# Endocan Overexpression in Pterygium

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**Purpose:** The aim of this study was to evaluate the possible role of endocan in the pathogenesis of pterygium.

**Methods:** The study was conducted on 33 patients with primary pterygium and 20 control subjects with normal bulbar conjunctiva. Patients with pterygium were graded into 3 groups as atrophic, fleshy, and intermediate, according to the Tan classification. Primary nasal pterygia and normal bulbar conjunctivas were surgically removed. Endocan expression was immunohistochemically investigated.

**Results:** Endocan expression in epithelial and endothelial cells was statistically significantly higher in pterygium tissues than control tissues ($P = 0.001$). No significant correlation was observed between pterygium classification groups and endocan expression in both epithelial and endothelial cells ($P > 0.05$).

**Conclusions:** The results suggest that endocan may have a role in the pathogenesis of pterygium.

**Key Words:** angiogenesis, endocan, inflammation, pterygium

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Pterygium is a common ocular surface disorder. It is characterized by corneal overgrowth of degenerative and proliferative conjunctival tissue. The pathophysiology is still unclear, but significant factors include chronic inflammation and angiogenesis.[1,2] Ultraviolet (UV) light exposure is the trigger factor, as the induced inflammatory cytokines can lead to events that precede pterygium development such as inflammation, cellular proliferation, and neovascularization.[3,4] The cytokines believed to play such a role include interleukin 1 (IL-1), IL-6, and IL-8, vascular endothelial growth factor (VEGF), tumor necrosis factor α (TNFα), platelet-derived growth factor, basic fibroblast growth factor, and transforming growth factor β (TGFβ).[3–8]

Endocan, derived from vascular endothelial cells, is a novel human endothelial cell–specific molecule.[9] It has a possible but unclear role in many conditions such as inflammation, endothelial dysfunction, and angiogenesis. Its regulation is also not clear, but cytokines and growth factors are believed to be involved. VEGF-A and VEGF-C have been shown to be actively involved in endocan expression.[10] Endocan is normally expressed in many human tissues such as kidney, lung, gastrointestinal tract, thyroid gland, epididymis, liver, and lymph nodes, and the expression increases in various diseases associated with inflammation and angiogenesis.[9–11]

We hypothesized that endocan could play a role in pterygium pathogenesis, as it is active in inflammation and angiogenesis. We are not aware of any other study evaluating endocan expression in patients with pterygium. Our aim was to evaluate the possible role of endocan in the pathogenesis of pterygium.

## MATERIALS AND METHODS

The study was conducted on 33 subjects with primary pterygium and 20 controls at the Ahi Evran University Training and Research Hospital. We excluded patients with recurrent pterygium; history of any other ocular disorder or surgery such as glaucoma, corneal trauma, corneal scarring, uveitis, or severe dry eye; any systemic disorder, systemic inflammation, or clinical condition; pregnancy; atopy; and those currently using any topical or systemic medication or antiinflammatory/antioxidant treatment. We obtained approval for the study from the local ethics committee and also voluntary informed consent from the subjects. The study adhered to the principles of the Helsinki Declaration.

The patients with pterygium were graded into 3 groups as atrophic, fleshy, and intermediate, according to the classification by Tan et al.[12] Grade 1 was the atrophic group in which the episcleral vessels under the pterygium were not obscured and could be clearly distinguished. Grade 3 was the fleshy group in which the episcleral vessels were totally obscured. Grade 2 was the intermediate group including all other pterygia that did not fall into the other 2 categories.

Surgical excision was performed on 33 primary nasal pterygia. Only the pterygium head was taken for immunostaining, and pterygial specimens were cut perpendicular to the axis of ptyerygia. We also obtained 20 normal conjunctival tissue segments as control tissue from the nasal bulbar conjunctiva near the limbus during cataract surgeries. Both pterygia and control tissues were fixed in 10% buffered formaldehyde solution and embedded in paraffin.

### Immunohistochemistry

The tissues were cut into 4-μm sections and then deparaffinized and labeled using a BenchMark XT automat device (Ventana Medical Systems, Tucson, AZ). A kit

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(ultraView Universal DAB Detection Kit; Ventana Medical Systems Inc) was used according to the recommended protocol. Immunohistochemistry investigations were performed using the anti-endocan mouse monoclonal antibody (ab56914; Abcam, Cambridge, MA) at a concentration of 3 μg/mL on formalin-fixed and paraffin-embedded tissues. Independent evaluation of the staining was performed by a pathologist masked to the clinical findings and the nature of the specimens. We considered all brown-stained endothelial and epithelial cells positive.

We evaluated immunoreactivity of endocan in both epithelial and endothelial pterygium cells and healthy conjunctival tissues. Endocan expression in epithelial cells was assessed with a previously described scoring system that combines (1) staining intensity (0: negative, 1: weak, 2: intermediate, and 3: strong staining) and (2) the percentile quadrants of positive cells (0: 0%, 1: 1%–25%, 2: 26%–50%, and 3: >50%) with a maximum score of 6.[5] Scores of 1 and 2 were weak, 3 intermediate, and 4 to 6 strongly positive. Endocan expression in endothelial cells was evaluated with a semiquantitative score, according to the previously described method as 0, no expression; +, focal expression; and ++, diffuse expression.[5]

## Statistical Analysis

Data analysis was conducted with SPSS software version 22.0, and the $\chi^2$ test was used to compare endocan expression levels between the groups. The relationship between endocan expression levels and pterygium classification groups was evaluated with the Spearman rho correlation test. The results were accepted as statistically significant when $P$ values were smaller than 0.05.

# RESULTS

Mean age was 44.3 ± 9.9 years in patients with pterygium and 47.9 ± 4 years in the control subjects. The patients with pterygium consisted of 17 men and 16 women, and the control subjects consisted of 14 men and 6 women. There was no statistically significant difference between both groups according to the age and sex distribution ($P = 0.133$ and $P = 0.186$, respectively). The pterygium group consisted of 8 eyes with atrophic, 14 with intermediate, and 11 with fleshy pterygium. Endocan expression in epithelial cells was weak in 1 and strong in 7 in the atrophic group, strong in 14 in the intermediate group, and weak in 1, intermediate in 1, and strong in 9 in the fleshy group. Endocan expression in endothelial cells was focal in 3 and diffuse in 5 in the atrophic group, focal in 6 and diffuse in 8 in the intermediate group, and focal in 2 and diffuse in 9 in the fleshy group. No significant correlation was observed between pterygium classification groups and endocan expression in both epithelial and endothelial cells ($P > 0.05$).

We found endocan expression in epithelial cells to be statistically significantly higher in pterygium tissues than control tissues ($P = 0.001$) (Fig. 1). Endocan expression in endothelial cells was also statistically significantly higher in pterygium tissues ($P = 0.001$) (Fig. 1). The endocan expression in epithelial and endothelial cells is presented in Table 1.

# DISCUSSION

Epidemiological studies have revealed a positive relationship between pterygium and UV light exposure.[13,14] It is believed that the characteristic localization of pterygium is the result of UV light that is focused on the medial limbus. UV light is known to produce inflammatory corneal changes that can be both acute and chronic.[4] It has been shown to act as the initial trigger activating epithelial cells for cytokine production (IL-1, IL-6, IL-8, TNFα, TGFβ, and VEGF), ultimately resulting in pterygium development.[3,4,15]

Pterygium pathogenesis is reported to include several proinflammatory cytokines and angiogenic growth factors together with their receptors.[3–8] These molecules also play a role in normal corneal wound healing. Proinflammatory cytokines such as TNFα and IL-1b can be produced by conjunctival epithelial cells in tissue cultures, and these then stimulate fibroblastic proliferation of the Tenon capsule.[16] Related immunoreactivity has been found in pterygium epithelial cells, vessel endothelial cells, epithelial and vessel basement membranes, fibroblasts, and infiltrating inflammatory cells.[7] A cascade of inflammation, proliferation, angiogenesis, and antiapoptosis is the result, leading to pterygium formation.[3] Fukuhara et al[17] found that lymphangiogenesis also played a role in pterygium pathogenesis. An interesting result was that vascular endothelial growth factor receptor-2 (VEGFR-2) levels in both epithelial and endothelial cells significantly correlated with the postoperative recurrence grade.[18] VEGF is a very important proangiogenic factor and may therefore have a significant role in pterygium pathogenesis.

The regulatory mechanisms for the production of endocan, a soluble chondroitin/dermatan sulfate proteoglycan, remain unclear, but many growth factors and cytokines

**TABLE 1.** Endocan Expression in Epithelial and Endothelial Cells

| Location | Expression | Pterygium (n = 33) | Control Tissues (n = 20) | *P* |
|---|---|---|---|---|
| Epithelial, n (%) | No expression | — | 1 (5) | 0.001 |
| | Weak | 2 (6.1) | 8 (40) | |
| | Intermediate | 1 (3) | 3 (15) | |
| | Strong | 30 (90.9) | 8 (40) | |
| Endothelial, n (%) | No expression | — | 2 (10) | 0.001 |
| | Focal | 11 (33.3) | 15 (75) | |
| | Diffuse | 22 (66.7) | 3 (15) | |

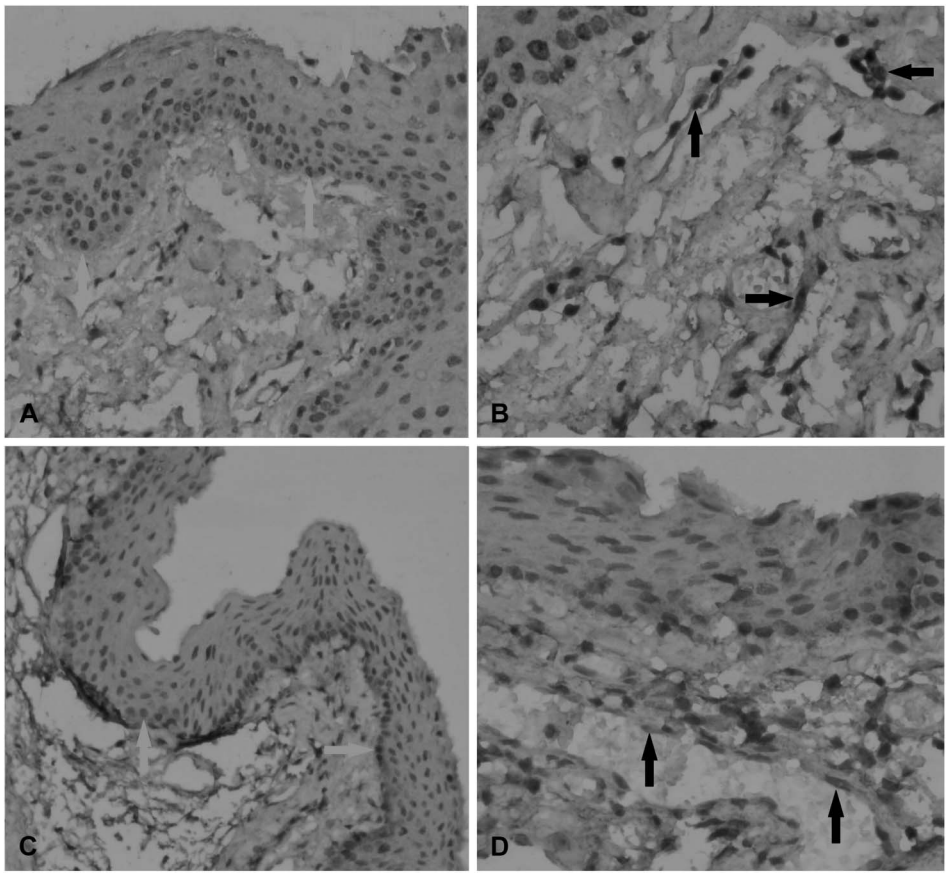


**FIGURE 1.** Immunostaining of epithelial and endothelial cells in pterygium (A, B) and normal conjunctiva (C, D) with mouse monoclonal endocan antibodies. A, (original magnification ×200): Strong immunoreactivity can be seen along with epithelial cells in pterygium (orange arrows). B, (original magnification ×400): Diffuse immunostaining is seen in endothelial cells of pterygium (black arrows). C, (original magnification ×200): Weak immunoreactivity is seen generally in basal epithelial cells of normal conjunctiva (orange arrows). D, (original magnification ×400): Right black arrow shows focal immunostaining, and left black arrow shows no immunostaining in endothelial cells of normal conjunctiva.

are believed to play a role.[9] Upregulation has been reported with VEGF-A, VEGF-C, IL-1, TNFα, TGFβ-1, and fibroblast growth factor–2 and downregulation with interferon-γ and phosphatidylinositide 3-kinases.[9,10,19] VEGF-A and VEGF-C increase endocan expression, and endocan increases their mitogenic and promigratory activities, an example of an autocrine positive feedback loop.[20] Endocan is known to play a role in the regulation of cell adhesion, migration, proliferation, and neovascularization. Endothelial cell activation and neovascularization, as seen in tumor progression and inflammation, have been reported with increased endocan tissue expression or serum levels.[10]

An increasing number of studies have focused on clarifying the physiopathological mechanisms of endocan in various disorders. Inflammation, angiogenesis, sepsis, and cancer are just some of the conditions and disorders with endocan overexpression.[9,21–25] Activation of hypoxia-inducible factor signaling leads to increased VEGF secretion in angiogenesis, and endocan is known to be active in mediating the effects of VEGF in promoting vascular growth.[10] Lung, colorectal, and hepatocellular cancer are just some of the cancers in which endocan messenger RNA levels have been found to indicate survival and tumor progression.[21–23] Endocan blood levels have been shown to reflect the patient's condition and prognosis in patients with sepsis.[24,25] Endocan was also suggested as a novel mediator of lymphangiogenesis. It was emphasized that it could be a potential target for the inhibition of pathologic lymphatic vessel growth induced by VEGF-A or VEGF-C.[20] In short, blood and tissue endocan levels have been shown to be biomarkers associated with angiogenesis and inflammation in various disorders.

Chronic inflammation, angiogenesis, and lymphangiogenesis play a major role at different levels in the pathogenesis of pterygium, with endocan having a significant role in all these processes.[1,2,9,10,17,20] Endocan is also closely associated with VEGF, for which increased expression has been reported in pterygium tissues with immunohistochemical studies.[5,8] Intralesional anti-VEGF injections for pterygium treatment have been found to reduce symptoms and vascularity in several studies.[26] VEGFR-2 expression is also correlated with postoperative pterygium recurrence.[18] VEGF is therefore a very important proangiogenic factor and plays a significant role in pterygium pathogenesis. However, some other cytokines and growth factors such as IL-1, TGFβ, and TNFα, which cause endocan upregulation, show more intensive immunostaining in pterygium tissues than in healthy conjunctivas.[4,7,8] We similarly found more endocan expression in pterygium tissues than in healthy conjunctivas.

Based on the current literature, endocan showed higher expression in the tissue when neovascularization and inflammation were present. We evaluated endocan expression in the epithelial and endothelial cells in pterygium tissues and compared the results with those of healthy conjunctivas.

Endocan was found to be overexpressed in both epithelial and endothelial cells. It is therefore possible for endocan to promote pterygium growth through interactions with cytokines and growth factors.

In conclusion, our results suggest that endocan may have a role in the pathogenesis of pterygium. However, the interactions between endocan and other cytokines and growth factors in pterygium development are still unclear, and their clarification requires further studies.

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