogenesis, classification, and targeted therapies.* Cancer control, 2009. **16**(1): p. 8-13.
15. Suzuki, A., et al., *High cancer susceptibility and embryonic lethality associated with mutation of the PTEN tumor suppressor gene in mice.* Current biology, 1998. **8**(21): p. 1169-1178.
16. Cristofano, A.D., et al., *Pten is essential for embryonic development and tumour suppression.* Nature genetics, 1998. **19**(4): p. 348-355.
17. Wu, H., V. Goel, and F.G. Haluska, *PTEN signaling pathways in melanoma.* Oncogene, 2003. **22**(20): p. 3113-3122.
18. Sun, L., et al., *Association between PTEN Gene IVS4 polymorphism and risk of cancer: a meta-analysis.* PloS one, 2014. **9**(6): p. e98851.
19. Wang, Y., et al., *Impact of PTEN IVS4 polymorphism (rs3830675) on cancer susceptibility: an updated meta-analysis.* Cancer Genomics & Proteomics, 2015. **12**(5): p. 263-269.
20. Canbay, E., et al., *Increased gastric cancer risk with PTEN IVS4 polymorphism in a Turkish population.* Genetic testing and molecular biomarkers, 2013. **17**(3): p. 249-253.