Original Article

Expression of CD44, PCNA and E-cadherin in pterygium tissues

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Purpose: Pterygium is a common ocular surface disease defined by fibrovascular conjunctival growth extending onto the cornea. However, its pathogenesis remains unclear. This study aimed to determine the role of CD44, proliferating cell nuclear antigen (PCNA), and E-cadherin in pterygium formation and recurrence. **Methods:** Sixty patients with pterygium participated in the study, and we collected conjunctival samples from 30 patients to form a control group. CD44, PCNA, and E-cadherin expressions in surgically excised pterygium were compared with tissue samples from the control group. **Results:** We observed that the percentages of CD44 and PCNA were statistically higher in the primary pterygium group and recurrent pterygium group than in the control group ($P < 0.001$ and $P < 0.001$, respectively). Conversely, E-cadherin values were statistically higher in the control group than in the primary and recurrent pterygium groups ($P = 0.013$ and $P < 0.001$, respectively). **Conclusion:** Cell proliferation and cell adhesion factors may play important roles in the pathogenesis of pterygium.

Key words: CD44, E-cadherin, immunohistochemistry, PCNA, pterygium

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Pterygium is a common ocular surface disease defined by triangle-shaped fibrovascular conjunctival growth extending onto the cornea.[1] In severe cases, it may extend into the central cornea, inducing irregular corneal astigmatism and causing loss of vision.[1] The pterygium usually occurs nasally in the palpebral fissure, but it may also occur temporally or bilaterally. Although the pterygium's head is firmly attached to the cornea, its body is loosely attached to the bulbar conjunctiva.

The pathogenesis of pterygium has yet to be fully elucidated. The main etiological factor for pterygium development is ultraviolet light,[2,3] and the prevalence of pterygium increases toward the equator. The other causative factors suspected in the pathogenesis of pterygium include human papillomavirus infection, inflammation, chronic irritation, angiogenesis, lymphangiogenesis, and genetics.[2-6] Pterygium tissue resembles tumoral tissues in that it invades the cornea, recurs after resection, and exhibits cell proliferation. With the progression of pterygium, patients develop foreign body sensation, redness and itching due to inflammation, blurred vision, and astigmatism due to its progression to the corneal surface toward the optic axis.[7]

Surgical excision is the preferred treatment model for pterygium.[8] The bare sclera technique used in the past is no longer preferred because of its high recurrence rate. Conjunctival autograft, limbal autograft, amniotic membrane graft, and the use of antifibrotic agents are the preferred methods of treatment.[3,9]

CD44 (phagocytic glycoprotein-1) is a member of a family of non-kinase, structurally changeable, and multifunctional transmembrane glycoproteins that are expressed to various extents in other cell types, including embryological stem cells, connective tissues, and bone marrow.[10] CD44 is involved in the organization of certain cellular processes, for instance, cell adhesion, division, and migration, by binding with its main ligand, hyaluronic acid.[11]

Proliferating cell nuclear antigen (PCNA) is a proliferation marker in the nucleus. PCNA plays a very important role in DNA synthesis, repair, and cell cycle regulation.[12] It is used as a proliferation marker in some cancer types.[13]

E-cadherin is a calcium-dependent transmembrane glycoprotein that plays a significant role in the protection of tissue integrity and cell-to-cell adhesion.[1] E-cadherin functions as a tumor suppressor protein by regulating cell activities.[14] Reduced expression of E-cadherin in many cancer types correlates with poor prognosis and the potential for metastasis.[15,16]

The present study aimed to investigate the expression patterns of CD44, PCNA, and E-cadherin in both control conjunctiva and pterygium tissue.

Methods

Sixty patients with pterygium participated in the study. The pterygia patients were divided into primary and recurrent

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groups, and we collected conjunctival samples from 30 patients to use as a control group. Tan *et al*.[17] graded pterygium according to tissue transparency and classified it into three types by using the visibility of episcleral vessels as a sign of translucency. Type 2 pterygiums were included in the current study based on the grading system of Tan *et al*.[17]

The patients were informed about the surgical procedures to be performed and provided written consent. The research adhered to the ethical principles stated in the Declaration of Helsinki. Prior to commencing the study, the necessary approval was sought and obtained from the Clinical Research Ethics Committee of …… University Faculty of Medicine. Autograft pterygium surgery was performed under local anesthesia. The pterygium tissue was removed from the cornea surface, and an autograft was taken from the superior bulbar conjunctiva. The graft was then sutured with 8-0 Vicryl onto the scleral bed. During autograft pterygium surgery, conjunctival samples were collected from the upper temporal fornix region of the patients in the control group.

Immunohistochemistry

The specimens were immersed in containers filled with 10% formalin and then embedded in paraffin. Thin sections measuring 4 µm were extracted from the samples and placed on glass slides with barcodes for identification. Following the process of deparaffinization and rehydration, the sections were subjected to histopathological evaluation through the use of hematoxylin and eosin staining.

We used primary antibodies against CD44 (1:50 dilution) and E-cadherin (1:200 dilution), and a human monoclonal antibody against PCNA (1:100 dilution) for antigen retrieval. Histopathological and immunohistochemical staining findings were reported using a Nikon light microscope and an image analysis system (Nikon Instruments Europe BV). At least 100 cells were counted, including immuno-positive cells, at a 20× magnification in three distinct microscopic areas of the pterygium tissue. The number of stained pixels reflects the intensity of staining of immunopositive cells and can be expressed as a percentage of the entire image pixel amount. The immunoreactivity level was evaluated based on the number of pixels, with fewer than 80 pixels indicating a weak (1+) reaction, 80–200 pixels indicating moderate (2+) reactivity, and more than 200 pixels indicating strong (3+) reactivity. The positivity ratio was evaluated for CD44 and E-cadherin. For CD44, cytoplasmic staining was semi-quantitatively scored from 0 to 3+. For E-cadherin, cell membrane staining intensity was estimated as negative, weak, moderate, or strong.

Statistical analysis

For continuous variables, the data were presented as either the mean ± standard deviation or the median with the range (minimum–maximum) displayed. We conducted the Kolmogorov-Smirnov goodness-of-fit test to analyze the normality of the continuous variables. We employed one-way ANOVA to assess the variables that were found to be normally distributed between the three groups and the Kruskal-Wallis test for those that did not conform to normal distribution. Pairwise forward analyses (*post hoc*) were performed with the Bonferroni-corrected Mann-Whitney U test to determine in which group significance originated. The categorical variables were evaluated using the Chi-square test. The analyses were conducted using IBM SPSS version 26.0 (IBM Corporation, Armonk, NY, USA). Cases in which the Type 1 error level was below 5% were deemed statistically significant. A statistical significance level of $P < 0.01$ was accepted for the Bonferroni-corrected Mann-Whitney U test.

Results

In the control group (group 1), we collected control conjunctiva from 30 patients (16 females and 14 males) with an age range of 48–70 years (mean: 56.66 ± 7.10 years). We collected primary pterygia from 30 patients (17 females and 13 males) aged 48–72 years (mean: 59.13 ± 7.43 years) (group 2) and collected recurrent pterygia from 30 patients (16 males and 14 females) aged 45–73 years (mean: 59.06 ± 7.37 years) (group 3). There were no notable variations observed between the groups regarding age and gender ($P = 0.334$ and $P = 0.956$, respectively).

We did, however, find significant differences in the percentage of CD44, E-cadherin, and PCNA between the groups ($P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively). We observed that the CD44 and PCNA percentages were statistically higher ($P < 0.001$ and $P < 0.001$, respectively) in experimental groups 2 and 3 than in control group 1. Conversely, E-cadherin values were statistically higher in group 1 than in groups 2 and 3 ($P = 0.013$ and $P < 0.001$, respectively). We also determined that the CD44, E-cadherin, and PCNA percentages ($P = 0.022$, $P = 0.025$, and $P = 0.074$, respectively) were not statistically significantly different between group 2 and group 3 [Table 1, Figs. 1–3].

Discussion

This study examined the levels of PCNA, CD44, and E-cadherin in pterygium tissue samples and control conjunctiva. Our results indicated a significant increase in positive PCNA and CD44 rates in the pterygium samples, while the E-cadherin level was significantly lower than in control conjunctival tissue samples. These findings suggest that cell proliferation and loss of cell adhesion are involved in the pathogenesis of pterygium, a disease with tumor-like growth characteristics.[6]

Despite being considered benign, pterygium exhibits properties similar to metastatic tissues, including dense cell proliferation, inflammation, cell migration, local angiogenesis, and epithelial-mesenchymal transition.[18-20] Our research also revealed a lower amount of E-cadherin in pterygium tissues than in control conjunctival tissue. Moreover, we observed no notable difference in E-cadherin levels between primary and

Table 1: Comparison of age, gender, and CD44, E-cadherin, and PCNA percentage values between groups

	Group 1 (*n*=30)	Group 2 (*n*=30)	Group 3 (*n*=30)	*P*
Age (years)	56.66±7.10	59.13±7.43	59.06±7.37	0.334*
Sex (f/m)	16/14	17/13	16/14	0.956**
CD44	0 (0–1)	1 (1–2)[a]	1.5 (1–3)[a]	<0.001***
E-cadherin	2 (0–3)	1 (0–2)[b]	1 (0–2)[a]	<0.001***
PCNA %	1 (0–5)	12 (5–51)[a]	15 (1–54)[a]	<0.001***

*One-way ANOVA. **Chi-square test. ***Kruskal-Wallis test (*post hoc*: Bonferroni-corrected Mann Whitney *U* test, [a]*P*<0.001 vs. group 1, [b]*P*=0.013 vs. group 1)

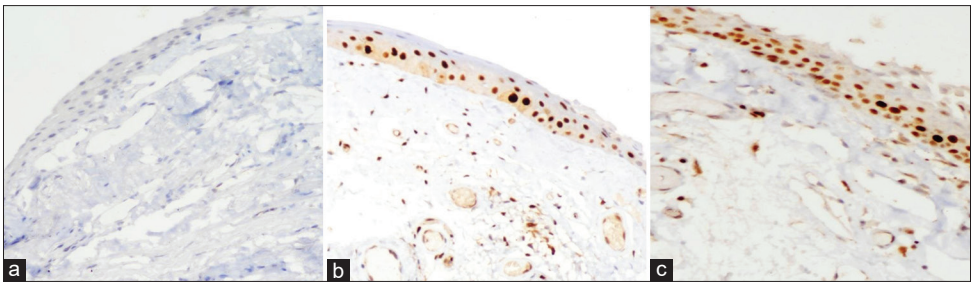

Figure 1: (a) At a magnification of 400×, the expression of PCNA can be observed in the nucleus of healthy conjunctival epithelial cells. (b) This is an image of primary pterygium tissue, showing the expression of PCNA in the nuclei of epithelial cells. The image was magnified to 200×. (c) At a magnification of 400×, it was observed that PCNA was expressed in the nuclei of epithelial cells in recurrent pterygium tissue

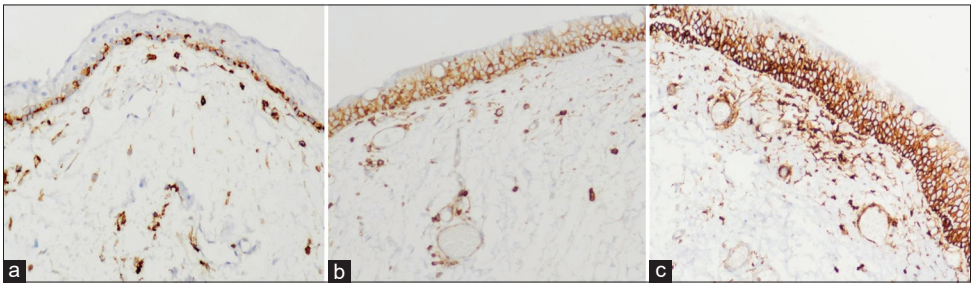

Figure 2: (a) CD44 is expressed on epithelial and stromal cells in normal bulbar conjunctiva (score 1) observed under 400 × magnification. (b) CD44 is expressed in both epithelial and stromal cells in primary pterygium (score 2, light brown) at a magnification of 400×. (c) CD44 is expressed in both epithelial and stromal cells of recurrent pterygium, shown as dark brown color and scored 3 at a magnification of 400×

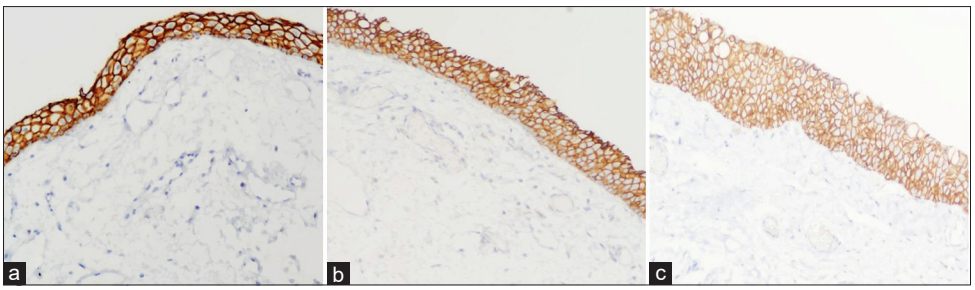

Figure 3: (a) E-cadherin is expressed in normal bulbar conjunctiva epithelial cells (score 3, dark brown, 400×). (b) The expression level of e-cadherin in primary pterygium epithelial cells is scored at 2, appearing as a light brown color when viewed at 400× magnification. (c) Expression of e-cadherin was scored 1 in light brown recurrent pterygium in both epithelial and stromal cells, observed at 400× magnification

recurrent pterygium cases. E-cadherin plays a crucial role in cell adhesion between epithelial cells, and its decreased expression can lead to tumor growth and proliferation, ultimately leading to metastasis.[21] Several studies have shown that low E-cadherin expression increases tumor progression in some types of cancer.[22,23]

PCNA is a protein associated with cell proliferation and is used as a tumor marker for some types of cancer.[24-26] Our study found that PCNA expression was significantly higher in pterygium samples than in control conjunctiva samples.[27] Cell proliferation plays a vital role in the pathogenesis of pterygium. In the study by Liang *et al*.,[27] the rates of PCNA and nuclear protein ki-67, another proliferation marker, were higher in pterygium tissue than in normal conjunctiva. PCNA was detected 72.7% in pterygium tissue and 30.4% in normal conjunctival tissue. Studies have shown that ki-67, an important component of the cell cycle, is more abundant in pterygium tissue than in normal conjunctival tissue.[28,29]

Similarly, CD44 is a protein that regulates cellular processes such as cell division, adhesion, and migration.[10] Aside from its usual cellular roles, CD44 has been identified as a significant indicator of the presence of cancer stem cells.[30,31] Studies have shown that CD44 contributes to tumor invasion and metastasis by promoting the adhesion of tumor cells to endothelial and fibronectin-enriched matrices.[32,33]

The present study had some limitations. As it was not considered ethically appropriate to take conjunctival samples from healthy eyes, we took the control group from the superior bulbar conjunctiva of eyes with pterygium. Another limitation is the small number of cases.

Conclusion

In conclusion, our research provides valuable insights into the pathogenesis of pterygium and the roles of PCNA, CD44, and E-cadherin in this disease. These findings can potentially be

used to develop new therapeutic strategies to treat pterygium and prevent relapses.

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Conflicts of interest: There are no conflicts of interest.

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