

The expression of vascular endothelial growth factor in pterygium tissue of atopic patients

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Abstract The exact pathogenesis of pterygium has not been completely elucidated. Growth factors have been considered to play a role in pterygium formation. Vascular endothelial growth factor (VEGF) is one of the principal mediators of angiogenesis, fibroblast stimulation and tissue remodeling in allergic conditions. The aim of this study was to compare the association between pterygium and VEGF gene expression between atopic and non-atopic individuals. At first visit, all patients with pterygium underwent blood tests, serum immunoglobulin E (IgE), serum cytokines including interleukin-4 (IL-4) and interferon- γ (IFN- γ) and peripheral blood eosinophil count. After

obtaining informed consents, questionnaires were used to obtain demographic and clinical data from patients who underwent pterygium excision surgery. Skin prick test was performed to confirm or rule out atopy in 30 patients with (case group) and 30 patients without (control group) atopy. Pterygium tissues were then removed by surgery. A semi-quantitative reverse transcriptase polymerase chain reaction was performed to determine VEGF gene expression in all patients. Our results illustrated that VEGF mRNA expression in atopic patients was significantly higher than in the non-atopic group ($P = 0.01$). Eosinophil count, serum IgE and IL-4 were also significantly higher in atopic patients than in the non-atopic group ($P = 0.03$, 0.001 and 0.001, respectively). However, no significant difference was noted in serum IFN- γ between the two groups ($P = 0.06$). The excessive expression of VEGF gene in pterygium tissue of patients with atopy suggests that growth factors may play a role in the pathogenesis of pterygium or accelerate its formation.

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Introduction

Pterygium is an invasive fibrovascular growth of the conjunctiva which is generally associated with ultraviolet (UV) light exposure [1]. Although environmental

factors, such as wind, dust, heat, infection, smoke, chemicals, pollens and eye dryness, are proposed to have a role in the pathogenesis of pterygium [2, 3], the exact etiology of this disease remains to be completely elucidated.

Some studies have suggested an allergic and immunological basis for the pathogenesis of pterygium [4, 5]. In addition, several growth factors, cytokines and metalloproteinase enzymes are detected in the cornea during the recovery period after photorefractive keratectomy. These biological factors, which are produced by migratory leukocytes, are considered to play a key role in pterygium formation [2]. It has also been shown that UV can activate signaling pathways in pterygium epithelial cells which result in cytokine and growth factor production [6]. A considerable body of evidence indicates that UV exposure enhances the tissue expression of vascular endothelial growth factor (VEGF) [7, 8].

Atopic patients experience both acute and chronic symptoms as a result of release of inflammatory mediators. VEGF along with immunoglobulin E (IgE), interleukins and other growth factors are strong mediators for allergic reactions in atopic patients [9, 10]. To the best of our knowledge, there are no previous reports in the literature investigating the association between pterygium and VEGF in atopic individuals. In this study, we evaluated VEGF mRNA gene expression in pterygium tissue of a group of atopic patients by using reverse transcriptase polymerase chain reaction (RT-PCR) and its probable association with pterygium formation. We aimed to determine whether VEGF overexpression in atopic patients can promote tissue remodeling in pterygium formation. In addition, we investigated the histopathological differences in pterygium between atopic and non-atopic patients.

Materials and methods

Study population

This study was in accordance with the Helsinki Declaration and approved by the Ethics Committee of Mashhad University of Medical Sciences. After obtaining informed consents, predesigned questionnaires were used to record demographic data and past medical history of pterygium patients referred to the

Ophthalmology Department of Mashhad Eye Hospital from June 2010 to May 2011. We aimed to choose most of the patients from spring until the middle of summer, as skin sensitivity increases after the pollen season. Consecutive pterygium patients with a history of at least one allergic condition, such as asthma, allergic rhinitis, atopic dermatitis, hives, angioedema or food allergies, underwent skin prick testing and blood tests including measurement of total serum IgE level and cytokines assay.

Thirty patients with atopic criteria were included in the case group. Similarly, 30 consecutive pterygium patients without any history of allergic reactions were enrolled in the control group. In this group, skin prick testing and measurement of total serum IgE level were performed to rule out atopy. The exclusion criteria were previous treatment with any kind of corticosteroids during the last 2 months, presence of immunodeficiency and absence of indication for excisional surgery according to the ophthalmology examination. Patients over 60 years of age were excluded from the study population due to the size of skin tests. In addition, we recommended to patients to withdraw at least 48 h before the skin test, because modulators may affect the allergic reactions. Subsequently, surgical excision of pterygium was performed in all 60 patients who were enrolled in this study.

Tissue processing

After the surgery, in order to obtain pterygium samples, tissues were divided into two parts: head of the tissue for histopathological evaluations and body/ the rest for RNA extraction. The tissues were fixed in 10 % formaldehyde, embedded in paraffin and sectioned into 3- μ m-thick pieces. The tissue sections were then stained with hematoxylin and eosin (H&E) for general histopathological investigation. For each tissue, five sections were analyzed, and their average scores were calculated. The tissues were evaluated in each case according to specific cytological contents including the number of eosinophils, mast cells and neutrophil infiltration.

Immunological assessment

For all patients, skin prick testing was performed on the inner forearm, using 19 common standard

domestic allergen extracts (Stallergen, France; Hollister-Stier, USA). In addition, histamine chlorohydrate at 10 mg/mL and phenol–glycerol saline solution were used in both groups simultaneously as positive and negative controls, respectively. We have chosen the most prevalent allergens in our region connected to VKC including tree pollens, grasses and weeds, fungal products and house dust mites, mammalian-derived allergens such as cat, dog and wool dander, and mosquito.

After 15 min, if present, the wheals were outlined and the markings transferred to mm-square paper using a tape. Wheal size was calculated by multiplying the long axis of the wheal by its perpendicular; $>3 \text{ mm}^2$ or any numbers greater than negative control were considered a positive result.

Blood samples, obtained by vein puncture, were allowed to clot at room temperature, and were centrifuged at $1,000\times g$ for 10 min. Aliquots of serum were stored at -70°C until being analyzed for IgE and cytokine assay. In addition, eosinophil count was determined using a Sysmex KX-21 N cell counter (Sysmex Corporation, Kobe, Japan). ELISA was used to determine serum IgE levels (Monobind Inc., USA). Interleukin-4 (IL-4) and interferon- γ (IFN- γ) were measured using an ELISA system from Amersham Bioscience (Buckinghamshire, UK).

Tissue RNA extraction and RT-PCR

After surgery, a piece of the excised tissue was immersed in RNAlater solution at -20°C until extraction. In addition, a clinical pathologist histologically confirmed all specimens as pterygium.

Total RNA was isolated from pterygium biopsy samples by RNeasy Total RNA isolation kit (Fibrous Tissue, Qiagen). The quality and quantity of the RNA were photometrically confirmed. cDNA was then generated with Oligo-dt₁₆ (Pars Tous, Iran). RT-PCR was performed in a total volume of 20 μL containing 2 μL of $10\times$ PCR buffer, 2 mmol/L magnesium chloride, 0.2 mmol/L dNTP mixture, 1.5 U Hot-Start Taq DNA polymerase, 0.5 mmol/L of each primer and 2 μL cDNA. A fragment of VEGF gene (91 bp) was amplified using the following PCR conditions: 35 cycles of 30 s at 94°C (denaturation), 30 s at 60°C (annealing) and 30 s at 72°C (extension) with the following primers: forward, 5'-ATGACGAGGGCCTGGAGTGTG-3' and reverse, 5'-CCTATGTGCTGGCCTTGGTGAG-3'.

To normalize the expression of VEGF mRNA, a 266-bp fragment length of GAPDH was amplified as follows: 35 cycles of 30 s at 94°C (denaturation), 30 s at 56°C (annealing) and 30 s at 72°C (extension) with the following primers: forward, 5'-GGC CAA GAT CAT CCA TGA CAA CT-3' and reverse, 5'-ACC AGG ACA TGA GCT TGA CAA AGT-3'.

PCR products were separated on 2.5 % agarose gels, visualized under ultraviolet illumination and stained with Green viewer (Pars Tous, Iran). The mRNA expression of each gene was determined using Kodak 1D Image Analysis Software (Kodak, Stuttgart, Germany). The band intensity was expressed as an absolute integrated optical density. The integrated optical density of each PCR product was normalized to that of GAPDH for the same biopsy sample.

Statistical analysis

Data were analyzed using SPSS version 18 (SPSS Inc., Chicago, IL, USA) and displayed as the mean $\pm$ standard deviation (SD). For all variables, the Kolmogorov–Smirnov (KS) test was applied to ascertain the normality of the data. Student's paired t test was performed for variables with normal distribution. To compare non-normal variables, the Mann–Whitney test was used. Statistical analysis for the distributions of optical densities in pterygium patients with and without atopy was carried out using the χ^2 test or Fisher's exact test. $P < 0.05$ was considered statistically significant.

Results

Mean $\pm$ standard deviation of age in patients with and without atopy were 52.2 ± 12.0 and 53.4 ± 16.6 years, respectively. In both groups, 21 patients were male (70.0 %) and 9 patients were female (30.0 %). There were no significant differences between the two groups in terms of age ($P = 0.76$) and gender ($P = 1$).

Serum IgE level was significantly higher in atopic patients ($P = 0.001$): $170.04 \pm 65.00 \text{ IU/mL}$ in allergic patients compared with $32.21 \pm 15.96 \text{ units/mL}$ in the control group (Fig. 1c). Similarly, blood eosinophil count was significantly ($P = 0.03$) higher in atopic patients ($1.87 \pm 0.73 \times 10^9/\text{L}$) than in patients without atopy ($1.47 \pm 0.51 \times 10^9/\text{L}$). As can be seen in Fig. 1, Student's paired t test indicated that serum IL-4 was significantly ($P = 0.001$) higher

Fig. 1 Comparison of serum cytokines IL-4 (a) and IFN- γ (b) and total IgE (c) levels between pterygium patients with and without atopy

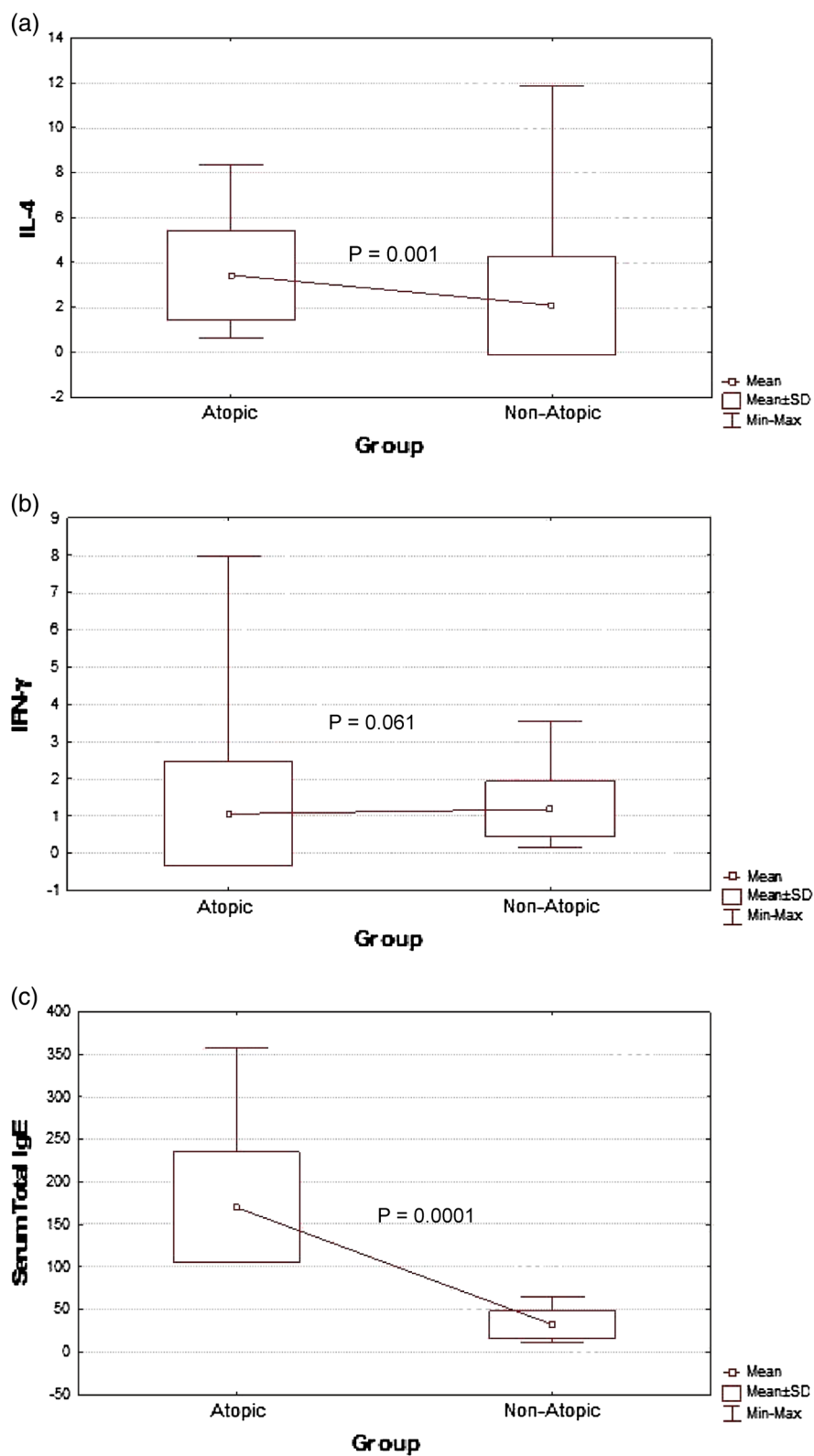
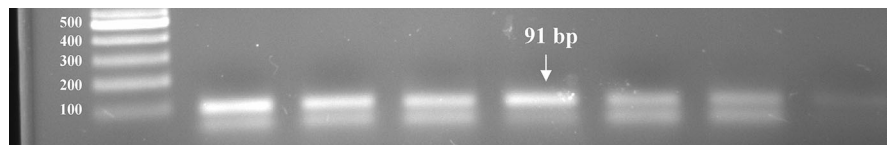


Fig. 2 Vascular endothelial growth factor gene expression compared to 100-bp ladder, measured using RT-PCR



in atopic patients (3.43 ± 1.98 pg/mL) than in non-atopic patients (2.09 ± 2.18 pg/mL). However, no significant difference was noted ($P = 0.06$) in the serum IFN- γ level between the two groups (1.06 ± 1.39 pg/mL in the case and 1.18 ± 0.74 pg/mL in the control group) (Fig. 1a,b).

According to the Mann–Whitney test, the mean relative expression level of VEGF mRNA was significantly ($P = 0.01$) higher in atopic patients (1.90 ± 0.74) than in non-atopic individuals (1.44 ± 0.52) (Fig. 2).

It should be noted that the increases in the number of eosinophils and mast cells in pterygium tissues of atopic patients did not reach statistical significance in any cytological contents of pterygium tissues of patients with or without atopy.

Discussion

In this study, we investigated the relationship between VEGF expression levels and pterygium formation in atopic and non-atopic patients. Although several factors have been proposed to play a role in the pathogenesis of pterygium, we found VEGF mRNA overexpression in pterygium tissue of atopic compared with non-atopic patients. Our data showed that the concentrations of IgE and IL-4 were significantly elevated in atopic patients with pterygium compared with non-atopic individuals with pterygium. To the best of knowledge, the current study is the first to demonstrate that VEGF mRNA is overexpressed in the pterygium tissue of atopic patients compared with non-atopic individuals. Therefore, we hypothesized that atopy may play a role in the mechanism of pterygium formation.

VEGF is involved in allergic, immunological and inflammatory processes such as allergic asthma. Allergic patients, such as asthmatic individuals, suffer from acute and chronic processes. Histamine and leukotrienes are instantly released from mast cells and eosinophils in response to allergens, hence inducing acute allergic conditions.

VEGF is a potent mediator of tissue remodeling in asthmatic patients. Chetta et al. [11] revealed that the

number of VEGF⁺ cells in bronchial tissue of asthmatic patients is significantly correlated with the number of vessels and mast cells, and basement membrane thickness (type IV collagen). In that study, it was concluded that VEGF leads to chronic airway inflammation and subepithelial tissue fibrosis through a pathway which is mediated by transforming growth factor- β_1 [11]. In addition, Lee et al. [12] demonstrated that VEGF is selectively elaborated by T-helper type 2 lymphocytes (Th2), but not Th1. They showed that VEGF enhances antigen sensitization and Th2 inflammation, since it is necessary for antigen-induced cytokine production by Th2 cells. Although many cytokines were produced by the subtype (where they are non-specific parameters for diagnosis of allergic disease), as shown previously, IL-4 is a hallmark Th2 cytokine and the Th2 cell-mediated pathway is implicated in IL-4 production and allergic reactions [13]. However, the Th1 pathway leads to IFN- γ production [14]. Thus, in this study, we aimed to measure IL-4 as a Th2 subtype. IL-4 was significantly elevated in atopic patients with pterygium compared with non-atopic with pterygium. As shown previously, the Th2 cell-mediated pathway is implicated in IL-4 production and allergic reactions. In addition, according to Huang et al. [15], IL-4 will stimulate VEGF production. It has been suggested that IL-4 and IL-5 promote airway hyperresponsiveness, neutrophilia and remodeling through the secretion of CXCL-8, E-selectin and VEGF from endothelial cells.

In our study, the higher level of serum IL-4 and the slightly lower level of serum IFN- γ , accompanied by VEGF overexpression, in the atopic compared with non-atopic patients suggests Th2 elevation. This may results in angiogenesis, tissue remodeling and matrix production through VEGF overexpression or a Th2-mediated pathway. However, more studies are needed to confirm the significance of hypersensitivity reactions in the pathogenesis of pterygium. Therefore, it is unclear to us whether the incidence or prevalence of pterygium is directly or indirectly correlated with the severity of atopy.

Although the pathogenesis of pterygium is still not well understood, some risk factors including UV

light exposure, immunoinflammatory processes, viral infections and DNA damage have been proposed to be responsible for pterygium formation [16]. In addition, some studies have suggested that pterygium may be considered a benign neoplastic disorder because of the local invasion, epithelial cell metaplasia and abnormal expression of p53 tumor suppressor gene which is found in pterygium tissue [17, 18]. Cells with mutations in p53 will become resistant to hypoxia-mediated apoptosis and produce VEGF. This pro-angiogenic cytokine facilitates tumor angiogenesis and oxygenation [19] and therefore increases cell proliferation and survival [20]. Since VEGF acts as a chemo-attractant for endothelial cells, it enhances cell migration and invasion [21, 22]. Our results have shown that inflammatory cells such as eosinophils and mast cells were increased in cytopathological evaluations of atopic patients; however, this increase was not significant. In addition, VEGF induces the expression of connective tissue growth factor (CTGF) and will result in angiogenesis, extracellular matrix production and fibrosis. It was previously reported that CTGF maintains vascular structure, promotes endothelial cell survival, exerts anti-apoptotic effects and may result in elastotic degeneration and neoplasm formation in the pterygial tissue [23]. Because VEGF induces the expression of CTGF and results in angiogenesis, extracellular matrix production and fibrosis [24], it could be considered as one of the key components of the pathophysiology of pterygium.

It has been revealed that VEGF plays an important role in wound healing [19]. Activated platelets, endothelial cells, monocytes and keratinocytes release VEGF at the site of injury to start up angiogenesis. In addition, the signals of VEGF recruit the circulating neutrophils and monocytes to assist in the normal inflammatory response [25]. Changes in permeability as a result of the effect of VEGF on cell junction proteins lead to the formation of granulation tissue [26]. In addition, VEGF is considered to be a potent mediator of angiogenesis and vasculogenesis: it gathers and stimulates endothelial progenitor cells of bone marrow to create new vessels [27] and stimulates pericytes to cover and maintain the vasculature. VEGF also activates receptors and causes endothelial cell proliferation and activation [25]. According to Pang and Rose [2], photoreactive keratectomy exacerbates the growth of pterygium, possibly because of the rise of several fibrogenic cytokines during recovery from

surgery. We therefore suggest that the high recurrence rate of pterygium [28] could be due to the stress-induced increase in growth factors such as VEGF after pterygium excision surgery that cause pathological wound healing and scar formation.

Recently, subconjunctival injection of anti-VEGF monoclonal antibodies, such as pegaptanib, bevacizumab and ranibizumab, has been widely used for the treatment of pterygium [29]. However, due to the potential systemic side effects of these reagents, and the possible inefficacy of bevacizumab in the improvement of refractive astigmatism and visual acuity, and its presumed inability to prevent recurrence [30, 31], anti-VEGF administration is suggested for selected patients with specific cytokine and growth factor profiles. For example, this drug may be utilized when certain cytokines and growth factors are detected in the molecular examination of each lesion [29]. Therefore, in the current study, the expression of VEGF mRNA in the pterygium tissues of atopic patients was significantly higher compared with non-atopic individuals. As a result, we suggest that anti-VEGF therapy may be an effective treatment in pterygium patients with atopy. In general, we have speculated whether the IgE-mediated mechanisms that cause allergic inflammation can predispose atopic individuals to development of pterygium. Further studies on atopic patients are required to evaluate the role of anti-VEGF therapy in success rates of pterygium surgery. Furthermore, the comparison of VEGF level in tears or conjunctival specimens between atopic patients with and without pterygium may help to understand the mechanism. We strongly believe that it is advisable to evaluate any differential response to anti-VEGF therapies between atopic and non-atopic patients for a few ocular conditions such as pterygium.

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References

1. Threlfall TJ, English DR (1999) Sun exposure and pterygium of the eye: a dose-response curve. *Am J Ophthalmol* 128(3):280–287
2. Pang Y, Rose T (2006) Rapid growth of pterygium after photorefractive keratectomy. *Optometry* 77(10):499–502
3. Abelson MB, Turner D (2003) A randomized, double-blind, parallel-group comparison of olopatadine 0.1 % ophthalmic solution versus placebo for controlling the signs and

- symptoms of seasonal allergic conjunctivitis and rhinoconjunctivitis. *Clin Ther* 25(3):931–947
4. Pinkerton OD, Hokama Y, Shigemura LA (1984) Immunologic basis for the pathogenesis of pterygium. *Am J Ophthalmol* 98(2):225–228
 5. Isaji M, Kikuchi S, Miyata H, Ajisawa Y, Araki-Inazawa K, Tsukamoto Y, Amano Y (2000) Inhibitory effects of tranilast on the proliferation and functions of human pterygium-derived fibroblasts. *Cornea* 19(3):364–368
 6. Di Girolamo N, Wakefield D, Coroneo MT (2006) UVB-mediated induction of cytokines and growth factors in pterygium epithelial cells involves cell surface receptors and intracellular signaling. *Invest Ophthalmol Vis Sci* 47(6):2430–2437
 7. Yano K, Kajiya K, Ishiwata M, Hong YK, Miyakawa T, Detmar M (2004) Ultraviolet B-induced skin angiogenesis is associated with a switch in the balance of vascular endothelial growth factor and thrombospondin-1 expression. *J Invest Dermatol* 122(1):201–208
 8. Trompezinski S, Pernet I, Mayoux C, Schmitt D, Viac J (2000) Transforming growth factor-beta1 and ultraviolet A1 radiation increase production of vascular endothelial growth factor but not endothelin-1 in human dermal fibroblasts. *Br J Dermatol* 143(3):539–545
 9. Park HK, Park HW, Jeon SG, Shin ES, Cho SH, Kim YY, Kim YK (2008) Distinct association of genetic variations of vascular endothelial growth factor, transforming growth factor-beta, and fibroblast growth factor receptors with atopy and airway hyperresponsiveness. *Allergy* 63(4):447–453
 10. Kanazawa H (2007) Microvascular theory of exercise-induced bronchoconstriction in asthma: potential implication of vascular endothelial growth factor. *Inflamm Allergy Drug Targets* 6(2):133–137
 11. Chetta A, Zanini A, Foresi A, D'Ippolito R, Tipa A, Castagnaro A, Baraldo S, Neri M, Saetta M, Olivieri D (2005) Vascular endothelial growth factor up-regulation and bronchial wall remodelling in asthma. *Clin Exp Allergy* 35(11):1437–1442
 12. Lee CG, Link H, Baluk P, Homer RJ, Chapoval S, Bhandari V, Kang MJ, Cohn L, Kim YK, McDonald DM, Elias JA (2004) Vascular endothelial growth factor (VEGF) induces remodeling and enhances TH2-mediated sensitization and inflammation in the lung. *Nat Med* 10(10):1095–1103
 13. Sauma D, Espejo P, Ramirez A, Fierro A, Roseblatt M, Bono MR (2011) Differential regulation of Notch ligands in dendritic cells upon interaction with T helper cells. *Scand J Immunol* 74(1):62–70
 14. Garssen J, Vandebruijn RJ, De Gruijl FR, Wolvers DA, Van Dijk M, Fluitman A, Van Loveren H (1999) UVB exposure-induced systemic modulation of Th1- and Th2-mediated immune responses. *Immunology* 97(3):506–514
 15. Huang H, Lavoie-Lamoureux A, Moran K, Lavoie JP (2007) IL-4 stimulates the expression of CXCL-8, E-selectin, VEGF, and inducible nitric oxide synthase mRNA by equine pulmonary artery endothelial cells. *Am J Physiol Lung Cell Mol Physiol* 292(5):L1147–L1154
 16. Detorakis ET, Drakonaki EE, Spandidos DA (2000) Molecular genetic alterations and viral presence in ophthalmic pterygium. *Int J Mol Med* 6(1):35–41
 17. Siak JJ, Ng SL, Seet LF, Beuerman RW, Tong L (2011) The nuclear-factor kappaB pathway is activated in pterygium. *Invest Ophthalmol Vis Sci* 52(1):230–236
 18. Chan CM, Liu YP, Tan DT (2002) Ocular surface changes in pterygium. *Cornea* 21(1):38–42
 19. Byrne AM, Bouchier-Hayes DJ, Harmey JH (2005) Angiogenic and cell survival functions of vascular endothelial growth factor (VEGF). *J Cell Mol Med* 9(4):777–794
 20. Royds JA, Dower SK, Qwarnstrom EE, Lewis CE (1998) Response of tumour cells to hypoxia: role of p53 and NFkB. *Mol Pathol* 51(2):55–61
 21. Grosskreutz CL, Anand-Apte B, Duplaa C, Quinn TP, Terman BI, Zetter B, D'Amore PA (1999) Vascular endothelial growth factor-induced migration of vascular smooth muscle cells in vitro. *Microvasc Res* 58(2):128–136
 22. Price DJ, Miralem T, Jiang S, Steinberg R, Avraham H (2001) Role of vascular endothelial growth factor in the stimulation of cellular invasion and signaling of breast cancer cells. *Cell Growth Differ* 12(3):129–135
 23. van Setten G, Aspiotis M, Blalock TD, Grotendorst G, Schultz G (2003) Connective tissue growth factor in pterygium: simultaneous presence with vascular endothelial growth factor—possible contributing factor to conjunctival scarring. *Graefes Arch Clin Exp Ophthalmol* 241(2):135–139
 24. Suzuma K, Naruse K, Suzuma I, Takahara N, Ueki K, Aiello LP, King GL (2000) Vascular endothelial growth factor induces expression of connective tissue growth factor via KDR, Flt1, and phosphatidylinositol 3-kinase-Akt-dependent pathways in retinal vascular cells. *J Biol Chem* 275(52):40725–40731
 25. Hoeben A, Landuyt B, Highley MS, Wildiers H, Van Oostrom AT, De Bruijn EA (2004) Vascular endothelial growth factor and angiogenesis. *Pharmacol Rev* 56(4):549–580
 26. Dvorak HF, Detmar M, Claffey KP, Nagy JA, van de Water L, Senger DR (1995) Vascular permeability factor/vascular endothelial growth factor: an important mediator of angiogenesis in malignancy and inflammation. *Int Arch Allergy Immunol* 107(1–3):233–235
 27. Takahashi T, Ueno H, Shibuya M (1999) VEGF activates protein kinase C-dependent, but Ras-independent Raf-MEK-MAP kinase pathway for DNA synthesis in primary endothelial cells. *Oncogene* 18(13):2221–2230
 28. Sarnicola V, Vannozzi L, Motolese PA (2010) Recurrence rate using fibrin glue-assisted ipsilateral conjunctival autograft in pterygium surgery: 2-year follow-up. *Cornea* 29(11):1211–1214
 29. Detorakis ET, Spandidos DA (2009) Pathogenetic mechanisms and treatment options for ophthalmic pterygium: trends and perspectives (Review). *Int J Mol Med* 23(4):439–447
 30. Razeghinejad MR, Hosseini H, Ahmadi F, Rahat F, Eghbal H (2010) Preliminary results of subconjunctival bevacizumab in primary pterygium excision. *Ophthalmic Res* 43(3):134–138
 31. Shenasi A, Mousavi F, Shoa-Ahari S, Rahimi-Ardabili B, Fouladi RF (2011) Subconjunctival bevacizumab immediately after excision of primary pterygium: the first clinical trial. *Cornea* 30(11):1219–1222