

Atopy and serum eosinophil cationic protein in 110 white children with vernal keratoconjunctivitis: differences between tarsal and limbal forms

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Abstract

Background A predominance of Th2 response has been suggested in vernal keratoconjunctivitis (VKC), and a high prevalence of IgE-sensitized (IgE-S) patients has been reported (positive skin prick test or serum-specific-IgE). Palpebral and bulbar VKC are considered to be expressions of the same disease and only occasional racial and histopathological differences are described between the two forms. Tear levels of eosinophil cationic proteins have been correlated with the severity of ocular symptoms; however, there is no published study that demonstrates the presence of serum markers of disease activity.

Objective This study was performed to evaluate the prevalence of IgE-sensitization in palpebral, bulbar and mixed VKC and to determine possible useful markers of disease activity in peripheral circulation.

Methods A total of 110 white VKC patients (mean age 8.3 years, range 3.2–18 years) were evaluated for ocular score in the active phase of the disease. Skin prick tests and serum-specific IgE for common allergens, serum-total IgE, peripheral blood eosinophil counts (PBECs) and serum eosinophil cationic protein (s-ECP) were determined. Fifteen age-matched non-IgE-S control children underwent the same determinations.

Results s-ECP, PBECs and s-total IgE were significantly higher in IgE-S than in non-IgE-S VKC patients and in non-IgE-S VKC patients than in controls. A lower prevalence of IgE-S patients was found in bulbar vs. tarsal ($P=0.050$) or mixed forms ($P=0.002$). The score of giant papillae was strongly correlated with s-ECP levels ($P<0.001$) and with PBECs ($P=0.001$).

Conclusions Our data suggest that an overall eosinophilic response is present in VKC independently of IgE-sensitization; bulbar forms, unlike tarsal and mixed forms, were associated with a low prevalence of IgE-sensitization. Serum ECP was a useful marker of disease activity in tarsal and mixed forms.

Keywords eosinophil cationic protein, giant papillae, IgE sensitization, Vernal keratoconjunctivitis

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Introduction

Vernal keratoconjunctivitis (VKC) is a chronic and potentially severe form of bilateral palpebral and/or bulbar conjunctivitis; the term 'keratoconjunctivitis' implies a possible involvement of the cornea and a subsequent compromise of vision [1]. It usually starts in the first decade of life and disappears in the second decade [2, 3]. It is widespread predominantly in warm climates, such as Mediterranean areas [3, 4], and the intensity of the disease usually increases in spring and, unlike seasonal allergic conjunctivitis, in summer.

Ocular subjective symptoms are itching, photophobia, tearing, foreign body sensation and burn sensation. The major conjunctival signs include hyperaemia, papillae and, in tarsal forms, giant papillae prevalently located in the upper tarsal region; in bulbar VKC, limbal papillar hypertrophy is present. Transient yellow-white points (Trantas' dots) may be found predominantly at the limbus. Punctate keratitis, corneal ulcers and late corneal neovascularization are possible complications. Limbus and tarsus are involved in VKC as in these sites the conjunctiva is firmly attached to the underlying structures containing lymphatics, vessels and nerves [5]. Limbal, tarsal and mixed forms have been considered to be expressions of the same disease; significant differences in the frequency of atopic conditions between palpebral and limbal VKC patients have not been reported [6]; occasionally, different epidemiological and histopathological data among these forms are described [5, 7].

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Recent studies have shown a prevalent local T-helper cell type 2 (Th2) response in VKC, with presence of Th2-like cells in tears and conjunctival biopsies [8, 9]; increased numbers of activated mast cells and eosinophils have been found in conjunctiva [2, 10], and IL-3, IL-5, IL-6 and granulocyte-macrophage colony-stimulating factor (GM-CSF) are particularly expressed in conjunctival eosinophils [10]. High levels of tear IL-5 and eosinophil cationic protein (ECP) have also been found in VKC patients [11, 12]. Moreover, increased levels of activated blood eosinophils, serum ECP and serum eosinophil protein X (EPX) have been detected, indicating a systemic involvement in VKC [13, 14]. In spite of a high prevalence of IgE-sensitized (IgE-S) patients, the causes of the disease remain obscure: skin test and serum-specific IgE for common inhalant and food allergens are often negative.

In the present study, a case series of 110 VKC patients has been investigated; ocular signs scores, serum and allergologic data and demographic features have been correlated; differences among limbal, tarsal and mixed forms, and possible markers of disease activity in the peripheral circulation are discussed.

Methods

Subjects

One hundred and nineteen consecutive patients with vernal keratoconjunctivitis were evaluated at the time of seasonal relapse in spring or summer. Nine among them did not show tarsal giant papillae or limbal involvement at the time of the evaluation and were withdrawn from the study. In 110 VKC children the following inclusion criteria were established: (i) symptoms and signs of VKC during the previous year, with or without remission in cold seasons; and (ii) a complete wash-out period from any type of topical or systemic drug of at least 2 weeks. Fifteen healthy non-atopic age-matched subjects were also evaluated for total serum IgE, serum-ECP (s-ECP) and peripheral blood eosinophil counts (PBEs).

Clinical scoring system

Each VKC patient was evaluated for ocular signs (conjunctival hyperaemia, papillae, giant tarsal papillae, limbal infiltrates); every parameter was graded as follows: 0 = absent; 1 = mild; 2 = moderate; 3 = severe. In detail, papillae were graded as previously reported [2] with slight modifications, depending on their number and size: 0 = absent; 1 = few papillae < 0.2 mm; 2 = papillae of 0.3 to 1 mm over the tarsal conjunctiva; 3 = papillae of 1 to 3 mm all over the tarsal conjunctiva. Giant cobblestone-like papillae of superior tarsal conjunctiva (> 3 mm) were also graded depending on their number and size: 0 = absent; 1 = few giant papillae 3 to 4 mm; 2 = giant papillae of 3 to 4 mm all over the tarsal conjunctiva, or few papillae of 4 to 6 mm; 3 = giant papillae of 4 to 6 mm all over the tarsal conjunctiva or papillae > 6 mm. The score of bulbar involvement depended on the number and size of limbal papillae: 0 = absent; 1 = few papillae of 0.1 to 1 mm; 2 = papillae of 0.1 to 1 mm around the limbus or few papillae of 2 to 3 mm; 3 = papillae of 2 to 3 mm around the limbus or papillae > 3 mm. Punctate keratitis, corneal ulcers and corneal neovascularization were not scored because they were considered to be

complications, and not a direct expression of VKC. A total objective score was assigned as the sum of hyperaemia, papillae, giant papillae and limbal involvement scores. Limbal VKC was differentiated from tarsal VKC, as previously reported [5], by the presence of papillae at the limbus without giant palpebral papillae. The tarsal forms were differentiated by giant papillae on the upper tarsal conjunctiva, and absence of limbal involvement; patients with mixed forms showed both limbal and giant tarsal papillae.

Biochemical and immunological parameters

Blood samples were collected at the moment of the clinical evaluation, for determination of white blood cells, peripheral blood eosinophil counts, serum ECP (UniCAP ECP; Pharmacia, Uppsala, Sweden), serum total IgE (UniCAP Total IgE; Pharmacia). All patients underwent skin prick tests (SPTs) for common inhalant and food allergens (house dust mites, horse, cat and dog dander, *Alternaria alternata*, *Cladosporium herbarium*, grass pollen, *Parietaria officinalis*, *Artemisia vulgaris*, plane, cypress and olive pollen, milk, egg, tomato, peanut, codfish, wheat, rice and soybean). Weal response of 3 mm diameter or more was considered to be positive [15]. Serum-specific IgE were determined for the same allergens (UniCAP Specific IgE, Pharmacia), and a result greater than 0.35 kU/L was considered to be positive. Patients were considered to be IgE-sensitized when at least one positive SPT response or serum-specific IgE determination (> 0.35 kU/L) was present [16, 17].

Statistical analysis

All data were elaborated using a commercially available statistical computer software package (SPSS, Chicago, IL, USA). Student *t*-tests for independent samples were used for the comparison of means. To assess the linear correlation between variables, Pearson's correlation coefficient was calculated. Probability (*P*) values less than 0.05 were considered statistically significant.

Results

The main clinical and allergologic features of the 110 VKC children are summarized in Tables 1, 2 and 3. All patients were white Italian children; most of them were from Tuscany (*n* = 62, 56.3%), eight (7.2%) were from other regions of central Italy, 36 (32.7%) were from the south, and four (3.6%) from the north. Eighty-one patients were affected by a seasonal VKC ranging from early spring to late summer (73.6%); 29 children had a perennial disease (26.4%), although a vernal-summer worsening was common.

The mean total ocular score was 10.7 ± 3.4 in limbal, 10.9 ± 3.5 in tarsal and 15.1 ± 4.0 in mixed forms. Corneal ulcers were found in 9/110 patients (= 8.1%), of whom four had tarsal, four mixed, and one had limbal VKC. The last was an 18-year-old girl evaluated for the first time.

The mean values $\pm$ SD of s-ECP levels were 51.6 ± 33.6 μ g/dL in IgE-S VKC patients, 31.4 ± 29.6 μ g/dL in non-IgE-S VKC patients, and 12.5 ± 7.2 μ g/dL in controls (Fig. 1). PBEs (mean $\pm$ SD) were higher in IgE-S than in non-IgE-S VKC patients (476 ± 219), and in non-IgE-S VKC patients

Table 1. Clinical features of 110 patients with vernal keratoconjunctivitis

	Bulbar	Tarsal	Mixed	Total VKC
Demographic features				
No. of patients	14	38	58	110
Mean age yr $\pm$ SD (range)	8.6 $\pm$ 3.7 (4.4–18.0)	8.5 $\pm$ 2.3 (3.8–14.3)	8.0 $\pm$ 2.4 (3.2–14.0)	8.3 $\pm$ 2.6 (3.2–18.0)
Mean age at onset	4.0 $\pm$ 2.2 (1.0–8.0)	4.6 $\pm$ 2.7 (0.3–12.0)	4.2 $\pm$ 2.4 (0.25–11.3)	4.4 $\pm$ 2.5 (0.25–12.0)
Males	11 (77.8%)	30 (79%)	40 (69%)	81 (73.6%)
Females	3 (22.2%)	8 (21%)	18 (31%)	29 (26.4%)
M/F	3.6/1	3.7/1	2.2/1	2.7/1
Clinical presentation				
Seasonal	8 (57%)	28 (73%)	14 (24%)	81 (73.6%)
Perennial	6 (43%)	10 (27%)	44 (76%)	29 (26.4%)

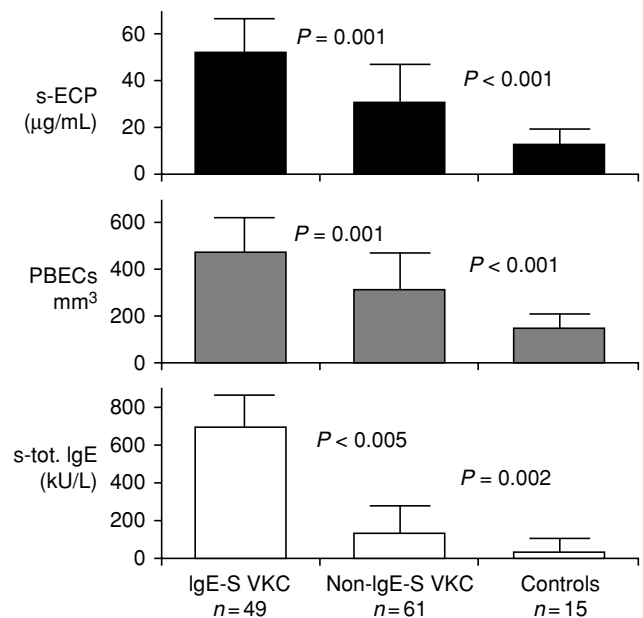
Table 2. Allergologic features of 110 patients with vernal keratoconjunctivitis

	Bulbar	Tarsal	Mixed	Total VKC
SPT and/or s-specific IgE				
Positive	2 (14.3%)	15 (39.5%)	32 (55.2%)	49 (44.5%)
Negative	12 (85.7%)	23 (60.5%)	26 (44.8%)	61 (55.5%)
Serum and blood values				
s-ECP μ g/dL (mean $\pm$ SD)	24 $\pm$ 16.2	37 $\pm$ 25.1	45.7 $\pm$ 38.5	40.3 $\pm$ 32
s-tot.IgE kU/L (mean $\pm$ SD)	65 $\pm$ 68.2	373 $\pm$ 574	417 $\pm$ 850	388 $\pm$ 891
PBECs/mm ³ (mean $\pm$ SD)	211 $\pm$ 112	410 $\pm$ 206	440 $\pm$ 226	397 $\pm$ 218
Associated atopic conditions				
Yes	0	11 (29%)	23 (40%)	34 (30.9%)
No	14 (100%)	27 (71%)	35 (60%)	76 (69.1%)
Atopy in 1st degree relatives				
Yes	4 (28%)	14 (36%)	21 (36%)	38 (34.5%)
No	10 (72%)	24 (64%)	37 (64%)	72 (65.5%)

Table 3. Sensitizing allergens in 49/110 VKC children

Allergen	SPT+	s-spec-IgE+	Total IgE sensitized
House dust mites	32	30	33 (30.0%)
Grass pollen	29	27	30 (27.0%)
Olive pollen	16	14	18 (16.0%)
Cat dander	18	17	18 (16.0%)
Dog dander	11	8	12 (10.9%)
<i>Parietaria off.</i>	10	10	12 (10.9%)
Soybean	4	6	6 (5.0%)
Peanut	2	3	3 (2.5%)
<i>Artemisia vulg.</i>	2	1	3 (2.5%)
Plane pollen	2	0	2 (1.8%)
Cypress pollen	1	1	2 (1.8%)
Egg	2	2	2 (1.8%)
Total patients	47	44	49 (44.5%)

than in controls (152 ± 68). Total serum IgE levels (mean $\pm$ SD) were 699 ± 1240 kU/L in IgE-S VKC, 130 ± 212 kU/L in non-IgE-S VKC, and 35.2 ± 34 kU/L in controls (Fig. 1).

**Fig. 1.** Levels of serum-ECP, PBECs and serum-total IgE (mean and 95% CI) in IgE-sensitized (IgE-S) VKC patients, non-IgE-S VKC patients and controls.

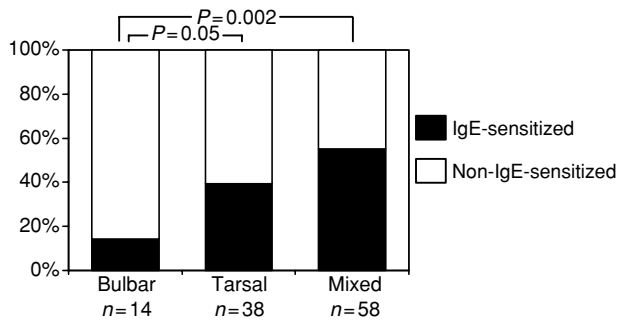


Fig. 2. Prevalence of IgE-sensitization (response to common allergen in SPT and/or serum-specific IgE) in bulbar, tarsal and mixed VKC patients.

Two children with bulbar form (14.3%), 15 with tarsal (39.5%) and 32 with mixed (55.2%) were IgE-S (Fig. 2). SPT results and s-specific IgE values of the sensitizing allergens are reported in Table 3. In bulbar forms the levels of s-ECP were also lower than in tarsal and mixed forms as well as the levels of s-total IgE, and PBECs (Table 2 and Fig. 3).

Thirty-four children (30.9%) with tarsal or mixed VKC suffered from other atopic conditions: asthma ($n=21$, 19%), atopic dermatitis ($n=13$, 11.8%), rhinitis ($n=7$, 6.3%) and food allergy ($n=3$, 2.7%); none of the bulbar VKC patients had other atopic manifestations besides conjunctivitis ($P<0.001$). The correlation between the total objective score and s-ECP, s-total IgE and PBECs was not statistically significant. The total score for giant papillae was strongly correlated with s-ECP levels ($P<0.001$, Fig. 4) and with PBECs ($P=0.001$). Hyperaemia, papillae and limbal infiltrates scores did not correlate significantly with any humoral parameter. The mean $\pm$ SD of s-ECP levels was 46 ± 38 in patients with associated atopic conditions and 37 ± 29 in patients with only VKC ($P=0.2$). The mean score of giant papillae was higher in IgE-S than in non-IgE-S children (3.65 vs. 2.67, $P<0.005$); the mean score of limbal papillae was 2.2 in IgE-S patients and 1.9 in non-IgE-S ($P=0.31$).

Age, sex and clinical presentation (seasonal or perennial) were not associated with the clinical or humoral parameters evaluated in the present study.

Discussion

Large series of VKC patients have been seldom reported in recent years [2, 5, 6] and non-homogeneous criteria distinguishing among tarsal, limbal and mixed forms have been referred. Bonini and colleagues [2] reviewed 195 patients from Italy, of whom 57.8% had a positive SPT and 52.2% had a positive radioallergosorbent test for common allergens; 83.6% of the patients had a 'prevalent' tarsal location of papillae, 7.5% a bulbar, and 8.7% a mixed location. A correlation between IgE sensitization and clinical forms was not evaluated; serum total IgE were higher in IgE-S VKC than in non-IgE-S VKC and controls. Montan and colleagues [6] classified a cohort of 62 VKC Swedish patients, of whom 59.7% were IgE-S (atopic); 78% had a palpebral form and 22% a limbal form (mixed disease was classified according to the site of the principal symptoms); the frequency of IgE-sensitization was 64.6% in palpebral and 42.9% in limbal forms ($P=0.25$), and this condition was less

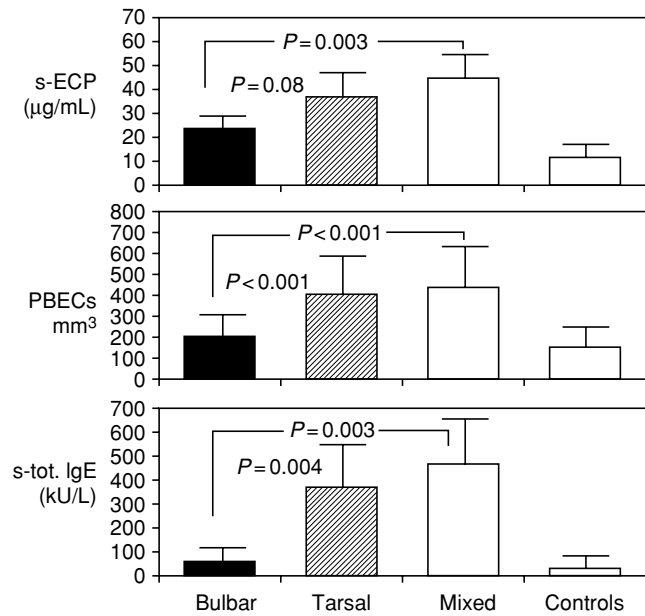


Fig. 3. Blood and serum determinations in bulbar, tarsal and mixed VKC patients.

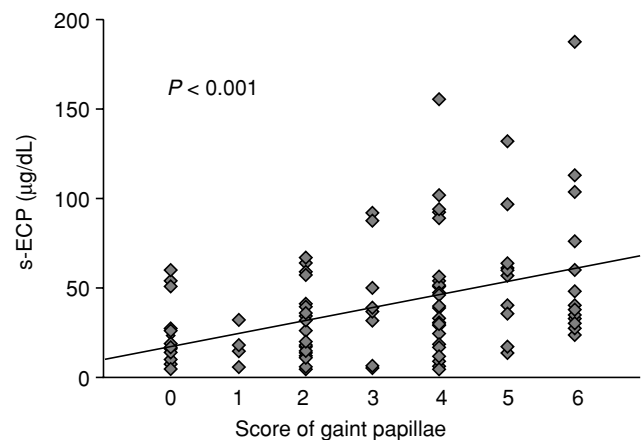


Fig. 4. Scatter plot of s-ECP levels against the score of giant papillae in 110 VKC patients.

common in children who originated from Asia than amongst Swedish children ($P=0.0001$). In a population of 120 VKC patients, Tuft and colleagues [5] found 32 limbal forms (papillae at the limbus without tarsal giant papillae), 54 tarsal (tarsal giant papillae without limbal hypertrophy) and 34 mixed forms. Fewer limbal patients had associated atopic diseases, but the mean tear and serum-specific IgE levels were not significantly different among limbal, tarsal and mixed VKC. In limbal VKC patients there was a higher prevalence of females ($P=0.01$) and a preponderance of blacks, Indians and Asians ($P=0.0001$). Recently Leonardi et al. [7], reported high tear levels of procollagen I and III in tarsal, but not in limbal, VKC.

Our study confirmed a low prevalence of bulbar forms in white children, but a higher number of mixed VKC has been observed. The overall prevalence of IgE-sensitization (44.5%) was similar to other reports [2, 5, 6], suggesting that this condition may represent a risk factor for VKC.

It is interesting to note that a very low prevalence of IgE-S children has been found in our bulbar VKC patients (14.3%) when compared with tarsal and mixed forms (39.9 and 55.2%, Fig. 2), as well as compared with a normal paediatric population [17]. Furthermore, in bulbar VKC children s-ECP, s-total IgE and PBEs were lower than in tarsal and mixed VKC patients (Fig. 3, Table 2). This strong evidence (the first reported to our knowledge) suggests that IgE sensitization is a predisposing factor for tarsal rather than limbal VKC.

In seasonal allergic conjunctivitis (SAC) mast cells but not eosinophils were recruited in ocular tissues [18], and s-ECP levels were not higher than in controls [19], showing lack of serum markers of eosinophil involvement in SAC.

Several studies indicate that eosinophils are the main effector cells in the ocular inflammation of VKC: these cells, together with mast cells and lymphocytes, were significantly increased in biopsies from both tarsal and bulbar VKC specimens [20]. Great amounts of cell surface antigen (ICAM1, CD4, IL-2R), indicating cell activation, were expressed by conjunctival eosinophils [10]; increased numbers of eosinophils in tears [21] and in peripheral circulation [22] have also been detected. IL-5, mainly derived from Th2 cells, induces eosinophil differentiation, recruitment, activation and survival [23]; increased levels of IL-5 in tears of VKC patients has been reported [24].

Eosinophil cationic protein (ECP), whose serum levels have been correlated with severity of atopic disease such as asthma and atopic dermatitis [25, 26], has been studied in VKC. ECP levels in tears were significantly higher in VKC patients than in controls and a positive correlation was found between tear-ECP levels and clinical signs and symptoms [12]. Serum ECP levels, together with serum eosinophil protein X (EPX) levels, were found higher in VKC patients than in controls [14] without a positive correlation between eosinophil protein levels and severity of ocular symptoms. A direct damage of eosinophil products on corneal epithelium has been suggested: eosinophil major basic protein (MBP) deposition was found in corneal ulcers of VKC patients [27], and *in vitro* dose-dependent damage on corneal epithelium resulted in presence of MBP and ECP [28].

In the present study s-ECP, as well as s-total IgE levels and PBEs, were significantly higher in VKC patients than in control subjects, even when skin tests or serum-specific IgE for common inhalant and food allergen were negative (Fig. 1); this suggests a prevalent Th2 response in VKC patients independently of IgE-sensitization (Fig. 2). The mean levels of s-ECP were not higher in patients with other atopic conditions (asthma, rhinitis, atopic dermatitis, food allergy) than in patients with only VKC ($P = 0.2$), indicating that the elevation of the serum protein was determined only by the ocular disease.

The levels of serum ECP, as already reported [14], were not significantly correlated with the total score of the objective ocular signs in our study group, but when each item of the score was evaluated separately, the total score of giant papillae was strongly correlated with s-ECP (Fig. 3) and PBEs; the other items calculated (hyperaemia, papillae and limbal papillae) did not show any significant correlation with the humoral parameters. We may speculate that giant papillae in VKC are a direct expression of eosinophil activation. Moreover, we can assert that serum-ECP is a useful marker of disease activity in tarsal and mixed forms.

In summary, some new data have emerged in our study. VKC is an ocular disease with a typical histopathological and

humoral Th2 response; in our patients s-ECP, s-total IgE and peripheral blood eosinophil counts were significantly higher in IgE-S than non-IgE-S VKC patients, and in non-IgE-S VKC patients than in control children; in the bulbar forms a significantly lower prevalence of IgE sensitization was found than in tarsal, mixed VKC, and in a normal paediatric population. This suggests that in the pathogenesis of this form of VKC, IgE sensitization is not a predisposing factor. A strong positive correlation between giant papillae score and serum ECP and PBEs suggests that eosinophil involvement in tarsal conjunctiva is a main pathogenic factor, and that serum ECP is a very useful marker of disease activity in tarsal and mixed forms of VKC. However, in spite of a high prevalence of IgE-S patients in tarsal and mixed VKC, skin test and serum-specific IgE for common inhalant and food allergens often are negative and the causes of the disease remain substantially obscure.

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