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# Investigation of the Profile Promotor Methylation of the E-Cadherin Gene in Patients with Pterygium

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**Abstract:** Pterygium is one of the most controversial ocular diseases whose pathology is not yet known. Although it is defined as a benign lesion, it behaves similar to tumor formation due to epithelial and fibrovascular overgrowth on the ocular surface. DNA methylation of specific genes as an epigenetic regulatory mechanism plays critical roles in the pathogenesis, progression, diagnosis and treatment of many cancers. The E-cadherin gene is involved in cell metabolism and is known to be associated with multiple tumor malignancies. In this study, we evaluated the possible relationship of the e-cadherin gene methylation profile with pterygium. E-cadherin gene promoter methylation profile was determined using methylation specific PCR (MSP) method in tissue samples of a total 36 patients with pterygium. According to the data obtained, the percentage of methylation of the E-cadherin gene in tissues with pterygium was statistically significant compared to the control group ($p<0,005$). Methylation in the E-cadherin gene is thought to be related to pterygium formation and development. 
**Keywords:** Pterygium; E-Cadherin; MSP; DNA Methylation.

## 1. Introduction

Pterygium is defined as the wing-shaped fibrovascular growth of the conjunctiva along the limbus on the cornea (1). The etiology of pterygium, which is a common disease in many parts of the world, is still unknown (2). It is seen that it is more common in sunny, hot, windy, dusty climates and tropical-subtropical regions. These locations are equatorial regions, which have been associated with greater exposure to ultraviolet (UV) radiation (3). Symptoms of pterygium include burning, irritation, eye redness, decreased vision due to the occlusion of the visual axis due to migration of pterygium tissue over the central cornea, and irregular astigmatism (4). Pterygium is a relatively benign formation. However, after removal, aggressive recurrence and locally invasiveness, various degrees of abnormalities ranging from mild dysplasia to cancer, and tumor-like features caused the disease to be referred to as a neoplastic-like growth disorder.

As a matter of fact, as a result of studies conducted by many researchers, it has been shown that preneoplastic lesions may be associated with pterygium (5-6). Pterygium is characterized by inflammation, angiogenesis, cell proliferation, fibrosis, disruptions in the extracellular matrix (ECM) of the conjunctiva, and increasingly corneal invasion (7-8).

E-cadherin, known as epithelial-cadherin, is encoded by the CDH1 gene. CDH1 gene is localized on the 16. chromosome (16q22.1) and covers an area of approximately 100kb (9). E-cadherin is expressed as a well known tumor suppressor gene (10). Abnormal methylations occurring in the promoter regions of tumor suppressor genes and silencing of related genes play crucial roles in elucidating the pathology of most human cancers (11). Hypermethylation of CpG islands in the promoter region of the E-cadherin gene (CDH1) is considered to be one of the factors contributing to the

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inactivation of E-cadherin (12). E-cadherin function as calcium-dependent cell adhesion molecules and mediates the formation of cell connections (13). For this purpose, in our study, we evaluated the methylation of the E-cadherin gene promoter region in terms of pterygium, a common eye condition that exhibits tumor-like behavior.

## 2. Material and Methods

*2.1 Collection of tissues*

36 patients (13 females, 23 males) who applied to Tokat Gaziosmanpaşa University Hospital Ophthalmology Outpatient Clinic and were diagnosed with pterygium were included in this study. Conjunctival tissue belonging to the same eye of the same patient was used as the control group. It was ensured that all patients did not experience any corneal discomfort such as glaucoma and uveitis in their medical history. With the permission and knowledge of the participants included in the study, tissue pieces taken during the surgery were appropriately labeled and quickly frozen in liquid nitrogen for DNA isolation and subsequently methylation studies and kept at -80 ° C until the time of study. A portion of tissue was separated for histopathological examination and the diagnosis of pterygium was confirmed.

The necessary permission for the study was obtained by the Tokat Gaziosmanpaşa University Clinical Research Ethics Committee at its meeting on 02.04.2019 with the project number 19-KAEK-090.

The demographic data of the patients are shown in Table 1.

**Table 1:** Demographic data of pterygium patients.

| | Pterygium (n = 36) |
|---|---|
| Age | 56±13,29 |
| Gender | 13 Women / 23 Men |
| Right / Left Eye | 24 Right / 12 Left Eye |

*2.2. Genomic DNA Isolation from Tissue*

GeneALL Clinic SV Mini (108-101) brand kit was used for DNA isolation from the tissue, and the protocol stipulated by the manufacturer was applied for the isolation steps. Measurements were made using ABP iQuant ™ dsDNA HS Assay Kit for purity and concentration of isolated DNA.

*2.3. Methylation-Specific PCR (MSP)*

The modification of the isolated and measured DNAs was carried out using the EZ DNA Methylation-Gold Kit (Zymo D5005 & D5006) in accordance with the manufacturer's instructions. From the modified DNAs, the E-cadherin gene promoter region was used with primers suitable for the MSP stage. Primary sequence information used for the study is given in Table 2 below. The applied PZR program was carried out as 2 minutes at 95 °C, 30 seconds at 95 °C, 30 seconds at 55 °C, 30 seconds at 72 ° C, 10 minutes at 72 °C, 45 cycles. PCR samples were run on a 2% agarose gel.

**Table 2:** E-cadherin gene primer sequences.

| | |
|---|---|
| **CDH1-M-F** | TTAGGTTAGAGGGTTATCGCGT |
| **CDH1-M-R** | TAACTAAAAATTCACCTACCGAC |
| **CDH1-UM-F** | TAATTTTAGGTTAGAGGGTTATTGT |
| **CDH1-UM-R** | CACAACCAATCAACAACACA |

*2.4. Statistical analysis*

According to the E-cadherin gene promoter region agarose gel electrophoresis images, the ratings of the tissue samples were expressed as fully methylated, semi-methylated and non-methylated. In determining the methylation percentage, the semi-methylated ones were calculated by adding them to the fully methylated ones. Statistical analysis of methylation data was done with the $\chi 2$ test. The statistical significance level was accepted as 0.05, if $p \leq 0.05$ was significant, and if $p > 0.05$, it was evaluated that there was no difference.

## 3. Results

*3.1. MSP gel images*

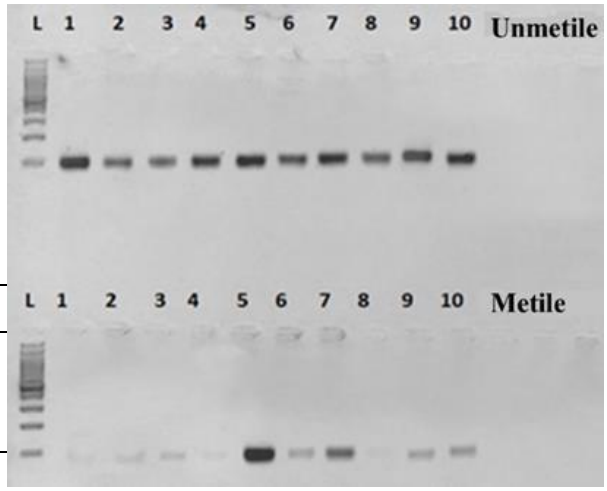


**Figure 1:** E-cadherin gene agarose gel image after MSP analysis. 2% agarose gel image after MSP analysis of the E-cadherin gene promoter region. L: ladder (100bp), wells 1-5: conjunctival tissue, wells 6-10: pterygium tissue.

When the methylation percentages of E-cadherin gene promoter region were compared with pterygium and control conjunctival tissues, 72.09% of the patients with pterygium have methylation, 27.9% of the patients with pterygium have no methylation. In conjunctival tissue, methylation was not observed in 95.14%, while methylation was observed in 4.85% (p=0.024). According to MSP analysis data, methylation percentage values between pterygium and conjunctival tissues were found to be statistically significant. When both pterygium and conjunctival tissues were compared within themselves, the methylation percentages for both groups were found to

be significantly lower. The percentage of methylation in tissues with pterygium was higher than in conjunctival tissue. Based on this, we see that the rate of methylation increases in tissues with pterygium compared to the conjunctival tissue (Table 3).

**Table 3:** Methylation percentages and statistical analysis of the E-cadherin gene promoter region.

| Tissue | Methylation percentage (%) | |
|---|---|---|
| | UM | M |
| Pterygium | 26 (72,09%) | 10 (27,9%) |
| Conjunctiva | 34 (95,14%) | 2 (4,85%) |
| *P* | ***0,024*** | |

The numbers and percentages of methylation degrees are given. Statistically significant data are expressed in bold. M: methylated UM: unmethylated.

## 4. Discussion

Molecular changes in pterygium are similar to molecular changes occurring in tumor cells. There are even opinions arguing that the mechanism of pterygium and oncogenesis is very similar. Methylation, a well-known epigenetic change, acts as a very important alternative to genetic differences in terms of gene inactivation. Abnormal methylations occurring in promoter regions of tumor suppressor genes and silencing of related genes play crucial roles in elucidating the pathology of most human cancers (11). Hypermethylation of CpG islands in the promoter region of the E-cadherin gene (CDH1) is considered to be one of the factors contributing to the inactivation of E-cadherin (12). E-cadherin promoter hypermethylation is involved in many different types of cancer events (14). In a study conducted by Young et al. In patients with pterygium, it was found that hypermethylation of the promoter region of the E-cadherin gene contributed to the decrease in E-cadherin protein expression (11). This situation causes abnormal promoter hypermethylation of tumor suppressor genes such as E-cadherin to turn the related gene into inactive form, causing the gene to deteriorate its function and thus progress in the process leading to cancer. Altered expression of E-cadherin can be cause loss of contact inhibition and abnormal cell proliferation. In this study, we evaluated the E-cadherin gene promoter region methylation in terms of pterygium, a common eye disorder that behaves similar to tumorigenesis. According to the data obtained, methylation percentage values between pterygium and conjunctival tissues were found to be statistically significant ($p = 0.024$). In other words, E-cadherin gene promoter methylation was observed to increase significantly in tissues with pterygium compared to the control group. Consistent with other cancer and its derivative studies, this increase in the rate of methylation in tissues with pterygium suggests a relationship between the disease and the gene E-cadherin. Because the E-cadherin gene is irregular promoter hypermethylations impair the expression of the gene. Abnormal expression of such a key tumor suppressor gene can lead to disruption of many important biological processes, including cell-cell connections, epithelial-mesenchymal transition (EMT), and cytoskeleton, resulting in cancer.

## 5. Conclusions

Hypermethylation of the promoter region of E-cadherin, a tumor suppressor gene, has been associated with pterygium disease exhibiting behaviors similar to cancer formation. In this sense, we believe that our study results shed some light on the molecular-based uncertainty of pterygium, whose pathology is still controversial.

**Conflict of Interests**

The authors declare no conflict of interest.

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**Author Contributions**

Ensari E, developed the concept and designed the manuscript: 70%; Aateş Ö, provided key information and help revise the manuscript: 30%.

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