



Review article

A comprehensive review on vernal keratoconjunctivitis with emphasis on proteomics



Lolly Pattnaik^{a,*}, Laxmikanta Acharya^{b,**}

^a Institute of Medical Sciences and SUM Hospital, Siksha O Anusandhan University, Bhubaneswar, Odisha, India

^b Centre of Biotechnology, Siksha O Anusandhan University, Bhubaneswar, Odisha, India

ARTICLE INFO

Article history:

Received 23 October 2014

Accepted 26 January 2015

Available online 3 March 2015

Keywords:

Allergic conjunctivitis

Chemokine receptor

Genomics

Interleukin

Matrix metalloproteinase

Proteomics

Vernal keratoconjunctivitis

ABSTRACT

Allergic conjunctivitis presents as a spectrum of different clinical entities, such as perennial allergic conjunctivitis, seasonal allergic conjunctivitis, atopic keratoconjunctivitis and vernal keratoconjunctivitis. Vernal keratoconjunctivitis (VKC) is a disorder that is often associated with allergens and is seen during the spring season. Herein, we focused on vernal keratoconjunctivitis (VKC) and reviewed its epidemiology, clinical presentations, ocular associations, available treatment options, and the progressive understanding of its histopathological features; we have also systematically elaborated on the various studies on proteomics. Initial theories of a solely IgE-mediated mechanism have been replaced by those considering IgE and non-IgE mechanisms. Developments in basic and clinical research will open novel diagnostic approaches for the early detection and cure of the disease.

© 2015 Elsevier Inc. All rights reserved.

Contents

Introduction	48
Demographics	48
Clinical features and diagnosis	48
Associated conditions	48
Risk factors	49
Etiology	49
Pathophysiology of vernal keratoconjunctivitis	49
Major gaps in knowledge	49
Proteomics of VKC	50
Histopathology	50
Cells in VKC	50
Mast cells	50
T cells	50
B cells	50
Eosinophils	50
Epithelial cells	51
Fibroblasts	51
Histamine	51
Adhesion molecules	51
Chemokines	51
Role of matrix metalloproteinase enzymes	51
Growth factors	51
Neuromediators	51

* Correspondence to: L. Pattnaik, IMS and SUM Hospital, Kalinganagar, Ghatikia, Siksha O Anusandhan University, Bhubaneswar, Odisha 751003, India. Tel.: +91 674 2386210.

** Co-corresponding author.

E-mail address: lollypattnaik@gmail.com (L. Pattnaik).

Recent developments	52
Treatment	52
Surgical treatment	52
Method of literature search	52
Disclosure	52
Conflict of interest	53
References	53

Introduction

Vernal keratoconjunctivitis (VKC) is a member of a group of allergic diseases that includes perennial and seasonal allergic conjunctivitis, atopic keratoconjunctivitis and giant papillary conjunctivitis [16,21,28]. The term vernal is a misnomer, as the disease can present throughout the year with seasonal exacerbations, and 60% of VKC cases have repeated recurrences throughout the year [74]. As a chronic disease, VKC can have a detrimental effect on the quality of life of patients and their families, affecting school, social interactions, lifestyle choices and overall personal development [27].

Demographics

VKC mostly presents bilaterally, and sometimes asymmetrically in young boys in the first decade of their lives, in 80% of the cases. Late-onset VKC-like disease has also been described in young adults [47]. VKC has a wide geographical distribution and is commonly found in warm, dry areas, including the Mediterranean regions, Central Africa, India and South America. In a large tertiary care center study in southern India, majority of the patients presented at a mean age of 12 years. Additionally, most of the patients presented with a mixed variety disease (72%), with adult onset of the disease (first onset at or above 20 years of age) occurring in only 12% of cases [67]. In a school in Rwanda, Central Africa, one study reported that 121 out of 3447 students had VKC (prevalence rate – 4%) [72]. A western European study reported a projected prevalence rate of 3.2/10,000 population, with the highest prevalence rates in Italy (0.4–4.8) [36]. An Italian multicenter study reported predominant limbal presentation (58.6%), and a study from Nigeria reported a predominance of the palpebral variety (82.6%) [42,81]. Though less frequently, cooler temperate regions such as the UK, Sweden, other northern European countries, Australia and North America have considerably added to our knowledge of VKC. Even Japan has a considerable number of VKC patients. The worldwide distribution of the disease indicates that factors apart from warm climate are involved in its pathogenesis [82].

Clinical features and diagnosis

The clinical features of VKC vary depending on the severity of the disease. Patients present with intense ocular itching, photophobia,

stringy mucous discharge and foreign body sensation in varying proportions. VKC primarily has two variants – limbal and tarsal, but it is not uncommon to find mixed appearances. The disease occurs commonly in young boys, with duration varying from four to eight years and gradual subsidence just before or after puberty. Adult onset VKC-like disease with no history of similar symptoms during childhood is found in a few young adults. Symptoms include infiltration of limbal subconjunctival tissue with formation of limbal papillae. At times, the limbus assumes a gelatinous, thickened appearance with chalky white excrescences on top of papillae; these are called Horner Trantas spots. The upper tarsal conjunctiva can show papillae ranging from 1 mm in size to giant cobblestone papillae. The hallmark of VKC that differentiates it from other allergic conjunctivitis is the presence of limbal and giant cobblestone papillae on the upper tarsal conjunctiva (Fig. 1) [16]. Severe cases may include corneal neovascularization with extensions of limbal vegetations affecting vision, shield ulcers, pseudogerontoxon, corneal scarring, and keratoconus. Prolonged presence of papillae at the limbus induces stem cell deficiency with subsequent corneal vascularization [68]. During exacerbations and in the absence of appropriate treatment, punctate epithelial keratitis may lead to macro erosions and shield ulcers.

Diagnosis is mostly based on the clinical findings of VKC. Laboratory investigations such as serum and tear IgE levels, abundance of eosinophils, mast cells in the conjunctiva, skin prick tests and response to anti-allergic treatment cannot conclusively confirm VKC or predict its prognosis [11]. Corneal scarring and vascularization, steroid-induced glaucoma and cataract, amblyopia, dry eye, and keratoconus are complications associated with the disease.

Associated conditions

VKC is associated with ectatic disorders, sex hormone-associated diseases, increased eyelash length and other atopic disorders (40%–75%) [39]. Excessive eye rubbing may play a role in the development of keratoconus in this category of patients [62]. In addition to keratoconus, which is the most common ectatic disorder associated with VKC, the disease is reportedly associated with other ectatic disorders, pellucid marginal degeneration, keratoglobus, and superior corneal thinning [25,38].

Sex-hormone-related diseases such as gynecomastia, polycystic ovary syndrome, mammary fibroadenoma, adiposogenital dystrophy

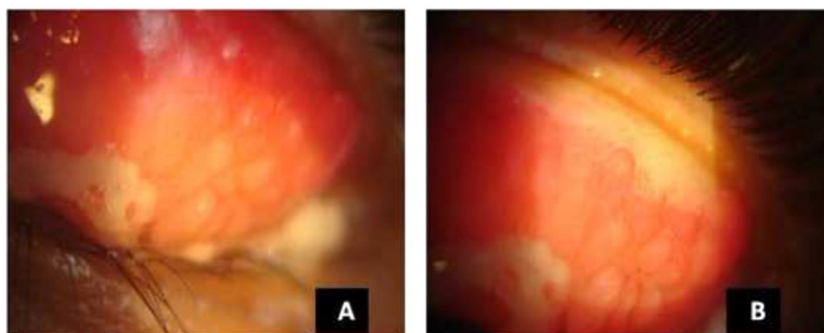


Fig. 1. Cobblestone papillae with stringy mucous discharge on upper tarsal conjunctiva in a VKC Patient. A: Normal view B: Magnified view.

and autoimmune diseases have been reported in 2% of patients with VKC [17]. A positive correlation has been found between eyelash length and severity of VKC [62]. It is postulated that long lashes could represent a possible protective mechanism against physical agents causing keratoconus. Multiple atopic disorders are associated with VKC, the commonest being asthma.

Risk factors

Exogenous allergens such as ultraviolet light, heat, fumes, and smoke are risk factors influencing the signs and symptoms of the disease [83]. Endocrine and genetic risk factors also reportedly play roles. The supporting evidence for the former are the gender disparity in VKC's prevalence, enhanced neuroreceptor expression in conjunctival epithelium, over-expression of estrogen and progesterone receptors in conjunctival eosinophils, and higher levels of growth factors, neuropeptides such as substance P and prostaglandins [18,20]. The role of helminthic infestation in VKC pathogenesis is controversial [40,54].

Etiology

The predominance of VKC during the pollen season lends credence to the hypothesis that the disease is an immunologically mediated hypersensitivity reaction to environmental antigens. (Fig. 2) There is a strong association between atopy and VKC in temperate climates, but this association is weaker in tropical regions [22,29,80]. The association between VKC and atopy in the published literature varies between 15% and 60%. There are a few isolated studies that have shown a higher incidence of allergic conjunctivitis in children with food allergies (8.9%) compared to those without (4.3%) [69,83]. The occurrence of VKC in cold climates could possibly be due to the migration of the susceptible population to these regions.

Pathophysiology of vernal keratoconjunctivitis

On the basis of immunohistochemical, genomic and proteomic analysis, it can be safely concluded that participation of the entire ocular surface in VKC is a consequence of tissue contiguity. This participation derives from a complex exchange of information between tissues, cell to cell communications, cytokines, adhesion molecules and chemical mediators [16]. A very recent confocal microscopy study has proved VKC to be a distinct corneal neuropathic process with involvement of all the nerve plexi [46]. Compared to normal controls, patients with VKC show higher cell diameter, cell reflectivity and cellular activity in superficial epithelial cells as well as decreased density of basal epithelial cells.

Like all allergic diseases, VKC is associated with elevated serum IgE levels and a hypersensitive response to normally innocuous antigen [15]. However, the initial idea that VKC was solely a type 1-mediated hypersensitivity reaction as advocated by Gell and Coombs has been replaced by a more complex, non-IgE dependent (type-4 hypersensitivity) pathogenic mechanism, [14,85]

Major gaps in knowledge

The major gaps in knowledge of VKC include the reasons for an increase in the number of cases, the mechanism responsible for spontaneous resolution at puberty, and the interaction between microbes, the immune system, the allergic disorder *per se* and therapeutic targets with a potential for complete cure [17]. An ideal approach for a comprehensive VKC study would be a combination of genomics and proteomics. However, tissues of the eye are not amenable to biopsy in living individuals, moreover if they are in the pediatric age group, to which majority of the VKC patients belong. A chronological sequence of the developments in proteomics has been outlined in Table 1.

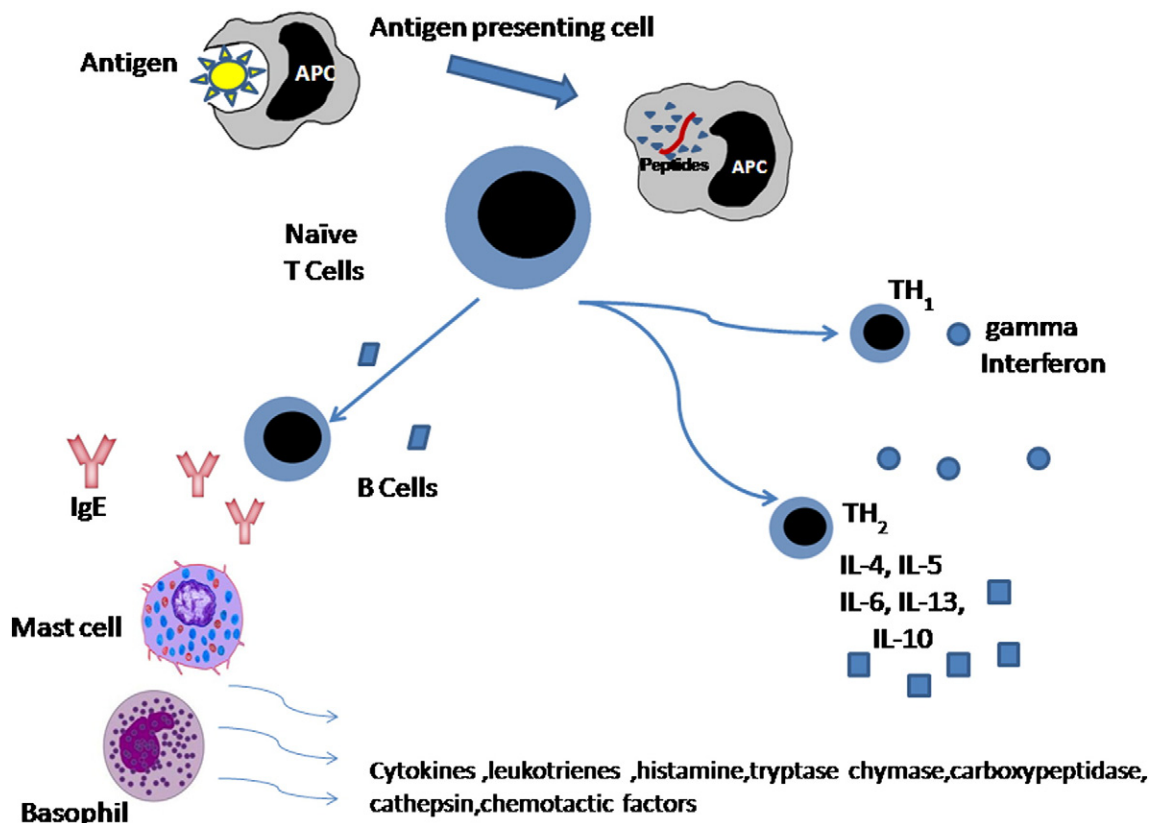


Fig. 2. Sequence of events following antigen challenge in VKC patients. IL: Interleukin, Th: T helper cells, IgE: Immunoglobulin E.

Table 1
Research and development in proteomics of VKC.

Sl No	Year	Author	Place of Study	Purpose	Result
1	2004	Ebihara et al. [30] 86	Japan	To determine the levels of mast cell chymase and tryptase activity in the tears of 38 VKC patients/8 healthy controls.	The degree of chymase activity in tears correlated significantly with VKC severity
2	2006	Asrar et al. [3] 87	Saudi Arabia	To investigate the role of growth factors (EDGF, VEGF, EGF, PDGF, FGF K i67) in 16 VKC patients/9 controls.	These growth factors are increased in chronic VKC that might mediate conjunctival remodeling.
3	2006	Motterle et al. [60] 46	Italy	To determine the presence of neurotransmitters and their receptors in 8 VKC/4 healthy subjects.	There is an alteration in muscarinic and beta1 adrenergic receptors, VIP, NGF indicating autonomic dysfunction in pathogenesis of VKC.
4	2006	Fukuda et al. [34] 88	Japan	To investigate the role of Th cytokines in fibroblast production and development of giant papillae	IL-4 and IL-13 inhibit fibroblast matrix degradation by inhibiting MMP-3
5	2008	Leonardi et al. [48] 89	Italy	Demographic and immunological data in 509 VKC patients have been elaborated.	Males outnumbered the females. Limbal form was commoner. Family history of allergy, increased IGE levels found in approximately half the cases.
6	2009	Micera et al. [57] 53	Italy	To investigate the role of NGF on TLR-EC in 7 VKC/5 healthy controls	NGF enhances TLR-EC expression in VKC indicating a different pattern of cell activation.
7	2009	Leonardi et al. [50] 90	Italy	To detect the presence of multiple mediators, growth factors in tears of VKC patients with active disease using stationary phase antibody arrays.	Many unreported factors including MMP-3 and MMP-10 and multiple proteases, growth factors and cytokines were identified.
8	2010	Manzouri et al. [53] 73	London, UK	Flow cytometry was used to assess dendritic cell (DC) induction of T cell proliferation.	Increased maturity of DCs in VKC patients, correlates with the heightened immune responsiveness associated with this disorder. Impairment of DC induction of T cell activation may contribute to the susceptibility of these patients to viral infection.
9	2011	Leonardi et al. [45] 73	Italy	To investigate the relationship between allergic inflammation and TGF- β /Smad signaling pathway, expression in VKC patients and in primary cultured conjunctival fibroblasts exposed to mediators found previously over-expressed in VKC.	The TGF- β /Smad signaling pathway is over-expressed in VKC tissues and modulated in conjunctival fibroblasts by histamine, IL-4, TGF- β 1 and TNF- α . These mechanisms may be involved in fibrillar collagen production, giant papillae formation and tissue remodeling

Proteomics of VKC

Histopathology

Immunohistochemical as well as *in-situ* hybridization studies of biopsied conjunctival tissue have been used to characterize and quantify the inflammatory cellular infiltrate in VKC. The majority of the identified cells were T-lymphocytes, with clustering of B-lymphocytes in limbal biopsy specimens [13,55].

Cells in VKC

VKC patients show increased levels of inflammatory cells (namely eosinophils), mast cells, T-lymphocytes, B-lymphocytes, and fibroblasts in the tears and conjunctiva. Additionally, patients show enhanced secretion of mediators such as histamine and enhanced expression of Toll-like receptors on epithelial cells and adhesion molecules on lymphocytes.

Mast cells

Central to the immune response are the numerous metachromatically staining mast cells. Expression of Fc [epsilon] RI receptor on the surface of mast cells enables IgE binding and the release of proinflammatory mediators that initiate the inflammatory cascade. The MC_T type mast cell secretes a single neutral protease and its action is dependent on T lymphocytes. This cell type is predominant in allergic conjunctiva, whereas the MC_{TC} subtype secretes both tryptase and chymase; the action of the MC_{TC} subtype is independent of the presence of T lymphocytes but dependent on fibroblast derived growth factors such as stem cell factor [59]. This mast cell heterogeneity leads to a functional heterogeneity in the response profile to various anti-allergic agents. Heterogeneity of eosinophils and other inflammatory cells could also possibly have an effect [12]. Tears have increased levels of tryptase and chymase, indicators of mast cell activation and conjunctiva mast cells show increased production of IL-4, which plays a key role in allergic inflammation [2]. Mast cell activation results in the release of

pro inflammatory mediators into the tears, which are responsible for the allergic response symptoms.

T cells

T lymphocytes are found in increased numbers in the conjunctiva and tears of VKC patients. These cells are found in the subconjunctival tissue, and the CD-4 type cell predominates over the CD-8 type [13,55]. In order to completely activate, co-stimulation is required in addition to the interaction between the T cells and specific antigen. Langerhans cells in the conjunctiva express co-stimulatory molecules B-7-2 (CD86) and B7-1 (CD80), which interact with CD28 on the surface of T lymphocytes and are useful in mounting an effective local Th2 response. Double immunohistochemistry has revealed a statistically significant higher count of B7-2⁺ expressed by CD1a⁺ Langerhans cells (in basal epithelial and superficial stroma) compared to normal controls. These cells interacted with CD28 expressed by CD3 T lymphocytes, mediating the inflammatory reaction in VKC by releasing numerous cytokines [7].

B cells

The B lymphocytes in the conjunctival lymphoid follicles may contribute to IgE synthesis by activating CD23, CD21 and CD40 on their cell surface [7].

Eosinophils

The level of eosinophils, which are considered to be powerful effector cells, is much higher in patients with VKC than in those with other ocular allergies. Eosinophils release arginine rich basic proteins like EDN (eosinophil derived neurotoxin), ECP (eosinophil cationic protein) and MBP (major basic protein) [2]. There are significantly more eosinophils in the tears, conjunctiva and serum of patients with active VKC. The tear and serum levels of the eosinophil products ECP and EPX (eosinophil protein X) / neurotoxin (EPX) are also higher in VKC patients when compared to normal subjects [41]. The serum ECP and blood eosinophil counts correlated strongly with the total score of

giant papillae [43]. There is overexpression of gelatinase-B enzyme in eosinophils of VKC conjunctiva, and eosinophilic MBP has been found in the base of VKC ulcers [7,78].

Epithelial cells

Tears in active VKC have increased amounts of cytokines, growth factors, neuropeptides and other soluble mediators that include nerve growth factor (NGF) [42,60]; [56,79]. Epithelial cells, which are the first line of defense, express transmembrane receptors named Toll like receptors (TLRs) [10,19,57]. In the inflamed conjunctiva of VKC patients, TLR-4 and TLR-9 are up-regulated and down-regulated, respectively, by NGF, which is one of the soluble mediators. Because commensal bacteria are thought to down-regulate Th2 responses, it had been hypothesized that regulation of the micro flora composition could treat or prevent Th2 diseases [71,76]. The cells, in addition to providing a mechanical barrier, also regulate allergic inflammation through expression of surface antigens such as adhesion/effector molecules ICAM-1, vascular cell adhesion molecule-1 and HLADR [41].

Fibroblasts

Conjunctival and corneal fibroblasts not only play an important role in inducing fibrosis that ultimately results in the formation of giant tarsal papillae and a gelatinous limbal thickening, which are hallmarks of the disease process, but also perpetuate the allergic process through the release of various cytokines. TGF β , nerve growth factor, and Th2 cytokines (IL-4 and IL-13) stimulate corneal fibroblasts and induce the formation of cytokines, eotaxins, vascular cell adhesion molecule-1 (VCAM-1) and thymus activation regulated chemokine (TARC) [41]. Further eosinophil adhesion to fibroblasts has been implicated in the formation of persistent corneal ulcers [63].

Histamine

Histamine released by degranulation of mast cells and basophils stimulates the conjunctival epithelial cells to produce cytokines, and thus plays a major role in tissue modeling [41]. There is not only an increased level of histamine in VKC but also an enhanced skin test reactivity and conjunctival hyper reactivity. This has been attributed to diminished levels of histaminases and increased production of histamine.

Adhesion molecules

Adhesion molecules found on circulating leukocytes bind to their ligands on vascular endothelial cells and on target cells [4,73]. The epithelial cells and vascular endothelium strongly express ICAM-1, whereas statistically significant numbers of ICAM, LFA-1 and VLA-4 molecules are found on the mononuclear cell infiltrate in the stroma in VKC patients. The authors have proposed that because adhesion molecules aid in sustaining the immunoinflammatory action in VKC, mononuclear antibodies directed against adhesion molecules may provide a new therapeutic modality for treating the disease.

Chemokines

Chemokines are a group of peptides that function as activators and chemo attractants for leukocyte trafficking and recruitment. They are produced by inflammatory cells and stimulated epithelial cells, fibroblasts and vascular endothelial cells in the conjunctiva. They accumulate in inflammatory sites and promote further inflammation.

It has been confirmed that all inflammatory cells expressing chemokines are CD68-positive monocytes/macrophages [77]. On activation, T lymphocytes express certain markers; among these markers, CD25 is reportedly the most numerous, followed by CD26 and CD62L.

All these markers are crucial for lymphocyte adhesion to endothelial venules and migration to inflammatory sites. The chemokines expressed by CD68 positive monocytes and macrophages help recruit naïve and activated T lymphocytes. Thus, the T lymphocyte activation markers (CD25, CD26, CD62L, CD71 and CD30) and the T lymphocyte chemo attractants (PARC, MDC, I-309) play a major role in the chronic inflammatory response seen in VKC. Yet other researchers have shown significantly higher numbers of cells with markers CD3, IgE, AA1, EG2, in IgE and non-IgE sensitized individuals [32]. It has been suggested that even IL-4 plays a role in VKC [33].

To further characterize the type of cytokines secreted, studies involving mRNA have depicted the preferential expression of mRNA for the cytokines IL-5 and IL-13 in IgE-sensitized VKC subjects [58]. Similar but insignificant trends were found in non-IgE subjects. [32] Interferon-gamma secreted by Th1 cells has been implicated in the inhibition of cytokine secretion by Th 2 cells; other studies, however, have found no evidence of this effect [35,75].

Increased levels of eotaxin and IL-8, as well as up-regulation of the (CXCR)-3 chemokine receptor, have been found in VKC patients [41]. There is a positive correlation between eosinophils levels in the conjunctiva and eotaxin, a chemokine, in the mucous of VKC patients. Additionally, increased numbers of polymorphonuclear cells and eosinophils in VKC conjunctiva were correlated with enhanced production of IL-8, which is a chemoattractant and activator of polymorphonuclear cells. Chemokine receptor (CXCR)-3 is greatly up-regulated and expressed abundantly on T lymphocytes in the conjunctiva of patients with active VKC [6,8].

Role of matrix metalloproteinase enzymes

Metalloproteinases are endopeptidases produced by conjunctival epithelial cells, eosinophils and fibroblasts. They selectively degrade components of the extracellular matrix. Along with the infiltration of inflammatory cells, there is limbal hyperplasia and the presence of giant tarsal papillae due to extracellular matrix hyperplasia and increased collagen deposition in active VKC. This is most likely due to an imbalance between MMP (matrix metalloproteinase-1) and TIMP-1 (inhibitor of matrix metalloproteinase-1). MMP-1 and MMP-9 tear levels are increased in VKC patients [44]. Thus, MMPs play a pivotal role in conjunctival tissue inflammation, remodeling and corneal changes.

Researchers have demonstrated deposits of collagen types I, III, IV, and V in the conjunctiva of patients with VKC [5]. Gelatinase-B is one of the membrane type matrix metalloproteinase enzymes that play a key role in extracellular matrix remodeling [32]. Double immunohistochemical analysis has confirmed the presence of significantly greater numbers of gelatinase-B positive cells in VKC patients when compared to normal controls. The majority of these cells were eosinophils, with a smaller number of monocytes/macrophages. Gelatinase-B degrades denatured collagens (gelatin), collagen types 1V, V, VII and X, elastin and fibronectin [61,70].

Growth factors

Increased levels of growth factors such as epidermal growth factor, fibroblast growth factor, transforming growth factor beta-1 (TGF β -1), and nerve growth factor are found in VKC. These factors increase fibroblast and procollagen formation. Substance P, which affects immune cells, has also been found in the tears and plasma of these patients [41].

Neuromediators

Neural involvement has been indicated due to hyper-reactivity to nonspecific stimuli such as heat, dust and cold [1,20]. Both neuromediators and inflammatory mediators act on neurotransmitter receptors. Among the inflammatory mediators, vasoactive intestinal

polypeptide and eosinophil major basic protein production levels are down-regulated in VKC epithelium [60]. This could most likely be due to increased secretion of these mediators from other sources. In contrast, these neuromediators were up-regulated in the stroma of VKC patients when compared to those of controls. The stickiness of the mucus has been attributed to a decrease in M1 receptors, which play a role in water output, as well as to the patchy distribution of M2 and M3 receptors. Nerve growth factor (NGF) can be produced by nerves, epithelial cells, immune cells and fibroblasts. Its preferential distribution in the limbal stroma could be related to tissue repair fibrous hyperplasia and neovascularization, which are typical of VKC.

Recent developments

In the past five years, MALDI TOF mass spectrometry has been utilized effectively for peptide profile analysis (qualitative and quantitative) of VKC tear samples [49]. A large number of previously undetected proteins with possible etiology in the pathogenesis of VKC have been quantified using iTRAQ (Isobaric tag for relative and absolute quantification) technology. Tear levels of albumin, transferrin, and hemopexin were found to be 100 times higher in VKC patients than controls. Leonardi et al. have used the more sophisticated multiplex bead analysis flow cytometry technique and report that limbal disease in adults has a more Th1-type cytokine profile compared to the tarsal form [47]. Moreover, the role of the TGF- β /Smad signaling pathway in giant papillae formation and tissue remodeling has been validated [45]. Dendritic cell maturity is associated with heightened immune responsiveness in VKC [53]. The presence and quantification of the acute phase protein hemopexin in the tears and in the conjunctival macrophages of VKC patients has been recently evaluated [65]. Hemopexin serves as a scavenger and transporter of extracellular haem, which is released during inflammation and protects against oxidative damage. Haem levels were higher in active VKC cases, and the levels correlated with the clinical severity of the disease process. A possible systemic source of hemopexin has been postulated, but the exact interaction between hemopexin and the other inflammatory mediators is still unclear. This novel finding could pave the way for use of hemopexin as a potential biomarker of disease diagnosis and progress monitoring. Levels of Alpha1 antitrypsin (ATT), another acute phase protein released during inflammation, are reportedly higher in the sera of active VKC patients in the Indian subcontinent [9]. This could possibly be due to over expression of chymase; a protease secreted by mast cells and kept under control by ATT [47].

Treatment

Treatment of VKC depends on the stage of the disease (Table 2). Milder cases can be treated with a combination of triggering factor avoidance, use of sunglasses, the application of cold compresses and the use of mild astringent antihistaminic eye drops or non-steroidal anti-inflammatory eye drops and mast cell stabilizers [26,51]. Severe varieties are treated with either steroid drops or immune-suppressants, such as cyclosporine and mitomycin-c eye drops [37,66]. The efficacy of currently available topical drugs has been evaluated by a meta-analysis, which recommended standard criteria for assessment of diagnosis and therapy based on severity [52].

Surgical treatment

In persistent and severe cases, supratarsal corticosteroid injection has been used [84]. Aggressive limbal variants with papillae encroaching the cornea can be treated by surgical excision [64]. Macro erosions and shield ulcers can be treated by debridement manually or by excimer laser phototherapeutic keratectomy to promote early re-epithelization. Further non-healing erosions and ulcers can be treated by amniotic membrane implantation and limbal stem cell transplantation [23]. Corneal scarring has been treated successfully using phototherapeutic keratectomy and Boston keratoprosthesis [24,31].

To recapitulate, VKC is an allergic disease of multifactorial etiology, occurring most commonly in young male boys with a gradual subsidence of symptoms with the onset of puberty. Both IgE (Type-1 hypersensitivity reaction) and non-IgE (Type-4 hypersensitivity) mediated mechanisms are responsible for its variegated response. With the advent of sophisticated immunohistochemical methodologies, light microscopy findings were replaced with extensive knowledge at the sub-cellular level. Among the diverse etiological factors responsible for VKC, a genetic role and neural involvement evidenced by hyper reactivity to environmental influences have recently interested investigators. At the proteomic level, the roles of cytokines, T-cells, adhesion molecules, gelatinase-B, and transmembrane receptors have been explored extensively (Table 1). A comprehensive integration of studies performed among ethnically diverse groups is bound to throw light on many unanswered questions. Once these are tackled and the elusive gold standard biomarker found, pharmacological modulations are possible at various locations to allow VKC patients to lead a symptom-less and effective life.

Method of literature search

Articles regarding vernal conjunctivitis were identified through a multistage systematic approach. First, we conducted a computerized search of the Medline database using PubMed (www.pubmed.com). All relevant English language literature published between 1989 and December 2013 was reviewed. A comprehensive search was performed encompassing the terms vernal keratoconjunctivitis, and allergic conjunctivitis followed by 'AND' and the following terms –history, demography, clinical features, ethical issues, children, adult, pathophysiology, proteomics, genomics, treatment, and surgical treatment. All the topics considered relevant to vernal conjunctivitis were included. Next, the references of these selected topics were further critically reviewed, and articles were included if they were considered to add additional data or refute a previous claim.

Disclosure

The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article.

Table 2
Therapeutic approach for different grades of VKC.

Clinical classification [17]	Treatment	Examples
Grades 1,2,3,4,5	Vasoconstrictors, non specific antihistaminics	Vasoconstrictors: naphazoline (0.05%), tetrahydrozoline (0.05%), Antihistaminics: pyrilamine, pheniramine (0.3%)
Grades 2, 3, 4.	Mast cell stabilizers	Cromolyn (4%), Nedocromil (2%), Iodoxamide (.1%)
Grades 1, 2,3,4,5	H1 receptor blockers	Emedastine(0.05%), levocabastine (0.05%),
Grades 1,2,3,4.	H1 receptor blocker and mast cell stabilizer	Olopatadine (.1%), Epinastine (0.05%), Ketotifen (0.05%), Azelastine (0.05%)
Grades 1,2,3,4,5.	Non steroidal anti inflammatory	Ketorolac (0.5%), Diclofenac (0.1%).
Grades 3,4.	corticosteroids	Dexamethasone (0.1%), Prednisolone (0.12%), Loteprednol etabonate (0.5%), Rimexolone (1%)
Grades 3,4.	Immunomodulators	Cyclosporin (1%), Mitomycin(.01%)

Conflict of interest

No conflict of interest exists between the authors.

References

- [1] M. Abelson, A. Leonardi, L.M. Smith, I. Fregona, M. George, A. Secchi, Histamine activity in patients with vernal keratoconjunctivitis, *Ophthalmology* 102 (1995) 1958–1963.
- [2] M.B. Abelson, Allergic diseases of the eye, Saunders, 2000.
- [3] A.M. Abu El-Asrar, S. Al-Mansouri, K.F. Tabbara, L. Missotten, K. Geboes, Immunopathogenesis of conjunctival remodelling in vernal keratoconjunctivitis, *Eye* 20 (2006) 71–79.
- [4] A.M. Abu el-Asrar, K. Geboes, S. al-Kharashi, K.F. Tabbara, L. Missotten, V. Desmet, Adhesion molecules in vernal keratoconjunctivitis, *Br. J. Ophthalmol.* 81 (12) (Dec 1997) 1099–1106.
- [5] A.M. Abu El-Asrar, K. Geboes, S.A. Al-Kharashi, An immunohistochemical study of collagens in trachoma and vernal keratoconjunctivitis, *Eye* 12 (1998) 1001–1006.
- [6] A.M. Abu El-Asrar, S. Struyf, A.A. Al-Mosallama, A. Missotten, J. Van Damme, K. Geboes, Expression of chemokine receptors in vernal keratoconjunctivitis, *Br. J. Ophthalmol.* 85 (2001) 1357–1361.
- [7] A.M. Abu El-Asrar, Langerhans' cells in vernal keratoconjunctivitis express the costimulatory molecule B7-2 (CD86), but not B7-1 (CD80), *Eye* 15 (2001) 648–654.
- [8] A.M.E.A. Abu El-Asrar, A.I. Van, S. Al-Mansouri, L. Missotten, G. Opendakker, K. Geboes, Gelatinase B in vernal keratoconjunctivitis, *Arch. Ophthalmol.* 119 (Oct 2001) 1505–1511.
- [9] A. Ahsan, K.A. Salman, S. Alam, et al., Alpha-1 antitrypsin, a diagnostic and prognostic marker of vernal keratoconjunctivitis, *J. Clin. Diagn. Res.* 8 (2014) 8–10.
- [10] S. Akira, K. Takeda, T. Kaisho, Toll-like receptors: critical proteins linking innate and acquired immunity, *Nat. Immunol.* 2 (2001) 675–680.
- [11] M.R. Allansmith, The eye and immunology, Mosby, St Louis, 1982.
- [12] D.F. Anderson, J.D. MacLeod, S.M. Baddeley, Seasonal allergic conjunctivitis is accompanied by increased mast cell numbers in the absence of leukocyte infiltration, *Clin. Exp. Allergy* 27 (1997) 1060–1066.
- [13] A.S. Bacon, S.M. Baddeley, J.L. McGill, Adhesion molecules and relationship to leukocyte levels in allergic eye disease, *Invest. Ophthalmol. Vis. Sci.* 39 (1998) 322–330.
- [14] Y.R. Baryshak, A. Zavaro, M. Monselise, Z. Samra, D. Sompolsky, Vernal keratoconjunctivitis in an Israeli group of patients and its treatment with sodium cromoglycate, *Br. J. Ophthalmol.* 66 (1982) 118–122.
- [15] L. Bielory, L.P. Frohman, Allergic and immunologic disorders of the eye, *J. Allergy Clin. Immunol.* 89 (1992) 1–15.
- [16] S. Bonini, M. Coassin, S. Aronni, A. Lambiase, Vernal keratoconjunctivitis, *Eye* 18 (4) (Apr 2004) 51–345.
- [17] S. Bonini, S. Bonini, A. Lambiase, et al., Vernal keratoconjunctivitis revisited. A case series of 195 patients with long-term followup, *Ophthalmology* 107 (6) (Jun 2000) 1157–1163.
- [18] S. Bonini, A. Lambiase, M. Schiavone, M. Centofanti, L.A. Palma, S. Bonini, Estrogen and progesterone receptors in vernal keratoconjunctivitis, *Ophthalmology* 102 (1995) 1374–1379.
- [19] S. Bonini, A. Micera, A. Iovieno, A. Lambiase, Expression of toll-like receptors in healthy and allergic conjunctiva, *Ophthalmology* 112 (2005) 1528.
- [20] S. Bonini, V. Todini, Conjunctival hyperresponsiveness to ocular histamine challenge in patients with vernal keratoconjunctivitis, *J. Allergy Clin. Immunol.* 89 (1992) 103–107.
- [21] J.M.F.C. Brody, Vernal conjunctivitis, in: J.S. Pepose, G.N. Holland, K.R. Wilhelmus (Eds.), *Ocular infection and immunity*, Mosby, St Louis, 1996.
- [22] R.J. Buckley, Vernal keratoconjunctivitis, *Int. Ophthalmol. Clin.* 24 (4) (1988) 303–308.
- [23] S. Burman, V. Sangwan, Cultivated limbal stem cell transplantation for ocular surface reconstruction, *Clin. Ophthalmol.* 2 (2008) 489–582.
- [24] J.A. Cameron, Shield ulcers and plaques of the cornea in vernal keratoconjunctivitis, *Ophthalmology* 102 (1995) 985–993.
- [25] A.K. Cingu, Y. Cinar, F.M. Turkcu, et al., Effects of vernal and allergic conjunctivitis on severity of keratoconus, *Int. J. Ophthalmol.* 6 (3) (Jun 2013) 370–374.
- [26] R.B. Couto, Treatment of vernal keratoconjunctivitis: a retrospective clinical case study, *Optom. Vis. Sci.* 68 (1991) 561–564.
- [27] S. De Smedt, G. Wildner, P. Kestelyn, Vernal keratoconjunctivitis: an update, *Br. J. Ophthalmol.* 97 (1) (Jan 2013) 9–14.
- [28] P.C. Donshik, W.H. Ehlers, Clinical immunologic diseases, in: G. Srolin, R.A. Thoft (Eds.), *The cornea*, 3rd ed., Little, Brown & Company, Boston, 1994.
- [29] D.L. Easty, External ocular disease in atopic patients, in: D.L. Easty, G. Smolin (Eds.), *External eye disease*, Butterworth and Co., London, 1985.
- [30] N. Ebihara, T. Funaki, S. Takai, M. Miyazaki, K. Fujiki, A. Murakami, Tear chymase in vernal keratoconjunctivitis, 2004. 417–420.
- [31] S. Egrilmez, S. Sahin, A. Yagci, The effects of vernal keratoconjunctivitis on clinical outcomes of vernal keratoconjunctivitis, *Can. J. Ophthalmol.* 39 (2004) 772–777.
- [32] A.M. El Asrar, S. Struyf, S.A. Al Kharashi, L. Missotten, J. Van Damme, K. Geboes, Expression of T lymphocyte chemoattractants and activation markers in vernal keratoconjunctivitis, *Br. J. Ophthalmol.* 86 (2002) 1175.
- [33] H. Fujishima, M. Takeyama, T. Takeuchi, I. Saito, K. Tsubota, Elevated levels of substance P in tears of patients with allergic conjunctivitis and vernal keratoconjunctivitis, *Clin. Exp. Allergy* 27 (1997) 372–378.
- [34] K. Fukuda, Role of corneal fibroblasts in the pathogenesis of ocular allergic diseases, *Nippon Ganka Gakkai Zasshi* 109 (2005) 717–726.
- [35] T.F. Gajewski, F.W. Fitch, Anti-proliferative effect of IFN-gamma in immune regulation. IFN-gamma inhibits the proliferation of Th2 but not Th1 murine helper T lymphocyte clones, *J. Immunol.* 140 (1988) 4245–4252.
- [36] B. Gignac, A. Leonardi, P. Pouliquen, et al., Prevalence of vernal keratoconjunctivitis: a rare disease? *Br. J. Ophthalmol.* 92 (2008) 1097–1102.
- [37] N. Gokhale, Samant, V. Sharma, Oral cyclosporine therapy for refractory severe vernal keratoconjunctivitis, *Indian J. Ophthalmol.* 60 (2012) 220–223.
- [38] Greiner JV PDBRAM, J.V. Greiner, D.G. Peace, R.S. Baird, M.R. Allansmith, Effects of eye rubbing on the conjunctiva as a model of ocular inflammation, *Am J. Ophthalmol.* 100 (1) (Jul 1985) 45–50.
- [39] A. Haagerup, T. Bjerke, P.O. Schoitz, H.G. Binderup, R. Dahl, T.A. Kruse, Allergic rhinitis – a total genome-scan for susceptibility genes suggests a locus on chromosome 4q24–q27, *Eur. J. Hum. Genet.* 9 (2001) 945–952.
- [40] M. Khatami, J.J. Donnelly, T. John, J.H. Rockey, Vernal conjunctivitis model studies in guinea pigs immunized topically with fluoresceinyl ovalbumin, *Arch. Ophthalmol.* 102 (11) (1984) 1683–1688.
- [41] S. Kumar, Vernal keratoconjunctivitis: a major review, *Acta Ophthalmol. (Copenh)* 87 (2) (Mar 2009) 133–147.
- [42] A. Lambiase, S. Minchiotti, A. Leonardi, A.G. Secchi, M. Rolando, G. Calabria, Prospective, multicenter demographic and epidemiological study on vernal keratoconjunctivitis: a glimpse of ocular surface in Italian population, *Ophthalmic Epidemiol.* 16 (2009) 38–41.
- [43] A. Leonardi, F. Borghesan, D. Faggian, M. Depaoli, A.G. Secchi, M. Plebani, Tear and serum soluble leukocyte, *Am J. Ophthalmol.* 129 (2000) 151–158.
- [44] A. Leonardi, P. Brun, G. Abatangelo, M. Plebani, A.G. Secchi, Tear levels and activity of matrix metalloproteinase (MMP)-1 and MMP-9 in vernal keratoconjunctivitis -, *Invest. Ophthalmol. Vis. Sci.* 44 (2003) 3052–3058.
- [45] A. Leonardi, A. Di Stefano, C. Vicari, L. Motterle, P. Brun, Histamine H4 receptors in normal conjunctiva and in vernal keratoconjunctivitis, *Allergy* 66 (2011) 1360–1366.
- [46] A. Leonardi, D. Lazzarini, M. Bortolotti, F. Piliego, E. Midena, I. Fregona, Corneal confocal microscopy in patient with vernal keratoconjunctivitis, *Ophthalmology* 119 (2012) 509–515.
- [47] A. Leonardi, D. Lazzarini, L. Motterle, et al., Vernal keratoconjunctivitis-like disease in adults, *Am J. Ophthalmol.* 155 (5) (May 2013) 796–803.
- [48] A. Leonardi, L. Motterle, M. Bortolotti, Allergy and the eye, *Clin. Exp. Immunol.* 153 (2008) 17–21 Suppl.
- [49] A. Leonardi, A. Palmigiano, E.A. Mazzola, et al., Identification of human tear fluid biomarkers in vernal keratoconjunctivitis using iTRAQ quantitative proteomics, *Allergy* 69 (2014) 254–260.
- [50] A. Leonardi, S. Sathe, M. Bortolotti, A. Beaton, R. Sack, Cytokines, matrix metalloproteases, angiogenic and growth factors in tears of normal subject, *Allergy* 64 (2009) 710–717.
- [51] A. Leonardi, Vernal keratoconjunctivitis: pathogenesis and treatment, *Prog. Retin. Eye Res.* 21 (2002) 319–339.
- [52] F. Mantelli, M.S. Santos, T. Pettiti, R. Sgrulletta, M. Cortes, A. Lambiase, Systematic review and metaanalysis of randomised clinical trials on topical treatments for vernal keratoconjunctivitis, *Br. J. Ophthalmol.* 91 (2007) 1656–1661.
- [53] B. Manzouri, M. Ohbayashi, A. Leonardi, D. Fattah, D.F. Larkin, S.J. Ono, Characterisation of the phenotype and function of monocyte-derived dendritic cells in allergic conjunctiva, *Br. J. Ophthalmol.* 94 (2010) 1662–1667 1662e1667.
- [54] J.L. McGill, S.T. Holgate, M.K. Church, D.F. Anderson, A. Bacon, Allergic eye disease mechanisms, *Br. J. Ophthalmol.* 82 (1988) 1203–1214.
- [55] D.P. Metz, A.S. Bacon, S.T. Holgate, Phenotypic characterisation of T-cells infiltrating the conjunctiva in chronic allergic eye disease, *J. Allergy Clin. Immunol.* 98 (1996) 686–696.
- [56] A. Micera, A. Lambiase, B. Stampaciachiere, et al., Nerve growth factor has a modulatory role on human primary fibroblast cultures derived from vernal keratoconjunctivitis-affected conjunctiva, *Mol. Vis.* 13 (2007) 981–987.
- [57] A. Micera, B. Stampaciachiere, Normando, A. Lambiase, S. Bonini, Nerve growth factor modulates toll-like receptor (TLR) 4 and 9 expression in cultured primary VKC conjunctival epithelial cells, *Mol. Vis.* 15 (Oct 13 2009) 2037–2044.
- [58] P.G. Montan, A. Scheynius, I. Van Der Ploeg, Similar T helper Th2-like cytokine mRNA expression in vernal keratoconjunctivitis regardless of atopic constitution, *Allergy* 57 (2002) 436–441.
- [59] S.J. Morgan, J.H. Williams, A.F. Walls, Mast cell hyperplasia in atopic keratoconjunctivitis. An immunohistochemical study, *Eye* 5 (1991) 729–735.
- [60] L. Motterle, Y. Diebold, A. Enríquez de Salamanca, et al., Altered expression of neuro transmitter receptors and neuro mediators in vernal kerato conjunctivitis, *Arch. Ophthalmol.* 124 (4) (Apr 2006) 462–468.
- [61] G. Murphy, M.I. Cockett, R.V. Ward, A.J. Docherty, Matrix metalloproteinase degradation of elastin, type IV collagen and proteoglycan. A quantitative comparison of the activities of 95 kDa and 72 kDa gelatinases, stromelysins-1 and -2 and punctuated metalloproteinase (PUMP), *Biochem. J.* 277 (Pt 1) (Jul 1991) 277–279.
- [62] P. Neri, E. Elío, E. Lombardi, M. Cristina, B. Roberto, C. Roberto, Long eyelashes in a case series of 93 children with vernal keratoconjunctivitis, *Pediatrics* 115 (AUG 2005) 86–91.
- [63] M. Okada, K. Fukagawa, Y. Takano, The implications of the upregulation of ICAM-1/VCAM-1 expression of corneal fibroblasts on the pathogenesis of allergic keratopathy, *Invest. Ophthalmol. Vis. Sci.* 46 (2005) 4512–4518.
- [64] G. Ping, K. Ahmad, Z. Wei-wei, Q. Lei, S. Xiao-li, Surgical resection and amniotic membrane transplantation for treatment of refractory giant papillae in vernal keratoconjunctivitis, *Cornea* 32 (6) (2013) 816–820.
- [65] J.F. Pong, C.Y. Chu, W.Y. Li, et al., Association of hemopexin in tear film and conjunctival Macrophages With vernal keratoconjunctivitis, *Arch. Ophthalmol.* 129 (2011) 453–461.

- [66] N. Pucci, E. Novembre, A. Cianferoni, Efficacy and safety of cyclosporine eye-drops in vernal keratoconjunctivitis, *Ann. Allergy Asthma Immunol.* 89 (2002) 298–303.
- [67] U.S. Saboo, M. Jain, J.C. Reddy, V.S. Sangwan, Demographic and clinical profile of vernal keratoconjunctivitis at a tertiary eye care center, *Indian J. Ophthalmol.* 61 (2013) 486–489.
- [68] V.S. Sangwan, V. Jain, G.K. Vemuganti, S.I. Murthy, Vernal keratoconjunctivitis with limbal stem cell deficiency, *Cornea* 30 (5) (May 2011) 491–496.
- [69] Y. Saraçlar, S. Yigit, G. Adalioglu, A. Tuncer, E. Tunçbilek, Prevalence of allergic diseases and influencing factors in primary-school children in the Ankara region of Turkey, *J. Asthma* 34 (1) (1997) 23–30.
- [70] R.M. Senior, G.L. Griffin, C.J. Fliszar, S.D. Shapiro, G.I. Goldberg, H.G. Welgus, Human 92- and 72-kilodalton type IV collagenases are elastases, *J. Biol. Chem.* 266 (12) (Apr 1991) 7870–7875.
- [71] R. Sgrulletta, S. Bonini, A. Lambiase, Allergy and infections: long-term improvement of vernal keratoconjunctivitis following viral conjunctivitis, *Eur. J. Ophthalmol.* 16 (2006) 470–473.
- [72] S.D. Smedt, J. Nkurikiye, J. Fonteyne, et al., Vernal keratoconjunctivitis in school children in Rwanda and its association with socio-economic status: a population-based survey, *Am. J. Trop. Med. Hyg.* 85 (2011) 711–717.
- [73] T.A. Springer, Adhesion receptors of the immune system, *Nature* 346 (1990) 425–434.
- [74] K.F. Tabbara, Ocular complications of vernal keratoconjunctivitis, *Can. J. Ophthalmol.* 2 (34) (Apr 1999) 88–92.
- [75] S. Till, S. Durham, R. Dickason, IL-13 production by allergen-stimulated T cells is increased in allergic disease associated with IL-5 but not IFN- γ expression, *Immunology* 91 (1997) 53–57.
- [76] H. Tlaskalova-Hogenova, R. Stepankova, T. Hudcovic, et al., Commensal bacteria (normal microflora), mucosal immunity and chronic inflammatory and autoimmune diseases, *Immunol. Lett.* 93 (2004) 97–108.
- [77] M. Toda, S.J. Ono, Genomics and proteomics of allergic disease, *Immunology* 106 (May 2002) 1–10.
- [78] S.D. Trocme, G. Kephart, W.M. Bourne, Eosinophil major basic protein deposition in human corneal shield ulcers, *Invest. Ophthalmol. Vis. Sci.* S33 (1992) 984.
- [79] E. Uchio, S.Y. Ono, Z. Ikezawa, Tear levels of interferon-gamma, interleukin IL-2, IL-4 and IL-5 in patients with vernal keratoconjunctivitis, atopic keratoconjunctivitis and allergic conjunctivitis, *Clin. Exp. Allergy* 30 (2000) 103–109.
- [80] I.J. Udell, G.J. Gleich, M.A. Allansmith, S.J. Ackerman, M.B. Abelson, Eosinophil granule major basic protein and Charcot-Leyden crystal protein in human tears, *Am J. Ophthalmol.* 92 (1981) 824–828.
- [81] C.U. Ukponmwan, Vernal conjunctivitis in Nigerians: 109 consecutive cases, *Trop. Dr.* 33 (2003) 242–245.
- [82] P. Vichyanond, P. Pacharn, U. Pleyer, A. Leonardi, Vernal keratoconjunctivitis: a severe allergic eye disease with remodeling changes, *Pediatr. Allergy Immunol.* (2014) 1–9.
- [83] Y. Yokouchi, M. Shibasaki, E. Noguchi, A genome-wide linkage analysis of orchard grass-sensitive childhood seasonal allergic rhinitis in Japanese families, *Genes Immunol.* 3 (2002) 9–13.
- [84] S. Zaouali, R. Kahloun, S. Attia, B. Jelliti, M. Trigui, Supratarsal injection of triamcinolone acetonide and childhood allergic keratoconjunctivitis, *Allergy Clin. Immunol.* 10 (2011) 478–485.
- [85] A. Zavaro, Y.R. Baryishak, Z. Samra, D. Sompolinsky, Extrinsic and idiopathic vernal keratoconjunctivitis? Two cases with a dissimilar immunopathology, *Br. J. Ophthalmol.* 67 (1983) 742–745.