

Chemokine CXCR-4 and cyclooxygenase-2 in the pathogenesis of pterygium

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Background/aim: This study aimed to investigate the expression of chemokine receptor 4 (CXCR-4) and cyclooxygenase-2 (COX-2) in the epithelium and stroma of pterygium tissue in comparison with healthy conjunctiva.

Materials and methods: The expression of CXCR4 and COX-2 was investigated by immunohistochemistry in the epithelium and stroma of the pterygium tissue of 29 eyes and compared with healthy conjunctival tissues. The correlation between CXCR4 and COX-2 expression as well as the correlation of these markers with the area of pterygium were evaluated statistically.

Results: COX-2 staining scores were 1.75 ± 0.63 in the epithelium and 1.20 ± 0.62 in the stroma of the pterygium tissue. Mean CXCR-4 staining in the epithelium was 0.069 ± 0.37 , whereas it was 5.0 ± 2.84 cells in the stroma. There was almost no staining of COX-2 and CXCR4 in the control samples. There was a strong positive correlation between the expression of CXCR-4 and COX-2 in the stroma of the pterygium.

Conclusion: CXCR-4 and COX-2 may play important roles in the pathogenesis of pterygium.

Key words: CXCR-4, cyclooxygenase-2, pterygium

1. Introduction

Pterygium is a fibrovascular tissue growing on the ocular surface. This disease demonstrates both hyperplastic and degenerative properties, as well as some tumor-like features. Irritation of the ocular surface by excessive ultraviolet (UV) light and repeated microtrauma seem to be the leading contributing factors to pterygium development in susceptible individuals (1). UV light exposure also plays an important role in skin tumors (2). Tumor-like characteristics of pterygium, such as a propensity to invade normal tissue, high recurrence rates following resection, epithelial proliferation, goblet cell hyperplasia, angiogenesis, inflammation, elastosis, stromal plaques, Bowman's membrane dissolution, inactivation of the *p53* tumor suppressor gene, and coexistence with ocular surface neoplasms, have been reported in previous studies (3–5).

Chemokines are polypeptides of 8–14 kDa in size that have the properties of signaling molecules. CXCR-4 is a member of the chemokine receptor family. The interaction between CXCR-4 and stromal cell-derived factor (SDF-1 α), its ligand, is known to play an important role in tumor

genesis, metastasis, and angiogenesis (6,7). This biological role is demonstrated in different types of tumors including malignant melanoma, glioblastoma, and lung, breast, pancreatic, and cholangiocellular carcinomas (8–12).

UV-induced abnormal prostaglandin synthesis is reported to be an important factor in the development of cancer. Cyclooxygenase (COX) is an enzyme that catalyzes the conversion of free arachidonic acid to prostaglandins. It has two isoforms, known as COX-1 and COX-2. COX-1 is found mostly in normal tissue and is required for physiological functions (13). COX-2 is expressed only under special conditions like hypoxia or by the influence of growth factors or cytokines. COX-2 is also known to be expressed in many neoplastic processes, like stimulation of cell division, angiogenesis, and inhibition of apoptosis (14,15).

Expression of COX-2 was reported in pterygium in previous studies (16) and there is only one article regarding CXCR-4 expression in pterygium (17). Although the relation of COX-2 and CXCR-4 was investigated in skin tumors (18), to our knowledge this relationship was not studied in pterygium

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We planned this study to investigate the expression of CXCR-4 and COX-2 in the epithelium and stroma of primary pterygium tissue to determine their importance in the pathogenesis of pterygium. We also evaluated the correlation between the area of pterygium and CXCR-4 and COX-2.

2. Materials and methods

This prospective study was approved by the ethics committee of Şifa University. All procedures were performed according to the tenets of the Declaration of Helsinki, and informed consent was obtained from all patients. The authors declare no conflicts of interest.

Prior to the operation, the pterygium area was measured as mm² with a slit lamp. Samples were collected from 29 eyes of 29 patients with primary pterygium who were operated on during the period of March 2013 to February 2014. Patients with a history of ocular trauma, surgery, inflammatory or malignant ocular surface diseases, chemical burns, use of systemic or topical steroids or any eye drops in the last 6 months before surgery, and recurrent pterygia were excluded from the study. The specimens were harvested intraoperatively from each eye during standard pterygium removal surgery. All patients had pterygium excision combined with limbal conjunctival autograft by the same surgeon (GB) using a standard technique (15). The limbal conjunctival autograft was taken from the upper bulbar conjunctiva. A piece was excised from the nasal edge of the conjunctival autograft from 11 patients to make the control group samples.

2.1. Histological evaluation of CXCR-4 and COX-2

Tissues were fixed in 10% formaldehyde solution and after the tissue processing procedure were embedded in paraffin blocks. Three sections of 4 µm in thickness were taken from each paraffin block. One section was stained with hematoxylin and eosin and the other 2 positively charged slides were stained by DAKO Autostainer 48 Link (Dako, Denmark) with COX-2 (DAKO, Clone CX-294, 1/100 dilution) and CXCR-4 (Abcam, Clone ab 2074, 1/50 dilution) antibodies. The slides were then evaluated under a light microscope by two pathologists. Cytoplasmic staining was accepted as positive for both antibodies.

Epithelial COX-2 expression was scored semiquantitatively according to the percentage of positive-staining cells: 0, negative staining; +, from 1% to 10%; ++, from 11% to 50%; and +++, more than 50% positive cells. COX-2 expression in the stroma was scored for the average number of cells expressing COX-2 with the most extensive staining in the high-power field: 0, no positive cells; +, 1 to 5 positive cells; ++, 6 to 10 positive cells; and +++, more than 10 positive cells, according to the method of Park et al. (19). Positively stained cells were counted in the most intensely stained high-power field for CXCR-4

evaluation for the stroma and the epithelium. There were very few positively stained cells in both compartments, not allowing semiquantitative scoring.

2.2. Statistical analysis

SPSS 11.6 (SPSS Inc., Chicago, IL, USA) was used. The differences of sex and the level of COX-2 between the groups were analyzed by chi-square test. The age and the level of CXCR-4 of the two groups were compared with independent samples t-test. Paired samples t-tests were used to compare the epithelium and stroma regarding CXCR-4 expression. The chi-square test was used to compare the epithelium and stroma regarding the expression of COX-2. The correlation of the area of pterygium with CXCR-4 and COX-2 expression and the correlation between CXCR-4 and COX-2 expressions were evaluated by Pearson correlation analysis.

3. Results

The patients consisted of 13 men (44.8%) and 16 women (55.2%). Mean age of the patients was 51.9 ± 12.9 (range: 26–79) years. Six of the control patients were males and five were females. Mean age in the control group was 55.5 ± 16.5 (range: 26 to 79) years.

The COX-2 staining of the epithelium ranged from 1 (+) to 3 (+++) (mean: 1.75 ± 0.63). The intensity was moderate (++) to strong (+++) in 67% of the cases. The COX-2 staining of the stroma ranged from 1 (+) to 2 (++) (mean: 1.20 ± 0.62). The staining was absent in 10% of the cases, whereas weak to moderate staining was observed in 90% of the cases. In the control group, the COX-2 staining of the epithelium was 0 (+) to 1 (+) (mean: 0.18 ± 0.40) and it was 0 ± 0.0 (+) in the stromal cells.

The COX-2 expression scores were higher in the epithelium and stroma of the primary pterygium group when compared with the control group ($P < 0.001$). The COX-2 cell staining intensity was higher in the epithelial cells than the stromal cells in the pterygium tissue ($P < 0.001$).

The CXCR-4 staining in the epithelium ranged from 0 to 2 cells (mean: 0.069 ± 0.37) in only one patient. The CXCR-4 staining in the stroma ranged from 1 to 10 cells (mean: 5.0 ± 2.84). The staining of CXCR-4 was 0.00 ± 0 in the epithelium and 0 to 1 cells (mean: 0.18 ± 0.40) in the stromal cells of the control group. There was no statistically significant difference between the primary pterygium and control groups regarding CXCR-4 expression in the epithelium ($P = 0.545$). The CXCR-4 expression in the stroma of the primary pterygium group was statistically higher than that of the control group ($P < 0.001$). The CXCR-4 cell staining intensity in the stromal cells was higher than in the epithelium ($P < 0.001$) in the pterygium tissue, and it was predominantly located in perivascular areas and fibroblastic cells (Table; Figures 1a–1l).

Table. Age, sex, and CXCR-4 and COX-2 levels in pterygium and control groups.

Age	Pterygium group	Control group	P-value: 0.463
	51.9 ± 12.9	55.5 ± 16.4	
Sex	13 men/16 women	6 men/5 women	-
Area of the pterygium (mm ²)	9.2 ± 6.3	-	-
CXCR-4 level in the epithelium	0.07 ± 0.37	0.0 ± 0.0	P-value: 0.545
COX-2 in the epithelium	1.7 ± 0.6	0.2 ± 0.4	P-value: 0.001*
CXCR-4 level in the stroma	5.0 ± 2.8	0.2 ± 0.4	P-value: 0.001*
COX-2 level in the stroma	1.2 ± 0.6	0.0 ± 0.0	P-value: 0.001*

The mean area of the pterygium was $9.17 \pm 6.27 \text{ mm}^2$ ($4\text{--}24 \text{ mm}^2$). The control tissues were cut into $1 \times 2 \text{ mm}$ pieces. The correlation between the area of pterygium and the CXCR-4 of the epithelium was not significant ($R = 0.159$, $P = 0.411$). There was a moderate positive correlation between the area of pterygium and CXCR-4 expression in the stroma ($R = 0.725$, $P < 0.001$). The relation of the area of the pterygium and the expression of COX-2 in the epithelium was not significant ($R = 0.288$, $P = 0.129$). There was a strong correlation between the area of the pterygium and the COX-2 in the stroma ($R = 0.560$, $P = 0.002$). There was no correlation between the levels of CXCR-4 and COX-2 in the epithelium ($R = 0.073$, $P = 0.707$). There was a moderate correlation between the expressions of CXCR-4 and COX-2 in the stroma ($R = 0.649$, $P < 0.001$).

4. Discussion

Although the exact pathogenesis of pterygium is still unclear, chronic inflammation, angiogenesis, and uncontrolled proliferation seem to be the key mechanisms in progression (1,5,20). Several inflammatory and angiogenic factors, such as VEGF, MMP, and TGF, were suggested to be related to its pathogenesis (21–23). In the present study, we investigated the cell expressions of COX-2 and CXCR-4 in pterygium tissue. The interaction between COX-2 and CXCR-4 has been demonstrated in various types of UV-exposed malignant skin tumors and cancers (19).

COX-2 expression in human pterygium has been previously studied. Chiang et al. found that more than 80% of pterygium tissue studied showed positive expression of COX-2 in the epithelium; however, they found no COX-2 expression in the stroma (24). Subsequent studies by Adigüzel et al. (25), Karahan et al. (26), and Maxia et al. (27) reported COX-2 expression in the epithelium of primary pterygia in 60%, 84.2%, and 67.7% of the cases, respectively. Karahan and Maxia also reported COX-2

expression in the stroma. Park et al. (17) reported more intense epithelial staining of COX-2 expression and they found that stromal COX-2 expression was increased with vascularity. In our study, COX-2 expression was observed in both epithelial and stromal cells, as in the studies of Park et al., Karahan et al., and Maxia et al. (19,26,27). The COX-2-positive cell intensity in the epithelial cells was higher than that of the stromal cells in the pterygium tissue. This may be related to the early and/or predominant superficial conjunctiva epithelial inflammatory response, which is prone to UV injury. As mentioned before, COX-2 expression was reported in UV-exposed skin tumors, and considering the tumor-like characteristics of pterygium tissue, the development of pterygium might have a similar pathway to that of skin tumors (18,28).

To the best of our knowledge, CXCR-4 expression in pterygium was investigated only in one study. Kim et al. (17) demonstrated a positive correlation between the severity of pterygium and SDF-1 expression. They postulated the possible contribution of SDF-1 and CXCR-4 interaction to myofibroblastic transformation, which can be possibly restored through the downregulation of the SDF-1/CXCR-4 axis. Myofibroblasts found at the site of tissue injury are considered to play a pivotal role in the healing process. By secreting ECM proteins and the contractile protein alpha-smooth muscle actin (α -SMA), myofibroblasts promote tissue repair (29). Additionally, these cells are also reported to exist in the fibrovascular tissue of pterygia (30). Indeed, various studies reported that the development of pterygium might result from reactive wound formation triggered by oxidative stress due to UV light exposure, ocular irritation, and/or inflammation on the ocular surface (17,31,32). In our series the expression of CXCR-4 was confined to the stroma and there was a significant correlation between the body surface and CXCR-4 and COX-2 expression in the stromal part of the pterygium, which is in close contact with the surface epithelium exposed to UV light. This might activate the

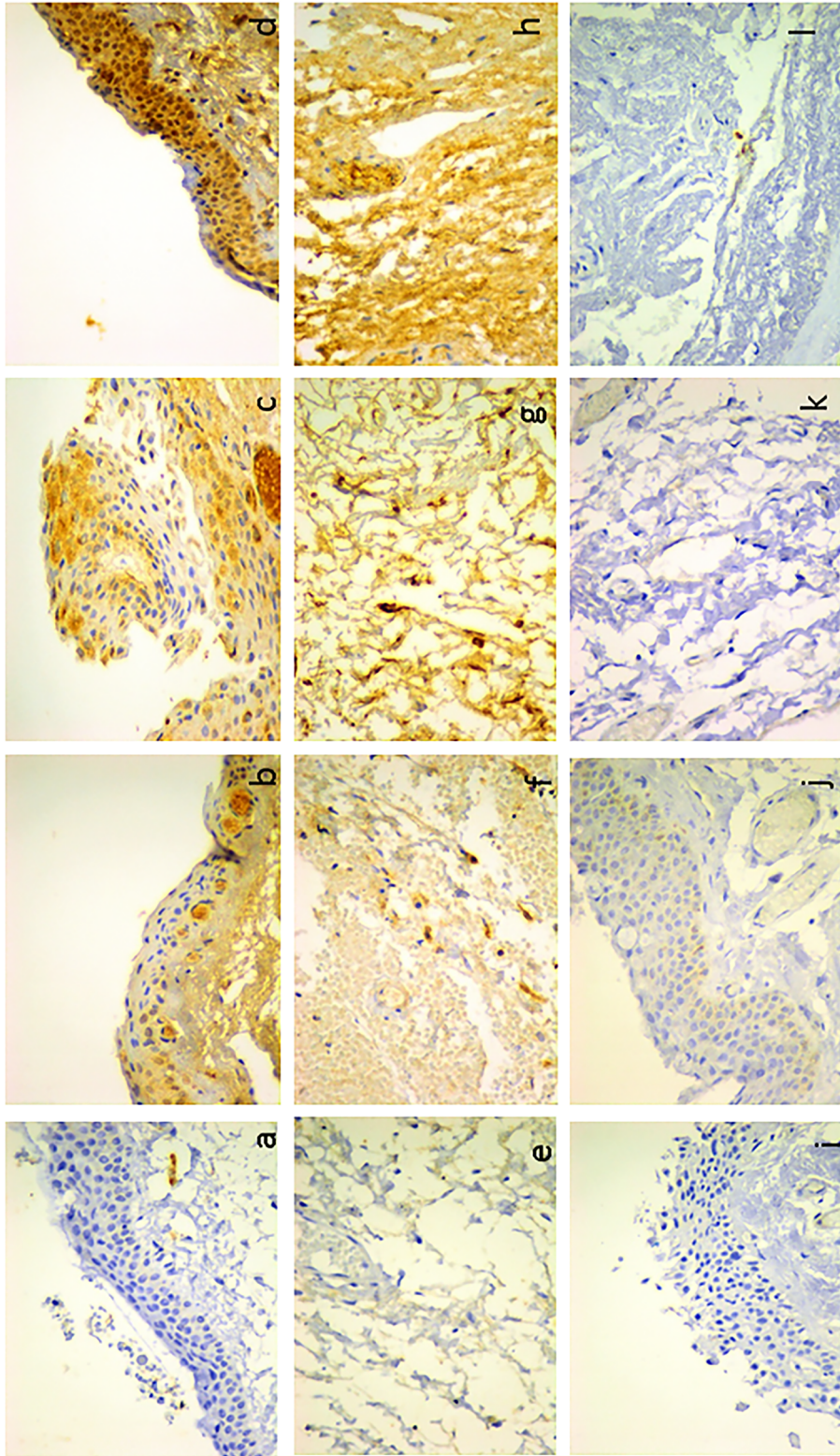


Figure 1. COX-2 and CXCR-4 expressions in pterygium stroma and epithelium. COX-2 expression in different cases: a, b, c, d- epithelium at -/+ +++/++++, respectively; e, f, g, h- stroma at -/+ +++/++++, respectively (IHC, COX-2, original magnification 40×). CXCR-4 expression: i- epithelium (-); j- stroma (-); k- positive in the epithelium; l- positive in the stroma (positivity in rare cells not allowing semiquantitative scoring and evaluated by cell counting) (IHC, CXCR-4, original magnification 40×).

transformation of fibroblasts to myofibroblasts and may promote the development of the stroma of the pterygium.

It was reported in a recent study that activities of COX-2/PGE2-EP3/EP4 signaling modulate tumor biology and it was shown that the CXCL-12/CXCR-4 axis could play a crucial role in tumor stromal formation and angiogenesis under the control of prostaglandins. It was postulated that PGE2-EP3/EP4 signaling in tumor stroma may promote tumor stromal formation and tumor growth mainly through the host CXCL-12/CXCR-4 axis by recruiting stromal fibroblasts (33). On the other hand, Obermajer et al. (34) demonstrated that COX-2 inhibition also blocked CXCL-12/CXCR-4 production in the ovarian cancer environment and its ability to attract MDSCs. These mechanisms may raise the possibility of new therapeutic targets to treat pterygia. Kim et al. (17) reported that cultured pterygial fibroblasts treated with CXCR-4 antagonist AMD-3100 demonstrated lower cellular expression of α -SMA, which promotes tissue repair.

Our study has its limitations. The small sample size is the first limitation. Another important point was the

evaluation of the apex and the body of the pterygium separately. The apex and the body of the pterygium may have different morphological and/or phenotypical characteristics and evaluation of the expression COX-2 and CXCR-4 separately in these sites may provide additional information.

To the best of our knowledge, there is no previous study investigating both COX-2 and CXCR-4 in the same pterygium series. The novel finding of this study is the moderate positive correlation between the expression of CXCR-4 and COX-2 in the stroma. The pterygium area is also moderately correlated with the stromal expressions of CXCR-4 and COX-2. These findings highlight the importance of the stroma and CXCR-4 and COX-2 in pterygium, which may lead to further therapeutic studies with inhibitors of CXCR-4 and COX-2. The “seed and soil” hypothesis proposed by Sir James Paget more than 100 years ago in favor of the importance of the stroma in tumor progression may be also important in the understanding and prevention of the growth of pterygium (35).

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