REVIEW ARTICLE

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Mechanisms and biomarker candidates in pterygium development

Mecanismos e candidatos a biomarcadores no desenvolvimento do pterígio

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ABSTRACT | Pterygium pathogenesis has been mainly associated with UV light exposure; however, this association remains quite controversial. The complete mechanism of pterygium also remains to be clarified. Factors such as inflammation, viral infection, oxidative stress, DNA methylation, inflammatory mediators, extracellular matrix modulators, apoptotic and oncogenic proteins, loss of heterozygosity, microsatellite instability, lymphangiogenesis, epithelial-mesenchymal cell transition, and alterations in cholesterol metabolism have been identified as causes. Several studies aimed to clarify the molecular mechanisms underlying the growth and proliferation of pterygium. Understanding its molecular basis provides new potential therapeutic targets for its prevention and treatment. A comprehensive search of the databases, namely, MedLine, EMBASE, and LILACS, was conducted with the following key words: pterygium, epidemiology, pathogenesis, biomarkers, and review. This review describes the epidemiology, clinical presentation, and current investigation of biological mediators involved in pterygium development.

Keywords: Pterygium/epidemiology; pathogenesis; Biomarkers; Review

RESUMO | A patogênese do pterígio tem sido relacionada, principalmente, à exposição à luz ultravioleta, mas esta associação permanece bastante controversa. O mecanismo completo do pterígio também permanece por esclarecer. Fatores como inflamação, infecção viral, estresse oxidativo, metilação do DNA, mediadores inflamatórios, moduladores de matriz extracelular, proteínas apoptóticas e oncogênicas, perda de heterozigose, instabilidade de microssatélites, linfangiogênese, transição celular epitelial-mesenquimal e alterações no metabolismo do colesterol tem sido identificados como causas. Diversos estudos visam esclarecer os mecanismos moleculares subjacentes ao crescimento e proliferação do pterígio. Entender sua base molecular fornece novos alvos terapêuticos potenciais para sua prevenção e tratamento. Uma busca abrangente nas bases de dados, a saber, MedLine, EMBASE e LILACS, foi realizada com as seguintes palavras-chave: pterígio; epidemiologia; patogênese; biomarcadores e revisão. Esta revisão descreve a epidemiologia, apresentação clínica e a atual investigação de mediadores biológicos envolvidos no desenvolvimento do pterígio.

Descritores: Pterígio/epidemiologia; patogênese; Biomarcadores; Revisão

INTRODUCTION

Pterygium is a nonneoplastic elastotic degeneration of subepithelial growth, originating from the bulbar conjunctiva that extends to the corneal surface, and even reached the visual axis in some cases. It is a common ocular surface disorder, especially in geographical areas near the equator. The exact cause of pterygium remains unclear; however, some risk factors are identified as causes, with long-term ultraviolet radiation exposure as the most important[1,2]. Although pterygium is generally regarded as a benign and cosmetic problem, it may result in significant visual morbidity or even potential blindness in severe cases if not properly treated[3]. It usually occurs in the nasal area, but can develop temporally or in both directions and may even occur bilaterally. Pterygium surgery is generally considered when symptoms do not respond to conservative treatment, when it induces visual disturbances, disability, or for cosmetic purposes[4,5].

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With regard to the mechanisms, genetic factors were suggested in some studies, noting that some genes associated with DNA repair play a crucial role in pterygium development. However, studies on genetic variant contributions are limited in sample size and should be cautiously interpreted. Chronic irritation with actinic damage is likely responsible for the typical fibrovascular reaction of pterygium[6]. Growth factors, cytokines, and matrix metalloproteinase are involved in the pathogenesis of pterygium and, along with UV exposure, may trigger the proinflammatory aspects[7].

This review describes the epidemiology, clinical presentation, and investigation of biological modulators found in recent literatures. Therefore, the following keywords were searched: pterygium, epidemiology, angiogenesis, proliferation, inflammation, gene, protein, pathogenesis, and tight junction proteins.

Epidemiology

The prevalence of pterygium has been investigated in several population-based studies. Rates widely vary depending on the studied population, ranging from 2.8% to 38.7%, as recent studies in South Korea and China found prevalences of 8.8%[8] and 9.84%[9], in contrast to 16% in Arizona (USA)[10] and 38.7% in Northwest Ethiopia[11].

In Brazil, no population data were conducted on pterygium prevalence throughout the country, and only a few studies investigated this subject even for specific regions. A survey in riverside communities at the Solimoes and Japura rivers, in Amazonas, showed that its prevalence in the general population was 21.2%[12]. Another survey, still in the Brazilian Amazon, reported that up to 36.6% prevalence of pterygium was observed in the indigenous population[13]. In the southeast region, a study in Botucatu City revealed a prevalence of 8.12%[14].

A large-scale survey on the rate of pterygium and other ocular diseases has not been conducted yet. A meta-analysis published in 2013, covering a total of 20 studies involving 12 countries with 900,545 samples, showed a combined pterygium prevalence rate of 10.2% (95% confidence interval [CI]: 6.3% to 16.1%), in the general population[15]. Table 1 summarizes the epidemiological information.

Pterygium more frequently occurs in young adults, rarely before aged 15 years. It is believed to have some inherited patterns; however, several risk factors have been reported, such as dust exposure, heat, inflammation, and eye surface infection, rural residency, advanced age, low educational levels, and outdoor activity[16].

The prevalence of pterygium has been described as higher in males[16]; however, other studies have shown that both genders have the same proportions[17] or even predominantly occurred in women[18]. Its relationship with smoking was also investigated, but remains inconclusive[15].

Table 1. Epidemiological studies

Country	Study	Prevalence	Latitude	Gender %
Brazil (Amazon)	Ribeiro et al., 2011[12]	21.2%	03º 06' S	Male 57.8
	Paula et al., 2006[13]	36.6%		Female 42.2
				Male 15.7
				Female 20.5
Brazil (Southeast)	Shiratori et al., 2010[14]	8.12%	22º 53' S	Male 10.4
				Female 6.5
South Korea	Pyo et al., 2016[8]	8.8%	37° 33' N	Male 3.1
	Rim et al., 2017[16]	1.9%-3.8%		Female 2.5
				Male 0.18
				Female 0.24
Northwest ethiopia	Anbesse et al., 2017[11]	38.7%	12º 31`N	Male 2.20
				Female 1.0
China	Song et al., 2017[9]	9.84%	35° 00' N	Male 10.26
				Female 9.46
Nepal	Shrestha & Shrestha, 2014[18]	12.4%-65.8%	27º 42`N	Male 8.8%-38.5
				Female 14.4%-40.7
USA (Arizona)	West S & Muñoz B, 2009[10]	16%	34° 30' N	Male 23.7
				Female 11.5

Clinical presentation

Pterygium is a conjunctival fibrovascular tissue that extends to the cornea and can lead to irritating symptoms, visual disturbance, recurrent inflammation, and aesthetic alterations in the ocular surface. Its diagnosis is confirmed with slit-lamp examination, in which the pterygium can be classified as grade 1, when the fibrovascular tissue reaches the limbus; grade 2, when it covers the cornea in approximately 2 mm; grade 3, when it reaches the pupil margin; and grade 4, when it exceeds the pupil (Figure 1). As regards its morphological features, pterygium can be classified as involutive or atrophic when it allows visualization of structures immediately below the lesion and as inflamed once the fibrovascular tissue is fleshy and prevents visualization of the structures below[19,20]. Recently, a functional classification using the corneal topographic data based on corneal higher-order irregularity was proposed by Miyata et al. to objectively evaluate pterygium severity. Hence, pterygium was graded based on corneal irregularity within the three zones: 1.0, 3.0, and 5.0 mm diameters. Thus, increased corneal irregularity within a 1.0-mm diameter was considered to highly result in the risk for visual function impact, and increased corneal irregularity within a 5.0-mm diameter was considered as mild severity with visual function influences[21].

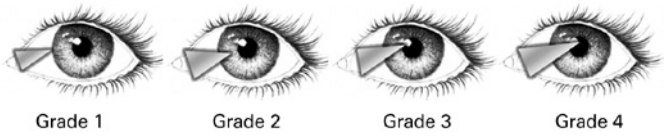

Figure 1. Pterygium classification.

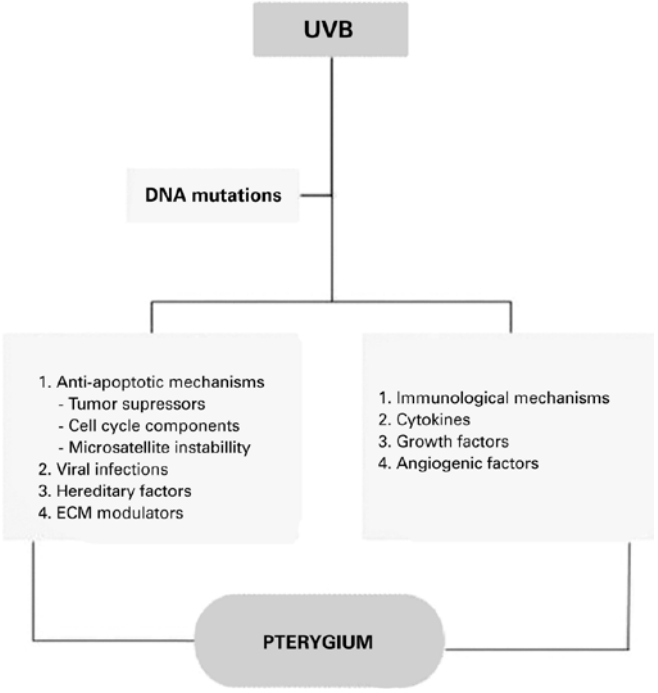

Figure 2. UV-related mechanism in pterygium development.

Visual impairment induced by pterygium growth occurs due to induced astigmatism and opacification in the visual axis, requiring surgical treatment, as well as recurrent inflammation that does not improve with topical treatment[20].

Its surgical procedure consists of dissecting the head of the pterygium from the cornea and resection of the conjunctiva and Tenon's capsule. Several surgical techniques have been used, with excision of the pterygium followed by autologous conjunctival grafts as the most common, showing lower recurrence rates. Recurrence after a surgical treatment can be identified by the growth of conjunctival vessels toward the limbal edge inducing fibrous tissue growth into the cornea and representing poor outcomes[22]. However, clinical features such as extensive size, inflammation, and recurrent lesions remain challenges during the surgical treatment[23,24].

Pathogenesis

UV radiation exposure

UV radiation from the sunlight is divided into three categories:

1. UVA (wavelength, 320-400 nm) has the longest wavelength and maximum penetration power; thus it is not attenuated by the ozone layer. Is an important inducer of pigmentation and contributes to premature skin aging, immunosuppression, and carcinogenesis[25].

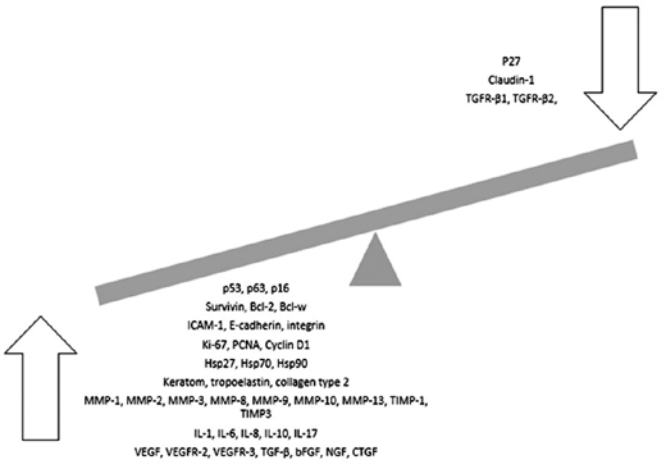

Figure 3. Possible biomarkers related to pterygium development.

2. UVB (wavelength, 280-320 nm) is absorbed by the ozone layer and comprises approximately 1%-10% of the total UV radiation that reaches the Earth's surface. It is responsible for various biological events, including sunburn, immunosuppression, and carcinogenesis[25].
3. UVC (wavelength, 200-280 nm) has the highest energy among the three UV rays and possesses strong mutagenic properties. It is almost completely absorbed by the ozone layer, thereby imposing negligible effects to human eyes[26].

UVB light exposure has been attributed as a major cause of pterygium. This kind of radiation can potentially harm and alter cells and tissues through direct phototoxic effects on the cellular DNA and generation of reactive oxygen species, which damage the cellular DNA. Wavelengths below 300 nm have been known as the most biologically active forms and are absorbed by the cornea. Exposure to UVB radiation causes oxidative stress, which may lead to upregulation of many potential mediators of pterygium growth[27-29] as shown in figure 2.

Viral infections

The polymerase chain reaction technique allowed examination of the alleged involvement of viral infections in the process of pterygium pathogenesis. Some reports demonstrated the presence of herpes simplex virus and human papilloma virus (HPV) in pterygium samples[30,31]. Viruses encode proteins that inactivate *p53*, leading to chromosomal instability and increasing the likelihood of cell progression to malignancy. HPV is most frequently found in the pterygium, with variable prevalence rates[32]. Its involvement as a cofactor in the pterygium pathogenesis is suggested, but remains controversial. If indeed HPV is involved in pterygium pathogenesis or recurrence, anti-viral medications or vaccination may be new options in pterygium therapy[30,33].

Molecular mechanisms

Many studies have proposed possible mechanisms of pterygium development, including oxidative stress, extracellular matrix modulators, apoptotic and oncogenic proteins, loss of heterozygosis, DNA methylation, inflammatory mediators, lymphangiogenesis, transition from mesenchymal epithelial cells, and cholesterol metabolism alterations. These studies show evidences that several molecules, such as matrix metalloproteinases (MMPs), growth factors, and interleukins (ILs), are related to proliferation, inflammation, angiogenesis, and fibrosis, as shown in figure 3 and detailed below[34-36].

Tumor suppressor genes

Tumor suppressor genes prevent cells from converting into cancer cells and regulate cell growth along with proto-oncogenes[37]. One of the tumor suppressor genes that have been extensively studied is *p53*. A survey[38] showed that >20% of all pterygium samples were positive for *p53* expression. Another immunohistochemical study[37] evaluated 13 pterygium samples and 2 normal conjunctiva samples, which showed that 54% of pterygium were positive for *p53* aberrant expression, whereas no pathological staining was observed in the normal conjunctiva. Therefore, the aberrant expression of *p53* is suggested to promote cell proliferation and slow down apoptosis, thereby accelerating the development of pterygium; besides, the possible growth of limbal tumors is also suggested to be caused by cellular DNA damage that causes mutations in other genes[39]. In addition to *p53*, other tumor suppressor genes, such as *p63*, *p16*, and *p27*, were possibly involved in the development of pterygium. *P63* is more expressed in the basal and parabasal layers in primary pterygium and in the total thickness of the epithelium in recurrent pterygium. Increased expression of *p16* protein was also observed in pterygium. Both *p63* and *p16* appeared to be rarely expressed in the normal conjunctiva[40]. *P27* gene showed low nuclear immunoreactivity in pterygium tissues, differing from other tumor suppressor genes[41].

Apoptosis-related proteins

Survivin is a protein encoded by the *BIRC5* gene in humans; it is a member of the apoptosis inhibitory gene family and is expressed in the pterygium epithelium[42]. The molecular mechanisms of survivin regulation are still not well understood; however, survivin regulation seems to be associated with the *p53* protein. Oxidative stress has been demonstrated to be caused by the activation of survivin leading to pterygium growth[43]. In addition, survivin has been found to be highly expressed in all pterygium tissues, but not in the normal human conjunctiva. Survivin was found to be closely related with COX-2 in primary pterygium, suggesting an antiapoptotic mechanism[44].

Bcl-2 is the founding member of the *Bcl-2* family of apoptosis regulatory proteins, which can induce or inhibit apoptosis. It is encoded by the *Bcl-2* gene in humans[45,46]. *Bcl-2* expression was noted in the basal epithelial layer of all pterygium epithelial cells, whereas the normal conjunctiva showed no evidence of the pro-

tein[39]. Decreased miR-122 expression in the pterygium can result in cell apoptosis abnormalities due to its regulation of *Bcl-w* expression, also a gene of the *Bcl-2* family, anti-apoptotic, and subsequently contribute to the development of pterygium[47].

Rapamycin complex 1 (mTORC1) is a central regulator of cell growth, proliferation, protein synthesis, autophagy, and transcription. The role of mTORC1 is to activate the protein translation. mTOR signaling is highly activated; therefore, aberrant apoptosis and cell proliferation were observed in pterygium samples. Activation of mTORC1 has been shown to inhibit apoptosis in pterygium by regulating *Beclin-1*-dependent autophagy by targeting *Bcl-2*. mTORC1 also negatively regulates the fibroblast growth factor receptor 3 (FGFR3) through the inhibition of *p73*, thereby stimulating cell proliferation in pterygium. This demonstrates that mTORC1 signaling is highly activated in pterygium and provides new pathways on its pathogenesis and progression[48].

Cell adhesion molecules

Cell adhesion molecules play an important role in various physiological and pathological phenomena. These proteins are located on the cell surface and are intrinsically involved in cell binding and other extracellular matrix related to cell adhesion, including selectin, and integrin[49]. The expression of intercellular adhesion molecule-1 (ICAM-1) is found to be present in pterygium and absent in the epithelium of a normal conjunctiva[50]. E-cadherin and beta-catenin have also been suggested to be concentrated in the pterygium tissue and are possibly involved in the epithelial proliferation and adhesion[51].

Proliferation-related proteins

Proliferation-related proteins such as Ki-67, cyclin D1, and nuclear proliferation antigen play a key role in the cell cycle. Ki-67 is an important marker of cell proliferation. An abnormal expression of ki-67 was found in pterygium samples when compared to a normal conjunctiva[52]. Proliferating cell nuclear antigen (PCNA) is a nuclear nonhistone protein necessary for DNA synthesis, and its expression may be used as a marker of cell proliferation. The expression of PCNA was significantly higher in pterygium than that in a normal conjunctiva[53]. Cyclin D1 is a well-known cell cycle control gene that promotes cell cycle progression. A study found that PCNA and cyclin D1 were overexpressed in the limbal part of pterygium epithelial cells as compared with normal conjunctiva samples, which might lead to hyperproliferation of epithelial cells[54]. Cyclin D1 protein expression in fleshy pterygium was found to be significantly higher than that in the atrophic ones. Another study indicated that β-catenin expressed in the nuclei/cytoplasm could increase cyclin D1 protein expression, which favors the proliferation of pterygium cells[55].

Heat shock proteins

Heat shock proteins (HSP) are a protein family produced by cells in response to exposure to stressful conditions. They were first described in relation to heat shock, but are recently known to be expressed during other stresses, including exposure to cold temperatures, UV light, and during wound healing or tissue remodeling[52]. The expression of HSPs, i.e., *Hsp27*, Hsp70, and Hsp90, and hypoxia-inducible factor-1α (HIF-1α) were increased in pterygium. The expression of Hsp27 was detected in the epithelial, endothelial, and vascular smooth muscle cells in pterygium, but only in the epithelium in normal conjunctiva[56]. Changes in HIF-1α and HSP levels in pterygium are believed to represent an adaptive process for cell survival under stressful conditions[57].

Tight junction proteins

Tight junction proteins represent a form of cell-to-cell adhesion in the epithelial or endothelial cell layers, forming continuous seals around the cells and also serving as a physical barrier to prevent solutes and water from passing freely through the paracellular space. Claudin family proteins are an important part of this functional and structural barrier and dysregulation on its expression may result in various diseases including cancer[58]. In normal cornea and conjunctiva, claudin-1 and claudin-4 positivity were demonstrated immunohistochemically[59].

Claudins are indispensable proteins for the formation and maintenance of tight junctions. A strong immunohistochemical expression of claudin-1 was found in epithelium conjunctiva samples, whereas its expression in the pterygium samples was low. The significant decrease in claudin-1 expression in the pterygium compared to the normal conjunctiva seems to be involved in the pathogenesis of pterygium[60].

Extracellular matrix proteins

The extracellular matrix (ECM) is a collection of extracellular molecules secreted by support cells that

provide structural and biochemical support to the surrounding cells[61].

The aberrant expression of extracellular matrix proteins is believed to may be directly associated with the proliferative growth of pterygium, because it is a fibrovascular tissue characterized by an excessive deposition of extracellular matrix and vascular growth. The extracellular matrix proteins contain keratin, elastin, collagen, and fibrin, among others. K8, K16, K14, and AE3 have been known to be present throughout the thickness in the pterygium epithelium but are absent in the normal conjunctiva[62]. In fact, pterygium samples showed a higher mRNA level and tropoelastin expression than the conjunctival tissue. Type II collagen expression was positive only in pterygium, whereas collagen types I, III, and IV were detected in both the pterygium and normal conjunctiva[63].

Matrix metalloproteinases and tissue inhibitors of metalloproteinases

Matrix metalloproteinases (MMPs), also known as matrixins, hydrolyze components of the extracellular matrix. These proteinases play a central role in several biological processes, such as embryogenesis, normal tissue remodeling, wound healing, and angiogenesis, and in diseases such as atheroma, arthritis, cancer, and tissue ulceration[64]. MMPs are a multigene family of >25 secreted and cell surface enzymes that process or degrade various extracellular matrices[65], which can be divided into five subgroups based on substrate preference: collagenases (MMP-1, MMP-8, MMP-13), gelatinases (MMP-2, MMP-9), stromelysins (MMP-3, MMP-10), membrane-associated MMPs (MT1-MMP, MT2-MMP), and others (e.g., MMP-12, MMP-19, MMP-20). Tissue inhibitors of metalloproteinases (TIMPs) bind to and prevent the activities of most MMPs. The relationship between pterygium and these two groups of proteins in the pathogenesis of pterygium has been studied[39].

MMP-1, MMP-2, MMP-3, TIMP-1, and TIMP-3 were detected in greater amounts in pterygium tissues, epithelial cells, and fibroblasts as compared to normal conjunctiva[66,67]. MMP-3 was positively regulated and located in the pterygium epithelium, which may help explain the various pterygium phenotypes[68]. A study showed that cyclosporin A can reduce MMP-3 and MMP-13 expressions in the pterygium fibroblast culture[69].

MMP and TIMP expressions vary at the different stages of pterygium. The balance break between MMPs and TIMPs may be considered to be responsible for the progression or recurrence of pterygium[39].

ILs

ILs are a group of cytokines, secreted proteins, and signal molecules first seen to be expressed by the white blood cells (leukocytes). These cells play vital roles in the inflammation process; thus, ILs can be closely related to pterygium[66].

The expression of IL-1α, IL-1β RA, and IL-1β precursor proteins in primary pterygium and normal conjunctival epithelium were detected via immunofluorescence. Enhanced levels of IL-1 family proteins were present in pterygium only. Likewise, IL-1α was found to be highly expressed not only in primary but also in recurrent pterygium[67].

IL-6 and IL-8 were strongly expressed in the pterygium epithelium as compared to the normal cornea, conjunctiva, and limbus. In addition, IL-6 and IL-8 proteins were significantly elevated in pterygium treated with UVB, suggesting that UVB could induce the secretion of these two ILs[70]. IL-8 can also induce corneal vascularization directly[71]. IL-10 had also been reported to be expressed more in pterygium than that in the normal conjunctiva. Recently, IL-17 was found to be upregulated in the ocular surface in inflammatory pathologies, such as pterygium[72].

Growth factors

A growth factor is a natural substance capable of stimulating cellular growth, proliferation, healing, and cellular differentiation. They are important in the regulation of various cellular processes, such as mitosis[73].

Numerous growth factors are thought to have a role in pterygium pathogenesis, such as the vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF-β), basic fibroblast growth factor (bFGF), insulin-like growth factor, nervous growth factor, and connective tissue growth factor (CTGF)[7]. The VEGF family has been extensively investigated in ophthalmology, because of its role in pathological angiogenesis and in increasing the vascular permeability in ocular diseases, such as pterygium and retinal diseases[74].

Increased expression of VEGF leads to angiogenesis and lymphangiogenesis, which may influence the normal metabolism of the connective cells and promote vascular growth. When compared to the normal conjunctiva, pterygium showed higher VEGF levels[75,76]. TGF-β regulates various processes common to tissue repair and

disease, including fibroblast proliferation, angiogenesis, synthesis, and degradation of extracellular matrix proteins[77]. TGF-β1 and TGF-β2 were found to be positively regulated, whereas transforming growth factor-beta receptor 1,2 (TGFR-β1, β2) was negatively regulated in pterygium[78,79].

Anti-VEGF drugs such as ranibizumab and bevacizumab have been widely used for the treatment and control of ocular diseases associated with vascular proliferation[80]. Although some studies suggest the use of anti-VEGF as an adjuvant therapy for surgery, studies conducted to characterize its use for the treatment of pterygium are lacking[81].

Understanding the etiopathogenesis and most relevant factors involved in pterygium may allow advances on strategies to prevent its onset and progression, which may even prevent surgical procedures in the future. Although various studies have already been conducted, important genes and proteins have probably not yet been discovered. In this sense, performing additional research to better understand the etiopathogenic mechanisms and, thus, promote more targeted and effective treatment options, especially in recurrent cases, may be interesting.

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