Graefe's Archive for Clinical and Experimental Ophthalmology (2019) 257:2743–2750
https://doi.org/10.1007/s00417-019-04463-3

INFLAMMATORY DISORDERS

Overexpression of fractalkine and its histopathological characteristics in primary pterygium

Meydan Turan [1] · Gulay Turan [2]

Received: 22 April 2019 / Revised: 23 August 2019 / Accepted: 4 September 2019 / Published online: 21 October 2019

Abstract

Purpose This study aimed to evaluate the differences in the expressions of fractalkine in normal bulbar conjunctiva and primary pterygium tissues.

Methods The study included 48 patients who had been operated on for primary pterygium. Histopathologically, the presence of epithelial atypia, epithelial hyperplasia, goblet cell hyperplasia, epithelial lymphocytic exocytosis, stromal inflammation, mast cell count, and stromal vascularity were evaluated in the primary pterygium tissues. An immunohistochemical fractalkine stain was applied to the primary pterygium tissue samples and normal bulbar conjunctival tissue samples.

Results Primary pterygium and normal bulbar conjunctival tissue samples were histopathologically analyzed. Epithelial atypia, epithelial hyperplasia, epithelial lymphocytic exocytosis, stromal inflammation, stromal vascularity, and mast cell count were found to be significantly higher in the primary pterygium ($p = 0.001$, $p = 0.002$, $p = 0.024$, $p = 0.007$, $p = 0.024$, and $p = 0.013$, respectively). When evaluated in terms of fractalkine expression, the epithelial, vascular endothelial, and inflammatory cells were significantly higher in the primary pterygium ($p \leq 0.001$, $p = 0.002$, $p = 0.001$, respectively). Moreover, compared to the normal bulbar conjunctiva, Ki-67 expression was significantly higher in the primary pterygium tissue samples.

Conclusion Fractalkine might play a key role in the etiopathogenesis of pterygium. Fractalkine may be important in developing new treatment approaches.

Keywords Pterygium · Immunhystochemistry · Fractalkine · Ki-67 · Conjunctiva · Histopathology

Introduction

Pterygium is a common disease of the ocular surface and is characterized by an overgrowth of triangular or winged conjunctiva toward the cornea. It is a fibrovascular neoformation consisting of loose connective tissue with epithelium and rich vascular structure [1]. In cases of overgrowth, the pterygium tissue sometimes closes the cornea substantially, which may lead to corneal astigmatism and loss of vision [2]. The pathogenesis of pterygium was for a long time unclear, and during this time, it was considered a degenerative disease. Chronic inflammatory infiltrate, which consists of T lymphocytes, macrophages, plasma cells, and mast cells, is present in pterygium [3, 4].

There are significant differences in both epithelium and connective tissue stroma in comparison with pterygium in normal bulbar conjunctiva [4]. Pterygia has similarities with tumors due to cell proliferation, corneal invasion, and recurrence after resection [5]. Kase et al. reported that epithelial proliferation is important in the growth and development of pterygium [6]. Other researchers have found that excessive cellular proliferation in the pterygium occurred in the fibrovascular layer [7]. The fractalkine (CX3CL1) is a chemokine member composed of low molecular weight proteins. It consists of two isoforms: dissolved (cytoplasm related) form and cell membrane bound (membrane bound CX3CL1) form [8]. To date, more than 60 cytokines have chemotactic properties [9]. Among them, the fractalkine is particularly noteworthy. Because of the regulation of the immune system and organization has multiple tasks [9], previous studies have shown that in many inflammatory conditions, such as atherosclerosis, rheumatoid arthritis, asthma, osteoarthritis, and diabetes

✉ Meydan Turan
meydanturan@gmail.com

[1] Department of Ophthalmology, Balikesir Ataturk City Hospital, Balikesir, Turkey

[2] Faculty of Medicine, Department of Pathology, Balikesir University, Balikesir, Turkey

mellitus, tissue expression and/or circulating levels of the fractalkine are increased [10–15]. Fractalkine is involved in the transport of T cells, natural killer cells, leukocytes, and monocytes from the blood to inflammatory sites in the presence of inflammation [16]. It is also involved in the control of the passage of angiogenesis [16]. Through the interaction of a specific receptor (CX3CR1), the fractalkine increases the transfer of inflammatory cells and tissue destruction through increased secretion of tumor necrosis factor alpha (TNF-α), matrix metalloproteinases, and interferon gamma (IFN-γ) [17].

We designed the current study to determine whether cellular inflammation participates in the pathogenesis of pterygia. This study aimed to evaluate the expression of fractalkine in normal bulbar conjunctiva and primary pterygia.

Methods

In this cross-sectional study, 48 patients were evaluated. Of the 48 patients, 21 were female and 27 were male. The median age was 46.5 ± 5.01 years (range: 37 to 55 years). The research was planned in accordance with the Declaration of Helsinki. Ethics committee approval was obtained from Balikesir University Medical Faculty before starting the study (Date: 05.09.2018/Decision no: 2018/144). All participants were informed before their participation in the study and their consent was obtained. The pterygium was graded with respect to the severity of redness: grade I, no redness; grade II, scattered areas have moderate redness; grade III, significant and common redness present [18].

The study included 48 patients who underwent primary pterygium excision including normal bulbar conjunctival tissue. Only patients with primary pterygium were included in the study. Recurrent pterygium was not included in the study. In addition, patients who had previously had ocular surgery, ophthalmic inflammatory or infectious diseases, systemic autoimmune disease, and those on topical or systemic medication were not included in the study. Pterygium size was determined before the operation using lamp biomicroscopy to measure the horizontal length from the limbus to the cornea. Excision of the pterygium was performed under local anesthesia using the naked sclera technique. All tissues were collected from the nasal side. The normal bulbar conjunctival specimen was excised from the adjacent conjunctiva while pterygium tissue was excised. Specimens were fixed with 10% neutral formalin and embedded in paraffin after tissue follow-up procedures. For staining with hematoxylin and eosin, sections of 4-μm thickness were taken and placed on microscope slides. Upon histologic examination, we evaluated the presence of epithelial atypia, epithelial hyperplasia, goblet cell hyperplasia, epithelial lymphocytic exocytosis, stromal inflammation, mast cell count, and stromal vascularity. We scored the vascularization of pterygium by light microscopy under 200× magnification. In the stroma of the tissue samples, the average mast cell count was obtained by counting in three separate regions under 400× light microscopy magnification. Both primary pterygium and normal bulbar conjunctival tissue samples were evaluated for epithelial atypia and epithelial hyperplasia and were defined as present or absent. The number of goblet cells was determined and graded as few or prominent. Epithelial lymphocytic exocytosis was classified as mild or moderate. Stromal vascularity was evaluated and grouped as moderate or severe. The presence of stromal inflammation was graded perivascular or diffuse according to localization. Mast cell count was determined and classified as mild or moderate.

Immunohistochemistry

Primary pterygium and normal bulbar conjunctival tissue samples were immunohistochemical stained for fractalkine and Ki-67. İmmunohistochemistry procedure: All samples were detected with 10% neutral formalin and the tissues were embedded in paraffin after tissue follow-up. Four-micrometer sections were taken to the microscope slides and allowed to dry for 12 h at 37 °C. Each sample was passed through xylene and ethanol solutions for deparaffinization. Samples were incubated with 1% H_2O_2 to prevent endogenous peroxidase activities. Each sample was washed in 0.1% tritonx-100 phosphate-buffered saline (PBS), then transferred to citrate buffer solution (pH 6) to provide antigen retrieval. Each sample was then washed again with PBS and placed in an immunohistochemistry container. Samples were blocked with serum reagent for 5 min before primary antibodies were added. Primary antibodies for anti-human CX3CL1/fractalkine antibody with a dilution of 1: 1000 (sc20730, Santa Cruz, CA, USA) and Ki-67 antibody dilution 1:50 (clone: polyclonal, ScyTek, Utah, USA) were added and cooled at 4 °C for 12 h. The samples were washed again with PBS and incubated with streptavidin peroxidase for 30 min. Next, samples were rinsed with PBS and incubated with chromogen aminoethylcarbazole substrate kit (AEC kit, Zymed Laboratories). After these steps, the slides were stained with hematoxylin for background staining and the slides were closed with Entellan®. The antibody used in this study was fractalkine and Ki-67 expression in normal bulbar conjunctival tissue samples and primary pterygium tissue samples was compared with staining intensity.

After immunohistochemical staining, the number of immuno-positive cells were determined using a microscope (Nikon retention Ni-U, Tokyo, Japan) and an image

Table 1 Histopathological findings in primary pterygium and normal bulbar conjunctiva

	Primary pterygium ($n = 48$, %)	Normal bulbar conjunctiva ($n = 48$, %)	p value
Epithelial atypia			
Present	11 (22.9%)	1 (2.1%)	
Absent	37 (77.1%)	47 (97.9%)	0.001
Epithelial hyperplasia			
Present	45 (93.8%)	34 (70.8%)	
Absent	3 (6.3%)	14 (29.2%)	0.002
Goblet cell hyperplasia			
Few	35 (72.9%)	37 (77.1%)	0.159
Prominent	13 (27.1%)	11 (22.9%)	
Epithelial lymphocytic exocytosis			0.024
Mild	37 (77.1%)	42 (87.5%)	
Moderate	11 (22.9%)	6 (12.5%)	
Stromal vascularity			
Moderate	35 (72.9%)	42 (87.5%)	0.007
Severe	13 (27.1%)	6 (12.5%)	
Stromal inflammation			
Perivascular	34 (52.1%)	39 (81.3%)	0.024
Diffuse	14 (47.9%)	9 (18.8%)	
Mast cell count			
Mild	28 (58.3%)	34 (70.8%)	0.013
Moderate	20 (41.7%)	14 (29.2%)	

Histopathological variables in primary pterygium and normal bulbar conjunctiva were evaluated and primary pterygium showed a significant increase in epithelial atypia, epithelial hyperplasia, epithelial lymphocytic exocytosis, stromal inflammation, stromal vascularity, and mast cell count ($p = 0.001$, $p = 0.002$, $p = 0.024$, $p = 0.007$, $p = 0.024$, and $p = 0.013$, respectively). However, there was no significant difference in the number of goblet cells between primary pterygium and normal bulbar conjunctiva ($p = 0.159$)

analysis system (Nikon Instruments Europe BV, Amsterdam, The Netherlands). This method used a pre-software system with color segmentation to perform quantitative color analysis. Brown color staining in the nucleus and the cytoplasm revealed the presence of fractalkine and Ki-67 expression. The number of pixels reflects the intensity of staining of immunopositive cells. In addition, the entire image pixel can be expressed as a percentage [< 80 pixels weak (1+), 80–200 pixels medium (2+), and > 200. Pixel shows strong (3+)] [9].

SPSS version 20.0 (Inc., Chicago, IL, USA) was used for statistical analysis. Differences were assessed by an independent sample t test. Kolmogorov-Smirnov test was used to determine whether the distribution of continuous variables was normal. Mann-Whitney U test was used to compare the mean values. The correlation coefficients and

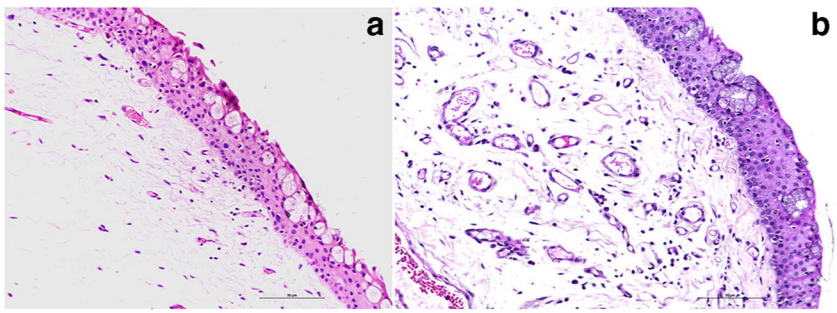

Fig. 1 Histopathological features, in normal bulbar conjunctiva and primary pterygium. **a** Normal bulbar conjunctival tissue specimen. Histopathological changes involving epithelial hyperplasia (eh), stromal inflammation (si), stromal vascularity (sv). Hematoxylin and eosin staining, 200×. **b** Primary pterygium tissue sample. Histopathological changes including increased epithelial hyperplasia (eh), stromal inflammation (si), stromal vascularity (sv). Hematoxylin and eosin staining, 200×

Table 2 Fractalkine expression in primary pterygium and normal bulbar conjunctiva tissue samples

Fractalkine expression	Primary pterygium ($n = 48$)			Normal bulbar conjunctiva ($n = 48$)			p value
Intensity	1+	2+	3+	1+	2+	3+	
Epithelium n (%)	8 (16.7%)	24 (50.0%)	16 (33.3%)	33 (68.8%)	12 (25.0%)	3 (6.3%)	< 0.001
Vascular endothelium n (%)	13 (27.1%)	21 (43.8%)	14 (29.2%)	18 (37.5%)	20 (41.7%)	10 (20.8%)	0.002
Inflammatory cells n (%)	12 (16.7%)	21 (41.7%)	15 (41.7%)	17 (37.5%)	23 (56.3%)	8 (6.3%)	0.001

The expression of the fractalkine in epithelial cells, inflammatory cells, and vascular endothelial cells was significantly higher in primary pterygium tissue samples than in normal bulbar conjunctival tissue samples ($p = 0.001$, $p = 0.002$, $p = 0.001$, respectively)

their significance were calculated using Spearman's rank correlation test. The Chi-square test was used to compare the categorical data. A p value of less than 0.05 was considered statistically significant.

Results

Fourteen patients (29.2%) had grade I, 23 patients (47.9%) had grade II, and 11 patients (22.9%) had grade III redness. The mean size of primary pterygium was 3.25 ± 0.44 mm. Morphological analysis with light microscopy evaluated epithelial atypia, epithelial hyperplasia, goblet cell hyperplasia, epithelial lymphocytic exocytosis, stromal inflammation, mast cell count, and stromal vascularity in primary pterygium and normal bulbar conjunctival tissue samples. Statistical analysis showed that epithelial atypia, epithelial hyperplasia, epithelial lymphocytic exocytosis, stromal inflammation, stromal vascularity, and mast cell count were significantly higher in primary pterygium samples ($p = 0.001$, $p = 0.002$, $p = 0.024$, $p = 0.007$, $p = 0.024$, and $p = 0.013$, respectively). However, there was no difference in goblet cell hyperplasia between primary pterygium and normal bulbar conjunctival tissue samples ($p = 0.159$; Table 1; Fig. 1a, b).

When the expression of the fractalkine was evaluated, both nuclear and cytoplasmic staining were observed in the epithelial cells and inflammatory cells, while only nuclear staining was observed in the vascular endothelium. The expression of the fractalkine was significantly higher in the primary pterygium tissues compared to the normal bulbar conjunctiva. Fractalkine expression was significantly higher in the epithelium, vascular endothelium, and inflammatory cells ($p = 0.001$, $p = 0.002$, $p = 0.001$, respectively) (Table 2; Fig. 2a–c). The fractalkine expression in primary pterygium epithelium had weak in 8 samples, had moderate in 24 samples, and had severe in 16 samples. The fractalkine expression in primary pterygium plasma cells, in lymphocytes and in mast cells in the stromal tissue, had weak in 12 samples, had moderate in 21 samples, and had severe in 15 samples. The fractalkine expression in primary pterygium endothelial cells had weak in 13 samples, had moderate in 21 samples, and had severe in 14 samples.

The primary pterygium and normal bulbar conjunctival epithelium showed positive staining with Ki-67, the proliferation protein. Ki-67 expression was significantly higher in primary pterygium than normal bulbar conjunctiva ($p = 0.001$) (Fig. 3a, b). The mean value of Ki-67 positive cells in the primary pterygium and normal bulbar conjunctival epithelium was $9.75 \pm 3.54\%$ and $1.81 \pm 0.67\%$, respectively (Table 3).

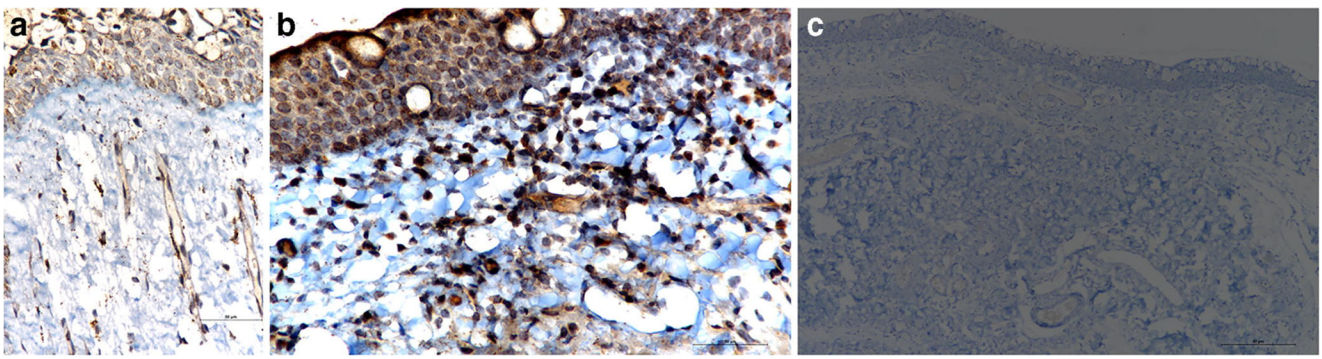

Fig. 2 Fractalkine expression, in normal bulbar conjunctiva and primary pterygium. **a** Poor expression of the fractalkine in epithelial, endothelial, and inflammatory cells in normal bulbar conjunctival tissue (e: epithelial cells; v: vascular endothelial cells; i: inflammatory cells), 400×. **b** Strong expression of the fractalkine in epithelial, endothelial, and inflammatory cells in primary pterygium tissue (e: epithelial cells; v: vascular endothelial cells; i: inflammatory cells), 400×. **c** Negative control: it was obtained from the patient with primary pterygium, 400×

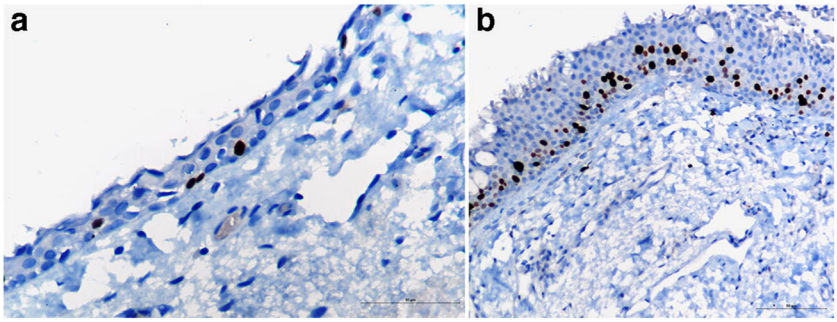

Fig. 3 Ki-67 expression, in normal bulbar conjunctiva and primary pterygium. **a** Low Ki-67 expression in epithelial cells in normal bulbar conjunctival tissue (yellow arrows), 400×. **b** High Ki-67 expression in epithelial cells in primary pterygium tissue (yellow arrows), 400×

Correlation analysis revealed a significant relationship between epithelial hyperplasia, stromal vascularity, stromal inflammation and fractalkine expression, which are histopathological variables in primary pterygium. In addition, clinical parameters such as pterygium grade and pterygium diameter correlated with fractalkine expression.

However, no correlation was found between the expression of the fractalkine in the normal bulbar conjunctiva and the histopathological variables. There was also no correlation between fractalkine expression and age and gender in primary pterygium and normal bulbar conjunctiva. Importantly, correlation was found between the fractalkine and Ki-67 expression in primary pterygium epithelium (Table 4). In addition, there was a significant positive correlation between fractalkine expression and grade III redness ($r = 0.78$, $p = 0.035$) (Table 5).

Discussion

This is a cross-sectional study. We investigated the fractalkine expression in primary pterygium tissue samples. According to our results, the primary pterygium tissue showed significantly increased fractalkine expression in the epithelial cells, endothelial cells, and inflammatory cells in comparison with the normal bulbar conjunctiva. Moreover, a significant correlation was found between the fractalkine expression of the primary pterygium and epithelial hyperplasia, stromal vascularity, stromal inflammation, and Ki-67 expression. To the best of our knowledge, this study is the first to investigate the fractalkine expression in the primary pterygium. Fraktalkine, which has many features such as adhesion and chemoattractant, is upregulated in vasculature in various inflammatory diseases [10, 19, 20]. Researchers have found that fractalkine has a role in the pathogenesis and progression of many inflammatory conditions and some malignancies [8]. In a study comparing asthmatic patients with healthy people, the amount of CX3CL1 was shown to increase in asthmatic patients. It can induce mast cell chemotaxis and increase the CX3CR1 function in Th2 cells [21]. CX3CL1/CX3CR1 is upregulated in chronic inflammatory conditions such as viral hepatitis [22]. The CX3CL1/CX3CR1 axis also plays an important role in inflammatory bowel diseases. In patients with Crohn's disease, when the inflamed and non-inflamed mucosa were compared, a significant increase in transcription of fractalkine in the inflamed colonic mucosa was found [23].

Zhou et al. [14] reported increased expression of fractalkine in small-cell lung cancer. For the first time, Matthew et al. described the expression of fractalkine in vascular endothelial and stromal cells obtained from human iris, retinal explants, and other ocular tissues. They considered the increased expression of fractalkine in the eye and other tissues and the role of mediating leukocyte extravasation in inflammatory conditions elsewhere in the body [24]. Studies on uveitis and retinitis have shown that both chemokines and adhesion molecules have abnormal expression. Based on this, they were thought to have an important role in the onset and progression of the disease [25, 26]. While interferon has an inducing effect on the expression of fractalkine in the ocular endothelial cells, interleukin (IL)-4 and -13 have an inhibitory effect. Thus, the fractalkine may have an effective role in the diseases of Th1-derived iritis and uveoretinitis [27–29]. Paolo et al. [30] reported that the increased fractalkine response in the iris and retinal endothelial cells was induced by stimulation of Th1 and Th2 cytokines. Recently, Enriquez-de-Salamanca et al. [31] reported that fractalkine concentration increased in tears of patients with dry eyes and this

Table 3 Ki-67 expression in primary pterygium and normal bulbar conjunctiva tissue samples

Tissue samples	Ki-67 positive cells (%)
Primary pterygium	9.75 ± 3.54
Normal bulbar conjunctiva	1.81 ± 0.67

Ki-67 expression was significantly higher in primary pterygium than normal bulbar conjunctiva ($p = 0.001$)

Table 4 Correlation analysis of fractalkine expression and clinicopathologic variables in primary pterygium and normal bulbar conjunctiva

Variables	Primary pterygium fractalkine expression	Normal bulbar conjunctiva fractalkine expression
Age (year)	$r = -0.359$	$r = 0.217$
	$p = 0.012$	$p = 0.139$
Gender	$r = 0.040$	$r = 0.168$
	$p = 0.788$	$p = 0.254$
Grade	$r = 0.757$	–
	$p < 0.001$	
Pterygium size (mm)	$r = 0.908$	–
	$p < 0.001$	
Epithelial hyperplasia	$r = 0.874$	$r = -0.428$
	$p < 0.001$	$p = 0.002$
Stromal vascularity	$r = 0.693$	$r = -0.406$
	$p < 0.001$	$p = 0.004$
Stromal inflammation	$r = 0.458$	$r = -0.406$
	$p = 0.001$	$p = 0.004$
Ki-67 expression	$r = 0.508$	$r = 0.147$
	$p < 0.001$	$p < 0.319$

Spearman's rank correlation

increase was correlated with the disease severity. Denoyer et al. [32] thought that the synthesis of epithelial fractalkine could have a significant effect on cytotoxicity due to inflammation in conjunctival immunity by acting on migration in immune cells. In light of this information, the evidence supports that chemokines/cytokines play a critical role in the pathogenesis of inflammatory events. In our study, the increased fractalkine expression of endothelial, epithelial, and inflammatory cells supports the fact that the fractalkine is a chemokine that may play a role in pterygium from ocular surface diseases.

One of the common symptoms of pterygium is inflammation and chronic inflammation may need to be permanent for this finding to occur. Researchers reported that there are inflammatory cells such as neutrophils [33], mast cells [34], lymphocytes, and plasma cells in the pterygium epithelium and stroma [4]. In addition, increased expression of many classical inflammatory factors, including TNF-α, IL-1, 4, and 8, has been shown in pterygia samples [35]. TNF-α, which is involved in the acute inflammatory response, activates the canonical nuclear factor kappa-light-chain-enhancer of activated B cell pathway, and thus controls cell proliferation, differentiation, migration, and apoptosis [36]. For example, UV irradiation stimulates secretion of TNF-α from the cornea and conjunctival epithelial cells and may then cause the proliferation of TNF-α fibroblasts [37].

In addition, only a few studies have addressed the relationship between inflammation and TNF-α in pterygium [38]. A recent study reported that fractalkine release was associated with TNF-α and IL-6 [13]. Consistent with previous studies [39], increased Ki-67 expression showed proliferation in primary pterygium. Furthermore, the expression of Ki-67 correlated with fractalkine expression.

In our study, the increased expression of lymphocytes, plasma cells, and mast cells with fractalkine in the primary pterygium stroma supports inflammation, which is thought to have a role in the etiology of pterygium. These findings suggest that fractalkine may have an effect on the pathological mechanisms of pterygium. Fractalkine is a molecule associated with inflammation. Therefore, we considered that investigating the role of fractalkine in pterygium pathogenesis could obtain new insights into the pathophysiological pathways and new treatment strategies for the disease. We found that an overexpressed fractalkine was strongly correlated with these pathological changes and proliferation in the pterygium.

In conclusion, our results suggest that fractalkine may play a role in pterygium pathogenesis. Further studies are needed to understand the etiopathogenesis of pterygium and to

Table 5 The relationship between the degree of redness in the primary pterygium and the intensity of fractalkine staining

Fractalkine intensity	Grade I ($n = 14$)	Grade II ($n = 23$)	Grade III ($n = 11$)
(1+)	8 (57.14%)	4 (17.39%)	2 (18.18%)
(2+)	6 (42.86%)	16 (69.57%)	5 (45.45%)
(3+)	0 (0%)	3 (13.04%)	4 (36.37%)

determine the role of fractalkine in developing new treatment approaches for this disease.

Acknowledgments The study was supported by Scientific Investigations Foundation of Balikesir University (Project no: BAP. 2018/159).

Funding information This study was funded by Scientific Investigations Foundation of Balikesir University (grant number 2018/159).

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

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 (ethical committee of Balikesir University Medical Faculty (Date: 05.09.2018/reference no: 2018/144)) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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

References

1. Di Girolamo N, Chui J, Coroneo MT et al (2004) Pathogenesis of pterygia: role of cytokines, growth factors and matrix metalloproteinases. Prog Retin Eye Res 23:195–228. https://doi.org/10.1016/j.preteyeres.2004.02.002
2. Sekundo W, Droutsas K, Cursiefen C (2010) Operative techniques for surgical treatment of primary and recurrent pterygia. Ophthalmologe 107:525–528. https://doi.org/10.1007/s00347-009-2099-6
3. Dzunic B, Jovanovic P, Zlatanovic G et al (2010) Comparative analysis of histopathological and clinical characteristics of pterygium. Vojnosanit Pregl 67:159–157. https://doi.org/10.4103/2008-322X.183917
4. Golu T, Mogoanta L, Streba CT et al (2011) Pterygium: histological and immunohistochemical aspects. Romanian J Morphol Embryol 52:153–158
5. Kase S, Osaki M, Jin XH et al (2007) Increased expression of erythropoietin receptor in human pterygial tissues. Int J Mol Med 20:699–702. https://doi.org/10.3892/ijmm.20.5.699
6. Kase S, Takahashi S, Sato I et al (2007) Expression of p27(KIP1) and cyclin D1, and cell proliferation in human pterygium. Br J Ophthalmol 91:958–961. https://doi.org/10.1136/bjo.2006.110387
7. Tan DT, Liu YP, Sun L (2000) Flow cytometry measurements of DNA content in primary and recurrent pterygia. Invest Ophthalmol Vis Sci 41:1684–1686
8. D'Haese JG, Demir IE, Friess H et al (2010) Fractalkine/CX3CR1: why a single chemokine-receptor duo bears a major and unique therapeutic potential. Expert Opin Ther Targets 14:207–219. https://doi.org/10.1517/14728220903540265
9. Usta A, Turan G, Sancakli Usta C et al (2018) Placental fractalkine immunoreactivity in preeclampsia and its correlation with histopathological changes in the placenta and adverse pregnancy outcomes. J Matern Fetal Neonatal Med 10:1–10. https://doi.org/10.1080/14767058.2018.1505854
10. Ruth JH, Volin MV, Haines GK et al (2001) Fractalkine, a novel chemokine in rheumatoid arthritis and in rat adjuvant-induced arthritis. Arthritis Rheum 44:1568–1581. https://doi.org/10.1002/1529-0131(200107)44:7<1568::AID-ART280>3.0.CO;2-1
11. Ruze A, Zhao Y, Li H et al (2018) Low shear stress upregulates the expression of fractalkine through the activation of mitogen-activated protein kinases in endothelial cells. Blood Coagul Fibrinolysis 29:361–368. https://doi.org/10.1097/MBC.0000000000000701
12. Sindhu S, Akhter N, Arefanian H et al (2017) Increased circulatory levels of fractalkine (CX3CL1) are associated with inflammatory chemokines and cytokines in individuals with type-2 diabetes. J Diabetes Metab Disord 16:15. https://doi.org/10.1186/s40200-017-0297-3
13. Siwetz M, Dieber-Rotheneder M, Cervar-Zivkovic M et al (2015) Placental fractalkine is up-regulated in severe early-onset preeclampsia. Am J Pathol 185:1334–1343. https://doi.org/10.1016/j.ajpath.2015.01.019
14. Zhou B, Xu H, Ni K et al (2016) Expression of chemokine XCL2 and CX3CL1 in lung cancer. Med Sci Monit 22:1560–1565. https://doi.org/10.12659/MSM.895985
15. Zou Y, Li Y, Lu L et al (2013) Correlation of fractalkine concentrations in serum and synovial fluid with the radiographic severity of knee osteoarthritis. Ann Clin Biochem 50:571–575. https://doi.org/10.1177/0004563213480494
16. Wojdasiewicz P, Poniatowski LA, Kotela A et al (2014) The chemokine CX3CL1 (fractalkine) and its receptor CX3CR1: occurrence and potential role in osteoarthritis. Arch Immunol Ther Exp 62:395–403. https://doi.org/10.1007/s00005-014-0275-0
17. Hannan NJ, Salamonsen LA (2008) CX3CL1 and CCL14 regulate extracellular matrix and adhesion molecules in the trophoblast: potential roles in human embryo implantation. Biol Reprod 79:58–65. https://doi.org/10.1095/biolreprod.107.066480
18. Safi H, Kheirkhah A, Mahbod M et al (2016) Correlations Between Histopathologic Changes and Clinical Features in Pterygia. J Ophthalmic Vis Res 11:153–158. https://doi.org/10.4103/2008-322X.183917
19. Robinson LA, Nataraj C, Thomas DW et al (2000) A role for fractalkine and its receptor (CX3CR1) in cardiac allograft rejection. J Immunol 165:6067–6072. https://doi.org/10.4049/jimmunol.165.11.6067
20. Raychaudhuri SP, Jiang WY, Farber EM (2001) Cellular localization of fractalkine at sites of inflammation: antigen-presenting cells in psoriasis express high levels of fractalkine. Br J Dermatol 144:1105–1113. https://doi.org/10.1046/j.1365-2133.2001.04219.x
21. Rimaniol AC, Till SJ, Garcia G et al (2003) The CX3C chemokine fractalkine in allergic asthma and rhinitis. J Allergy Clin Immunol 112:1139–1146. https://doi.org/10.1016/j.jaci.2003.09.041
22. Efsen E, Grappone C, DeFranco RM et al (2002) Up-regulated expression of fractalkine and its receptor CX3CR1 during liver injury in humans. J Hepatol 37:39–47. https://doi.org/10.1016/S0168-8278(02)00065-X
23. Kobayashi T, Okamoto S, Iwakami Y et al (2007) Exclusive increase of CX3CR1+CD28-CD4+ T cells in inflammatory bowel disease and their recruitment as intraepithelial lymphocytes. Inflamm Bowel Dis 13:837–846. https://doi.org/10.1002/ibd.20113
24. Silverman MD, Zamora DO, Pan Y et al (2007) Constitutive and inflammatory mediator-regulated Fractalkine expression in human ocular tissues and cultured cells. Invest Ophthalmol Vis Sci 44:1608–1615. https://doi.org/10.1167/iovs.02-0233
25. Rosenbaum JT, Becker MD, Smith JR (2000) Toward new therapies for ocular inflammation [in Spanish]. Arch Soc Esp Oftalmol 75:511–514
26. Magone MT, Whitcup SM (1999) Mechanisms of intraocular inflammation. Chem Immunol 73:90–119. https://doi.org/10.1159/000058742

27. Egwuagu CE, Sztein J, Mahdi RM et al (1999) IFN-gamma increases the severity and accelerates the onset of experimental autoimmune uveitis in transgenic rats. J Immunol 162:510–517
28. Singh VK, Rai G (2001) Cytokines in posterior uveitis: an update. Immunol Res 23:59–74. https://doi.org/10.1385/IR:23:1:59
29. Lacomba MS, Martin CM, Chamond RR et al (2000) Aqueous and serum interferon gamma, interleukin (IL) 2, IL-4, and IL-10 in patients with uveitis. Arch Ophthalmol 118:768–772. https://doi.org/10.1001/archopht.118.6.768
30. Fraticelli P, Sironi M, Bianchi G et al (2001) Fractalkine (CX3CL1) as an amplification circuit of polarized Th1 responses. J Clin Invest 107:1173–1181. https://doi.org/10.1172/JCI11517
31. Enriquez-de-Salamanca A, Castellanos E, Stern ME et al (2010) Tear cytokine and chemokine analysis and clinical correlations in evaporative-type dry eye disease. Mol Vis 16:862–873 <http://www.molvis.org/molvis/v16/a96>
32. Denoyer A, Godefroy D, Celerier I et al (2012) CX3CL1 expression in the conjunctiva is involved in immune cell trafficking during toxic ocular surface inflammation. Mucosal Immunol 5:702–711. https://doi.org/10.1038/mi.2012.43
33. Chui J, Coroneo MT, Tat LT et al (2011) Ophthalmic pterygium: a stem cell disorder with premalignant features. Am J Pathol 178:817–827. https://doi.org/10.1016/j.ajpath.2010.10.037
34. Butrus SI, Ashraf MF, Laby DM et al (1995) Increased numbers of mast cells in pterygia. Am J Ophthalmol 119:236–237. https://doi.org/10.1016/S0002-9394(14)73882-6
35. Zhou WP, Zhu YF, Zhang B et al (2016) The role of ultraviolet radiation in the pathogenesis of pterygia (review). Mol Med Rep 14:3–15. https://doi.org/10.3892/mmr.2016.5223
36. Pahl HL (1999) Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene 18:6853–6866. https://doi.org/10.1038/sj.onc.1203239
37. Kennedy M, Kim KH, Harten B at al. (1997) Ultraviolet irradiation induces the production of multiple cytokines by human corneal cells. Invest Ophthalmol Vis Sci 38:2483–2491
38. Dong Y, Kase S, Dong Z et al (2016) Regulation of vascular endothelial growth factor-C by tumor necrosis factor-α in the conjunctiva and pterygium. Int J Mol Med 38:545–550. https://doi.org/10.3892/ijmm.2016.2647
39. Ljubojevic V, Gajanin R, Amidzic L et al (2016) The expression and significance of p53 protein and Ki-67 protein in pterygium. Vojnosanit Pregl 73:16–20. https://doi.org/10.2298/VSP140428124L