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ORIGINAL PAPER

YKL-40 expression in pterygium: a potential role in the pathogenesis

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Abstract
Purpose The aim of the study was to evaluate whether YKL-40 (chitinase 3-like 1 protein) plays a role in pterygium pathogenesis.
Methods We included 42 primary pterygium patients and 24 control subjects with normal bulbar conjunctiva in the study. The pterygium patients were classified into the atrophic, fleshy, and intermediate groups according to the Tan classification. We then surgically removed the primary nasal pterygium and normal bulbar conjunctiva from the patients and immunohistochemically investigated YKL-40 expression.
Results YKL-40 expression was statistically significantly higher in the epithelial, endothelial, and stromal cells of the pterygium tissues than in the control tissues ($P = 0.009$, $P = 0.003$, $P = 0.002$, respectively). There was no significant correlation between the pterygium subgroups and YKL-40 expression ($P > 0.05$).
Conclusions We believe YKL-40 may play a significant role in pterygium pathogenesis.

Keywords Angiogenesis · Inflammation · Pterygium · YKL-40

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Introduction

Pterygium is a common ocular surface disorder characterized by the overgrowth of degenerative and hyperplastic conjunctival tissue onto the cornea [1]. Despite its frequency, the pathogenesis is not fully understood but chronic ultraviolet (UV) exposure is known to be the triggering factor [2]. Various proinflammatory cytokines such as interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor-α (TNF-α), together with growth factors such vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), basic fibroblast growth factor (b-FGF), and transforming growth factor-β (TGF-β), lead to chronic inflammation, and also proliferation and the activation of the angiogenesis cascades on the ocular surface, with the effect of chronic UV light, resulting in pterygium formation [3, 4].

YKL-40 is from the family of chitinase-like proteins as it is a 40 kDa highly conserved chitin- or heparin-binding glycoprotein and is also called chitinase 3-like 1 protein (CHI3L1) [5]. In addition to cancer cells, it is also secreted by macrophages and neutrophils, chondrocytes, fibroblasts, vascular endothelial and smooth muscle cells, epithelial cells, and hepatic stellate cells [6]. It participates in various processes, both physiological and pathological, including inflammation, differentiation, cell proliferation, angiogenesis stimulation, and extracellular tissue remodeling regulation [7]. It also interacts with various cytokines, with its production stimulated by many cytokines including IL-13, IL-6, TNF-α, IL-1β, and IFN-γ but suppressed by IL-4 [6, 8]. Serum YKL-40 levels can increase following sustained inflammation as in rheumatoid arthritis, asthma, Crohn's disease, and chronic pancreatitis, and it is also known to increase tumor angiogenesis in some human cancers such as colorectal cancer, breast cancer, and glioblastoma [5, 7–9]. Immunohistochemical studies have demonstrated YKL-40 overexpression in and its association with many disorders where inflammation and angiogenesis play a role in the pathogenesis [5–9].

The relationship of some molecules associated with inflammation and angiogenesis has been shown in pterygium pathogenesis but the primary action of many of the others has not been elucidated. We believe the YKL-40 molecule may play a role in pterygium pathogenesis as it is active in many processes such as inflammation, angiogenesis, and proliferation. We are not aware of any previous study on YKL-40 expression in pterygium tissue and therefore aimed to evaluate this expression in the current study.

Methods

The study was performed prospectively at the Ahi Evran University Training and Research Hospital. We included 42 primary pterygium patients in the patient group and 24 age- and gender-matched subjects in the control group. All patients underwent a comprehensive ophthalmic examination including pupillary dilation before surgery. Exclusion criteria were recurrent pterygium, history of any other ocular disorder or surgery (including uveitis, severe dry eye, eye trauma, glaucoma, pseudoexfoliation syndrome), any systemic disorder (such as diabetes, hypertension, hypercholesterolemia, asthma or chronic obstructive pulmonary disease), systemic inflammation (inflammatory bowel disease, hepatitis B or C), pregnancy, and the current use of any topical or systemic medication or anti-inflammatory/antioxidant agent. The local ethics committee provided approval for the study, and we adhered to the ethical aspects of the Helsinki declaration.

The pterygium patients were divided into three grades according to the Tan classification as atrophic, fleshy, and intermediate. Grade 1 was the atrophic group where the pterygium did not obscure the episcleral vessels underneath so that they could be clearly seen. These vessels were totally obscured in Grade 3, the fleshy group, while Grade 2 cases fell between these two categories [10].

All pterygium patients underwent surgery with the limbal conjunctival autografting technique. Surgical excision was performed on 42 primary nasal pterygia. We removed the pterygium head for immunostaining and cut the pterygial specimens perpendicular to the pterygium axis. The control tissue used was 24 normal conjunctival tissue segments excised during cataract surgery from the nasal bulbar conjunctiva near the limbus. All tissues were fixed in 10% buffered formaldehyde solution and then embedded in paraffin.

Immunohistochemistry

The tissues were first cut into 4-μm sections. We then used a Benchmark XT automat (Ventana Medical System, Tucson, AZ, USA) for deparaffinization and labeling. The ultraView Universal DAB Detection Kit (Ventana Medical Systems Inc, USA) was used according to its recommended protocol. Anti-YKL-40 mouse monoclonal antibody (ab86428, Abcam, Cambridge, UK) was used at a concentration of 5 μg/ml for immunohistochemistry evaluation of the formalin-fixed and paraffin-embedded tissues. A pathologist blinded to the clinical findings and to the nature of the specimens provided independent staining evaluation. All endothelial, epithelial, and stromal cells with brown cytoplasmic staining were considered to be positive. The specificity of the anti-YKL-40 antibody was validated by pre-incubation of recombinant YKL-40 with the antibody to prevent its interaction with tissue-derived YKL-40.

YKL-40 immunoreactivity was evaluated in epithelial, endothelial, and stromal pterygium cells in

addition to conjunctival healthy tissues. The epithelial cell expression was assessed with a scoring system that was previously defined. The system combined values of (1) staining intensity (0: negative, 1: weak, 2: intermediate, 3: strong staining) and (2) positive cell percentile quadrant (0: 0, 1: 1–25%, 2: 26–50%, 3: $>$ 50%). The maximum score possible was 6.[11] We graded a score of 1 or 2 as weak, 3 as intermediate, and 4–6 as strong positive. A semiquantitative score, again previously described, was used to grade YKL-40 expression in the stromal and endothelial cells with 0 indicating no expression; +, focal expression; and ++, diffuse expression [11].

Statistical analysis

Data analysis was conducted with SPSS software version 22.0. The independent t test was used to compare age between the groups. The Chi-square test was used to compare gender between the groups in addition to YKL-40 expression levels. Spearman's rho correlation test was used to determine the relationship between YKL-40 expression levels and pterygium subgroups. A P value smaller than 0.05 was accepted as statistically significant.

Results

The mean age was 45.76 $\pm$ 8.8 years in the pterygium patients and 49.20 $\pm$ 5.0 years in the control subjects. The pterygium group consisted of 26 males and 16 females, and the control subjects of 16 males and eight females. There was no statistically significant difference between the two groups for age or gender distribution (P = 0.085 and P = 0.699, respectively). We found statistically significantly higher YKL-40 expression in the epithelial cells of the pterygium samples than in control tissues (P = 0.009) (Fig. 1). Endothelial cell YKL-40 expression was also statistically significantly higher in pterygium samples (P = 0.003) (Fig. 1). YKL-40 expression in the stroma was similarly statistically significantly higher in pterygium samples (P = 0.002) (Fig. 1). Table 1 presents the YKL-40 expression in epithelial, endothelial, and stromal cells in the pterygium and conjunctival tissue samples. The pterygium subgroup distribution is depicted in Table 2. No significant correlation was observed between the pterygium subgroups and YKL-40 expression in the endothelial or stromal cells ($P > 0.05$) (Table 2). We did not perform correlation analysis for epithelial cells as YKL-40 expression was strong in all groups.

Discussion

Pterygium etiopathogenesis is not fully understood but genetic and environmental factors are known to play a role in the development of this multifactorial disorder [2]. The strong relationship between pterygium and UV exposure is well known [2, 12]. It is characterized by an inflammatory infiltration that includes mast cells, neutrophils, and lymphocytes. Excessive cytokine (IL-1, IL-6, IL-8 TNF-α) and growth factor (VEGF, PDGF, b-FGF, and TGF-β) production in epithelial and endothelial cells, fibroblasts, and leukocytes that can be triggered by UV radiation increases these effects [2, 3, 12]. These activated inflammatory cells and also increased cytokines, and growth factors cause cascades of proliferation, angiogenesis and inflammation, and finally the development of a pterygium [2, 12, 13]. Stromal fibroblast activity is believed to have a major influence on the severity and post-excisional recurrence of the pterygium [13, 14]. YKL-40 is a molecule associated with inflammation, proliferation, and angiogenesis. We therefore thought that investigating the role of YKL-40 in pterygium pathogenesis may enable obtaining new insights into the pathophysiological pathways and new treatment strategies for the disease.

Despite the various strategies developed for pterygium treatment, recurrence continues to be a major problem for both ophthalmologists and patients. Various treatment methods in addition to surgery have been tried. An encouraging development in recent years has been the use of anti-VEGF agents that have a molecular effect on the clinical aspects of pterygium such as angiogenesis and cellular proliferation [15, 16]. Intralesional anti-VEGF injections used for pterygium treatment have been shown to decrease both symptoms and vascularity [15–17]. The preoperative injection of combined bevacizumab and Mitomycin-C beneath the pterygium has also been shown to decrease postoperative primary pterygium recurrence [18]. In addition, bevacizumab treatment has been found to decrease the migration and invasion of fibroblasts in the pterygium [19]. Taking into

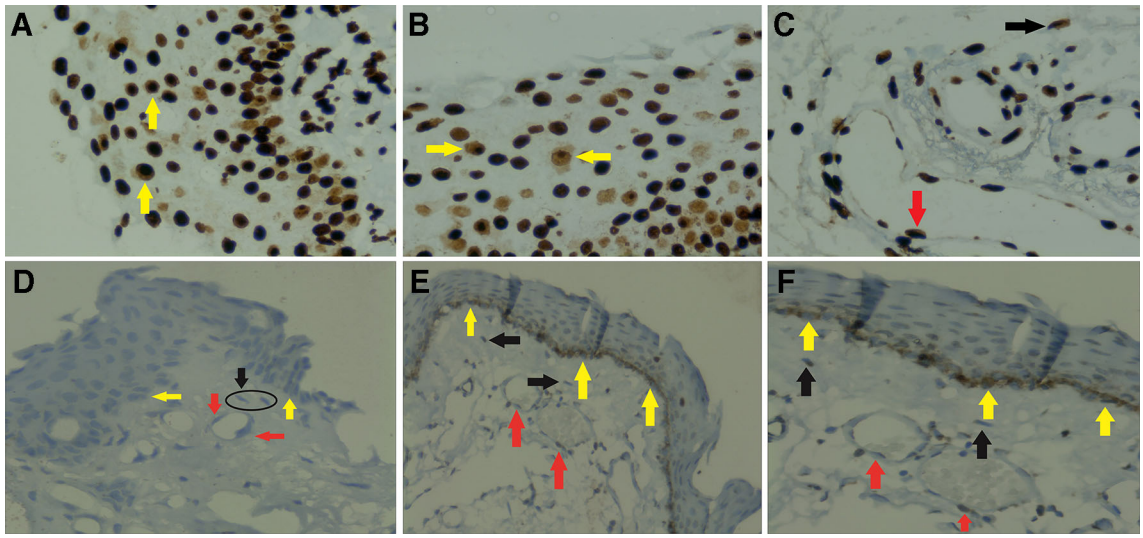

Fig. 1 Epithelial, endothelial, and stromal cell immunostaining with mouse monoclonal YKL-40 antibodies in pterygium (**a**–**c**) and normal conjunctiva (**d**–**f**). **a**, **b** (original magnification ×400): Strong immunoreactivity can be seen in epithelial cells (yellow arrows) in the pterygium sample. **c** (original magnification ×400): Diffuse immunostaining is seen in endothelial (red arrow) and fibroblast cells (black arrow) in the pterygium sample. **d** (original magnification ×200): No immunoreactivity is seen in basal epithelial cells (yellow arrows), endothelial cells (red arrows), or fibroblast cells (black arrow) in the normal conjunctival sample. **e** (original magnification ×200): Weak immunoreactivity is seen in basal epithelial cells (yellow arrows), and focal immunostaining is seen in endothelial (red arrows) and fibroblast cells (black arrows) of the normal conjunctival sample. **f** (original magnification ×400): Weak immunoreactivity is seen in basal epithelial cells (yellow arrows), and focal immunostaining is seen in endothelial (red arrows) and fibroblast cells (black arrows) of the normal conjunctival sample

Table 1 YKL-40 expression in epithelial, endothelial, and stromal cells

Location	Expression	Pterygium (n = 42) (%)	Control tissues (n = 24) (%)	P value
Epithelial	No expression	–	1 (4.2)	0.009
	Weak	–	1 (4.2)	
	Intermediate	–	4 (16.7)	
	Strong	42 (100)	18 (75)	
Endothelial	No expression	–	2 (8.3)	0.003
	Focal	11 (26.2)	14 (58.3)	
	Diffuse	31 (73.8)	8 (33.3)	
Stromal	No expression	–	2 (8.3)	0.002
	Focal	12 (28.6)	15 (62.5)	
	Diffuse	30 (71.4)	7 (29.2)	

consideration of previous studies, YKL-40 and other inflammatory cytokines and growth factors may also be a target for the treatment of pterygium.

The complicated mechanisms of pterygium pathogenesis are still not clear. Many recent studies have attempted to elucidate the mechanisms of inflammation, angiogenesis, and oxidative stress in the disorder and to evaluate the roles of various molecules [20–23]. We similarly investigated the role of endocan expression as it has a close relationship with VEGF and found that it was overexpressed in pterygium [23]. However, many other new molecules thought to be related to the inflammation, proliferation, and angiogenesis processes may also play a role in the disorder.

YKL-40 was first reported in the early 1990s [7, 24]. It is a 40-kDa 'mammalian chitinase-like protein' and can be secreted by many different cells such as macrophages and fibroblasts, vascular

Table 2 YKL-40 expression in epithelial, endothelial, and stromal cells in Tan's classification groups

Location	Expression	Atrophic (n = 12) (%)	Intermediate (n = 18) (%)	Fleshy (n = 12) (%)	P
Epithelial	Strong	12 (100)	18 (100)	12 (100)	*
Endothelial	Focal	3 (25)	3 (16.67)	5 (41.7)	0,365
	Diffuse	9 (75)	15 (83.33)	7 (58.3)	
Stromal	Focal	4 (33.33)	3 (16.67)	5 (41.7)	0,661
	Diffuse	8 (66.67)	15 (83.33)	7 (58.3)	

There was no 0 expression, weak and intermediate expression in epithelial cells and 0 expression in endothelial and stromal cells. We therefore excluded the related lines from the table

* The correlation analysis for epithelial cells was not performed as YKL-40 expression was strong in all groups

endothelial and smooth muscle cells, epithelial cells, and cancer cells [7, 24, 25]. Its physiological and biological functions have not yet been clarified but it is believed to play a role in various processes such as inflammation, cell proliferation, apoptosis protection, extracellular tissue remodeling, and angiogenesis stimulation [7]. It is also thought to be active in malignant disorders, autoimmune disorders, and inflammatory conditions such as infections and liver disease [7, 8, 25]. An attempt has been made to use it as a biomarker of some autoimmune disorders and pathology involving fibroblast activation as a result of these presumed roles [8]. It is also thought to be a new angiogenic factor due to its stimulation of vascular development and endothelial cell migration in cancer that has been found to occur independently of VEGF activity [25]. YKL-40 has been found to induce tumor angiogenesis by increasing VEGF expression in glioblastoma and human breast cancer, and by increasing IL-8 secretion in colorectal cancer [26, 27]. Its expression has been reported in various cancer tissues such as human breast cancer, colorectal cancer, ovarian cancer, and brain cancer, and high serum levels in these patients have been associated with a short survival and metastasis, indicating a possible role as a new cancer biomarker [25]. Many cytokines are able to influence YKL-40 levels with induction by IL-13, IL-6, TNF-α, IL-1β, and IFN-γ and suppression by IL-4 [6, 8].

Another molecule with an important role in pterygium pathogenesis is IL-1, which is itself activated by UV radiation. It is also active by mediating many other cytokines and chemokines' expression [1–4, 13]. TNF-α also plays a similar role in UV-induced inflammation leading to pterygium and is similarly involved in the expression of many cytokines and growth factors such as IL-6, IL-8, VEGF, and b-FGF. Another factor induced by UV radiation is IL-6, which is expressed by resident cells [3, 13]. TNF-α and IL-6 have an indirect effect on neovascularization, exerted by inducing VEGF. VEGF is already known to play a significant role in pterygium pathogenesis [1, 3, 13]. VEGFR-2 has been reported to be correlated with postoperative recurrence development [28]. Our molecule of interest, YKL-40, has a close relationship with all these cytokines [6, 8]. It also increases VEGF expression in various cancers [26]. In the present study, we found YKL-40 overexpression in epithelial cells, endothelial cells, and stromal cells. It is therefore possible that YKL-40 is involved in the inflammatory, proliferative, and remodeling phase of pterygium through interactions with the abovementioned cytokines and growth factors. However, there was no significant correlation between YKL-40 expression and pterygium subgroups in our study, possibly indicating a role for other pathophysiological mechanisms.

We evaluated YKL-40 expression in both pterygium tissues and healthy conjunctivas in this study and found it to be overexpressed in epithelial, endothelial, and stromal cells. In conclusion, the results suggest that YKL-40 may play a role in pterygium pathogenesis. However, the pathogenesis remains unclear and further studies are needed for clarification of the pathophysiological mechanisms of the disorder.

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Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. This study was approved by the ethics committee of Ankara Numune Training and Research Hospital with the date of January 25, 2017 and number 1111/2016.

Informed consent Informed consent was obtained from all individual participants included in the study.

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