

SHORT REPORT

New insights into childhood Vernal keratoconjunctivitis-associated factors

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Abstract

The purpose of this study was to test for detectable serum levels of antibodies usually associated with immune-related diseases in children with Vernal keratoconjunctivitis (VKC) and seek for their family history of allergies and autoimmune disorders. The association of human leukocyte antigens (HLA) with VKC was also investigated. We enrolled 181 VKC children and assessed total and specific IgE, antithyroglobulin (AbTG), antithyroidperoxidase (AbTPO), antitransglutaminase (tTG), and antinuclear antibodies (ANA) by standard procedures. Class I and II HLA typing was also carried out following standard protocols, and it was compared with that of healthy subjects. Patients were positive for AbTG (22%), AbTPO (14.6%), and ANA (15.8%), and AbTG positivity was associated with VKC severity (mean ocular score $\pm$ SD positive vs. negative: 6.56 ± 2.1 vs. 4.82 ± 2.1 ; $p = 0.03$). We found that 12.2% of VKC cases had a positive family history for psoriasis, 6.4% for other cases of VKC, and 5.2% for thyroiditis, while 50.2% of them were atopic. The expression of HLA class I B37 was significantly higher in VKC patients than in controls (7.1% vs. 2.1%, $p = 0.04$), although not confirmed after multiple antigens testing analysis. Our study suggests a role of common components associated with immune-based diseases in the clinical expression of VKC.

Introduction

Vernal keratoconjunctivitis (VKC) is a seasonal ocular inflammatory disease, involving tarsal and/or bulbar conjunctiva, with greater prevalence in boys living in warm climates (1). VKC is considered to be primarily, but not solely, an immunoglobulin (Ig)E-mediated disease (1). Eosinophils along with mast cells, T cells, and neutrophils are the main effector cells in this ocular inflammation (1, 2). Conjunctival epithelial cells also play an active role in VKC inflammatory response by expressing surface adhesion molecules and chemokine receptors human leukocyte antigens (HLA)-DR positive, as well as acting as a mechanical barrier preventing the entry of noxious substances into the eyes (3). Based on the multifactorial and complex pathogenesis of the VKC, several molecular drugs with different mechanism of action, such as anti-histamines, mast cell stabilizers, and immunosuppressive molecules, have been used to control signs and symptoms (1, 4, 5). In line with another Italian study (4), we have previously demonstrated the efficacy and safety of low-

dose cyclosporine eyedrops in the treatment of children with severe VKC, supporting the hypothesis that this ocular disorder may have an immune-based inflammatory component (5).

The aim of this study was to investigate whether a family history for conditions other than atopy and serological immune-related biomarkers was associated with VKC severity in Caucasian children. For the first time, the assessment of HLA markers expressed in VKC children was also carried out.

Methods

We studied 181 (M/F ratio 3:1) consecutively enrolled children, aged 2–12 years, with diagnosis of VKC made by an ophthalmologist by scoring each eye for objective signs and symptoms, as previously described (5). Children were defined as having severe VKC, if the ocular score (OS) was ≥ 3 points for one eye (5). The family history of atopy and immune-related disorders was recorded by the specialist pediatric allergologist at the time of the first visit of each patient. The

family history of atopy was recorded, by asking parents about the presence of rhinitis and/or asthma and/or atopic dermatitis and/or allergic sensitization. Autoimmune diseases specifically queried include rheumatoid arthritis, type 1 diabetes, psoriasis, systemic lupus erythematosus (SLE), inflammatory bowel disease, multiple sclerosis, celiac disease, thyroiditis, scleroderma, vasculitis, myasthenia gravis, polymyositis, and dermatomyositis. The autoimmune diseases considered in this study were characterized by specific symptoms that would generally bring the subject to medical attention.

All children underwent total IgE and specific IgE (CAP-RAST) assessments, and they were considered atopic in the presence of elevated specific (>0.35 kU/l) and total IgE (>100 kU/l). In a subgroup of 95 (52.5% of the total) severe VKC patients, antibodies, antithyroglobulin (AbTG), antithyroidperoxidase (AbTPO), and antitransglutaminase (tTG) were assessed by ELISA, while antinuclear antibodies (ANA) were measured by indirect immunofluorescence assay. Class I HLA typing (HLA-A, HLA-B, and HLA-C) was performed by microlymphocytotoxicity test using a panel of commercial antisera (6), whereas class II HLA typing (HLA-DR and HLA-DQ) was carried out by polymerase chain reaction sequence-specific oligonucleotide, by means of a commercial kit (7). The HLA distribution in our study population was compared with that of healthy bone marrow donors from the same geographic area (M/F 0.9:1; aged 18–65 years), comprising a total of 6156 and 1183 individuals, for HLA class I and II, respectively. An informed consent was obtained by the parents of the children, and the study has been approved by the local Ethic Committee.

All analyses were performed using SPSS for Windows software (SPSS Inc., version 15.0, Chicago, IL, USA; Prentice-Hall, Upper Saddle River, NJ, USA). Values are expressed as means and standard deviation (SD). The Mann–Whitney *U*-test was computed to analyze continuous variables, and the chi-square test was used to test categorical data between groups. A corrected Bonferroni's probability value (*P_c*) was calculated for multiple comparisons between HLA. Probability (*p* and *P_c*) values <0.05 were considered significant.

Results

Patients were mainly affected by a mixed form, tarsal and bulbar, of VKC (65.6%). The tarsal form was diagnosed in 22.3% of the examined children, while the solely bulbar form was present in 12.1% of our cases. As expected, subjects bearing the mixed VKC had significantly severe OS than those with the tarsal or the bulbar ones [(mean OS $\pm$ SD): 6.5 ± 2.1 vs. 4.5 ± 2.1 ; $p = 0.0001$]. Ninety-one (50.2%) children were positive for allergen sensitization and 90 (49.7%) resulted negative. The mean values $\pm$ SD of total IgE was 198.4 ± 293.8 kU/l. There were no differences in mean score values between atopic and non-atopic children [(mean OS $\pm$ SD): 5.8 ± 2.3 vs. 5.9 ± 2.1 ; $p = 0.9$]. None of the children was affected by a concomitant systemic chronic disorder.

Data on family (parents and/or siblings) history of atopy and immune-mediated diseases were recorded in 172 (95.0%)

patients. Among those, 62 (36.0%) VKC children had a positive family history for allergies, 21 (12.2%) for psoriasis, 11 (6.4%) for other cases of VKC, 9 (5.2%) for thyroiditis, 7 (4.0%) for celiac disease, one case (0.6%) for Crohn's disease, one (0.6%) for SLE, and another one (0.6%) for multiple sclerosis (Fig. 1). Having relatives either atopic or affected by immune-related diseases did not affect the VKC clinical expression in our population (data not shown).

Patients positive for AbTG antibodies (22%) showed mean OS values significantly more severe than those of patients negative for the same marker [(mean OS $\pm$ SD): 6.56 ± 2.1 vs. 4.82 ± 2.1 ; $p = 0.03$] (Fig. 2). There was not a significant difference in means of OS between subjects positive (14.6%) for AbTPO and those negative [(mean OS $\pm$ SD): 5.93 ± 2.2 vs. 5.37 ± 2.3 ; $p = 0.8$]. Similarly, ANA-positive patients (15.8% with title $\geq 1:160$) showed no differences in mean OS values when compared with those ANA negative [(mean OS $\pm$ SD): 6.00 ± 2.4 vs. 5.58 ± 2.2 ; $p = 0.7$]. Positive tTG antibody subtypes were found only in two of 95 (2.1%) children.

We could compare the class I and II HLA frequencies of 42 of 95 (44.2%) severe VKC children with those of controls, and we found that some antigens tended to be differently expressed in patients than in the healthy population (Table 1). Particularly, the HLA class I B37 resulted significantly more expressed in VKC patients as compared with controls. However, significance was lost after Bonferroni's correction.

Conclusions

Results emerging from the present study suggest that either the personal or the family history for atopy and immune-related disorders seem to affect the severity of VKC. Familial aggregation of allergic and autoimmune diseases likely reflects shared pathogenic factors between different disorders. In the present study, we have documented an high prevalence of positive family history of allergies in

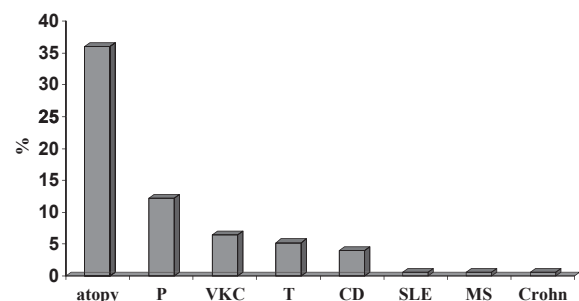


Figure 1 Distribution of family history of atopy and autoimmune diseases in the study population. Children with Vernal keratoconjunctivitis (VKC) had mainly a positive family history for atopy and psoriasis (P), but there were also some cases of VKC, thyroiditis (T), celiac disease (CD), systemic lupus erythematosus (SLE), multiple sclerosis (MS) and Crohn's disease in their family (see text for details).

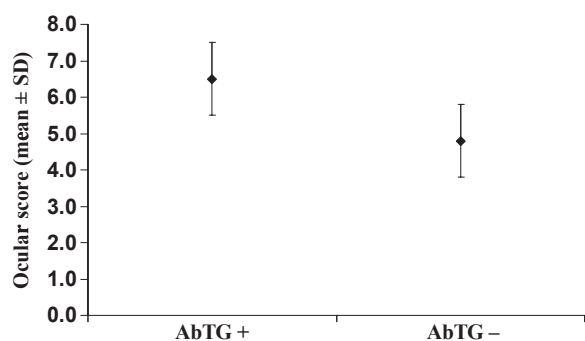


Figure 2 Percentages of antithyroglobulin antibodies (AbTG) between groups of children with Vernal keratoconjunctivitis (VKC). Patients positive for AbTG showed a more severe form of VKC than those negative for the same autoantibodies.

VKC patients, and in line with other studies, 50% of our patients were atopic (4, 5). Recently, Bertelsen *et al.* have confirmed that conjunctivitis is the most prevalent allergy related comorbidity of rhinitis and that rhinoconjunctivitis might also be associated with asthma and bronchial hyper-responsiveness, supporting the concept of a 'united airway disease' (8). We have also investigated the close link between nasal inflammation and VKC in a previous study including part of our VKC patients, showing that the same inflammatory cells are involved in the two organs, affecting the severity of rhinoconjunctivitis (3). However, the nasal characteristics of patients with VKC differed from those associated with the 'classic' allergic rhinoconjunctivitis (3). Since now, among immune-mediated disorders, it has been observed an high frequency of allergic conjunctivitis only in myasthenia gravis (9). We found that 5.4% of our young VKC patients concomitantly suffered for an immune disease and that some autoimmune disorders were also

Table 1 Distribution of HLA class I and II frequencies in Vernal keratoconjunctivitis patients and controls

HLA	Patients (n = 42) N (%)	Controls (n = 6156) N (%)	HLA	Patients (n = 42) N (%)	Controls (n = 6156 or 1183)* N (%)
A1	14 (33.3)	1368 (22.2)	B53	1 (2.4)	104 (1.7)
A11	2 (4.7)	707 (11.5)	B55	2 (4.7)	228 (3.7)
A2	14 (33.3)	2498 (40.6)	B57	2 (4.7)	486 (7.9)
A23	1 (2.4)	341 (5.5)	B58	1 (2.4)	135 (2.2)
A24	14 (33.3)	1508 (24.5)	B60	1 (2.4)	172 (2.8)
A25	1 (2.4)	104 (1.7)	B62	3 (7.1)	258 (4.2)
A26	3 (7.1)	664 (10.8)	B63	3 (7.1)	154 (2.5)
A28	4 (9.5)	523 (8.5)	Cw1	2 (4.7)	289 (4.7)
A29	1 (2.4)	381 (6.2)	Cw2	2 (4.7)	609 (9.9)
A3	13 (30.9)	1373 (22.3)	Cw3	6 (14.3)	572 (9.3)
A30	1 (2.4)†	726 (11.8)	Cw4	11 (26.2)	1914 (31.1)
A32	7 (16.7)	708 (11.5)	Cw5	4 (9.5)	425 (6.9)
A33	1 (2.4)	265 (4.3)	Cw6	7 (16.7)	1619 (26.3)
A66	2 (4.7)‡	89 (1.4)	Cw7	12 (30.0)	2462 (40.0)
B7	5 (11.9)	658 (10.7)	DR1	2 (4.7)§	186 (15.7)
B8	2 (4.7)	394 (6.4)	DR4	11 (26.2)	171 (14.5)
B13	2 (4.7)	585 (9.5)	DR7	7 (16.7)	259 (21.9)
B18	7 (16.7)	1403 (22.8)	DR9	2 (4.7)	29 (2.5)
B27	1 (2.4)	166 (2.7)	DR10	2 (4.7)	46 (3.9)
B35	11 (26.2)	1791 (29.1)	DR11	21 (50.0)	624 (52.8)
B37	3 (7.1)¶	141 (2.3)	DR12	2 (4.7)	57 (4.8)
B38	5 (11.9)	498 (8.1)	DR13	10 (23.8)	229 (19.4)
B39	3 (7.1)	246 (4.0)	DR14	6 (14.3)	145 (12.3)
B41	4 (9.5)	351 (5.7)	DR15	1 (2.4)	141 (11.9)
B44	5 (11.9)	942 (15.3)	DR16	8 (19.0)	110 (9.3%)
B49	6 (14.3)	517 (8.4)	DQ1	21 (50.0)	712 (60.2)
B51	10 (23.8)	1046 (17.0)	DQ2	9 (21.4)	344 (29.1)
B52	3 (7.1)	375 (6.1)	DQ3	17 (40.0)	745 (63.0)

HLA, human leukocyte antigens.

*Number of controls for HLA class I and II are 6156 and 1183, respectively; ns = not significant.

†RR = 0.17, p = 0.05, Pc = ns.

‡RR = 3.4, p = 0.07, Pc = ns.

§RR = 4.8, p = 0.05, Pc = ns.

¶RR = 3.2, p = 0.04, Pc = ns.

present in their parents. The concordance between family history of a specific organ involvement with the implication of the same organ in the child has been recently investigated by Garcia-Marcos and coworkers for asthma and rhinoconjunctivitis comorbidity (10). In a population of atopic children, they observed that the organs (nose and/or lung) that were implicated in parents also tended to be involved in their children. We found that 6.4% of our VKC cases had a family history positive for the same ocular disorder. So far we have firstly investigated the distribution of HLA markers in children with VKC, and we observed that the B37 HLA class I tended to be more expressed in these patients compared with healthy controls from the same geographic area. This antigen has been associated with psoriasis in a Korean population (11). The percentage of psoriasis cases (12.2%) within the family of our VKC patients was significative, considering the prevalence of psoriasis in the general population ranging between 0.6% and 4.8% (12). Taken together, these data might indicate that some HLA markers (likely including B37) involved in the susceptibility for immune-mediated diseases, such as psoriasis, may contribute to VKC development. The same B37 HLA class I was also significantly present in Tunisian subjects affected by autoimmune thyroid diseases (13). We found that 5.2% of our VKC cases had parents suffering from thyroiditis and that 22% of the

patients had circulating AbTG antibodies, which correlated with VKC severity. Although ANA antibodies can be usually detected in healthy people, we also observed that 15.8% of our VKC subjects were ANA positive, supporting the hypothesis of the role of immune-mediated mechanisms in the pathogenesis of this ocular disease. Further investigations into a bigger panel of immunologic biomarkers in a larger cohort of VKC patients and a long-term clinical follow-up for the concomitant appearance of other immune-mediated disorders in these children are needed for a better understanding on possibly immune or genetic features associated with VKC. The rate of detection of autoantibodies in an age-matched population of healthy individuals is also warranted. There is an ongoing discussion about when a disease should be considered autoimmune, leading to different criteria such as Witebsky's postulates (14). Our report supports the notion that VKC is an immune-based disorder, most likely with a genetic predisposition, but many questions about its pathogenesis remain still unanswered.

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