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Serum allergen specific immunoglobulin E levels in patients with allergic conjunctivitis

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PURPOSE. *To evaluate serum allergen specific immunoglobulin E (IgE) levels in patients with various types of allergic conjunctivitis.*
METHODS. *Twenty-five patients with seasonal allergic conjunctivitis (SAC), 17 patients with perennial allergic conjunctivitis (PAC), and 10 patients with vernal conjunctivitis (VC) were included in the study. Specific IgE levels to Dermatophagoides pteronyssinus (Dp), Dermatophagoides farinae (Df), mixed grass pollens, and animal epithelia were measured using Pharmacia CAP system (Pharmacia Diagnostic AB, Uppsala, Sweden).*
RESULTS. *The percentage of subjects with specific IgE against Dp and Df was statistically higher in VC (30%) compared to PAC (5.9%) and SAC (0%) (p=0.03). Specific IgE against mixed grass pollens was found in 30% of VC and 40% of SAC, whereas 10% of VC and 8% of SAC patients were found to be hypersensitive to animal epithelia.*
CONCLUSIONS. *Allergic reaction against house dust mites and pollens was common in VC, whereas specific IgE against grass pollens was remarkable in SAC. IgE levels specific to various antigens might be measured by UNICAP system, which is a rapid and practical technology. (Eur J Ophthalmol 2008; 18: 675-9)*

KEY WORDS. *Allergic conjunctivitis, Serum specific IgE, UNI-CAP system*

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INTRODUCTION

Allergic reaction can occur at various sites, including the skin, respiratory tract, and eye, with accompanying symptoms ranging from mildly irritating to severe and chronic. Conjunctiva is one of the most frequently affected sites in an allergic reaction. There are various types of allergic conjunctivitis, including seasonal allergic conjunctivitis (SAC), perennial allergic conjunctivitis (PAC), vernal conjunctivitis (VC), atopic conjunctivitis (AC), and giant papillary conjunctivitis (GPC) (1-3). The incidence of ocular allergy varies markedly among different geographic regions, being more likely in individuals living in warm climates.

SAC and PAC represent the majority of ocular allergic disorders. They are caused by direct exposure of the ocular mucosal surfaces to the allergens, followed by reaction of these allergens with specific immunoglobulin (Ig) E antibodies bound to the surface of conjunctival mast cells resulting in degranulation and release of chemical mediators, including histamine, prostaglandins, leukotrienes, tryptase, and cytokines (4-7). SAC is most commonly related to atmospheric pollens, whereas PAC is related to animal dander, dust mites, or other environmental year-round allergens (8, 9). VC is a rare condition which typically develops in childhood and is more common in males (10, 11). Previous studies (12, 13) have demonstrated increased IgE levels specific for house dust, dust mites, fungi, animal epithelia, and grass antigen in patients with VC.

As allergic conjunctivitis is a growing social concern worldwide, it is crucial to detect the causal allergens to

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prevent and treat the disease. The purpose of this study is to determine the serum allergen specific IgE levels in subjects with various allergic conjunctivitis using Pharmacia CAP system (Pharmacia Diagnostic AB, Uppsala, Sweden).

MATERIALS AND METHODS

Patients with signs and symptoms of allergic conjunctivitis were recruited into the study from March to September. The procedures used in this study conformed to the tenets of the Declaration of Helsinki and the Ethics Committee of Hacettepe University Faculty of Medicine approved the research. Informed consent was obtained from the participating individuals following an explanation of the nature and possible consequences of the study. A complete eye examination including visual acuity testing, refraction, slit-lamp biomicroscopic examination, and fundus examination was performed. Demographic data, including age at inclusion, age at onset, gender, association with concomitant or previous systemic allergic conditions, medications, and family history were evaluated.

The diagnostic criteria of SAC patients were bilateral involvement, acute attack, watery discharge, itching, papillary hypertrophy, and alteration of symptoms with seasons (14), whereas PAC occurs throughout the year. The main features of VC are itching, photophobia, mucoid discharge, and giant papillae on the upper tarsal conjunctiva. VC was classified as palpebral if the patient had giant papillae on the upper tarsal conjunctiva, as limbal if the patient had limbal papillae and Trantas dots, and as mixed if the patient had both palpebral and limbal signs. Patients with blepharitis and contact lens wearers were excluded from the study.

Twenty-five patients (17 male, 8 female) with SAC, 17 patients (7 male, 10 female) with PAC, and 10 patients (8 male, 2 female) with VC, who had been treated at the Hacettepe University School of Medicine, Department of Ophthalmology, were included in the study. The mean ages of subjects were 13.5±4.6 years, 19.2±13.53 years, and 18.2±12.6 years in VC, SAC, and PAC groups, respectively (p=0.56). In the VC group, 2 patients had limbal VC, 6 patients had palpebral VC, and 2 patients had mixed type VC.

Ten milliliters peripheral blood was taken from the antecubital vein and centrifuged, and serum samples were collected and stored at –20°C. UniCAP specific IgE assay is an in vitro quantitative assay used for the measurement of allergen specific IgE in human serum or plasma. UniCAP specific IgE assay is used with the instrument ImmunoCAP 100/UniCAP 100. The allergen of interest, covalently coupled to ImmunoCAP, reacts with the specific IgE in the patient sample. After washing away non-specific IgE, enzyme-labeled antibodies against IgE (enzyme-anti-IgE) are added to form a complex. After incubation, unbound enzyme-anti-IgE is washed away and the bound complex is then incubated with a developing agent. After stopping the reaction, the fluorescence is measured. The specific IgE assay was calibrated according to WHO standard (standard WHO 75/502), in the range of 0.35–100 kilounits per liter (kU/L) and the levels were read from a standard curve. According to Pharmacia CAP system instructions, a value greater than 0.35 kU/L was defined as a positive result. The values 0.35–0.69 kU/L were grouped in class 1, 0.70–3.49 kU/L in class 2, 3.50–17.49 kU/L in class 3, 17.5–49.99 kU/L in class 4, and 50.0–100 kU/L in class 5. One kU/L corresponds to 2.4 ng IgE/mL. In all patients specific IgE levels against grass pollen (mixed grass pollen, gx1, g3, g4, g5, g6, g8; *Dactylis glomerata, Festuce elator, Lolium perenne, Phleum pratense, Poa pratensis*), house dust mites (*Dermatophagoides pteronyssinus* [dp, d1], *Dermatophagoides farinae* [df, d2]), and mixed animal epithelia (ex1-cat, horse, cow, dog dander) were measured.

In statistical analysis, age at inclusion was compared using Student *t*-test, while gender, family history, associated allergic diseases, and positive IgE against allergens were analyzed using chi-square test and Fisher exact test and a p value < 0.05 was considered as statistically significant.

RESULTS

The age at onset was before 10 years in 70% of VC, 72% of SAC, and 64.7% of PAC subjects (p>0.05). Family history was present in 80% of VC, 58.8% of PAC, and 32% of SAC (p=0.03). The percentages of associated allergic rhinitis, asthma, and atopic dermatitis in each group can be seen in Table I. There were no statistically significant differences in the incidence of these diseases among the three groups (p>0.05).

The percentage of subjects with specific IgE against dp and df was statistically higher in VC (30%) compared to PAC (5.9%) and SAC (0%) (p=0.03). Specific IgE against

mixed grass pollens was found in 30% of VC, 40% of SAC, and none of the subjects with PAC, whereas specific IgE against animal epithelia was found in 10% of VC, 8% of SAC, and none of the subjects with PAC. The distribution of positive subjects according to five classes is given in detail in Table II. In subjects with associated allergic disorders, the IgE positivity was 33.3% in VC group, 40% in SAC group, and 20% in PAC group.

DISCUSSION

The diagnosis of allergic conjunctivitis is mainly based on history and clinical examination. Laboratory tests may be useful to confirm the clinical diagnosis (15) and determination of serum IgE levels is one of the useful tests. UNICAP system is a solid-phase immunoassay, used for the titration of serum specific IgE. Ewan et al (16) compared the Pharmacia CAP system and radioallergosorbent test (Phadebas RAST) for five inhalant allergens, dp, timothy grass pollen, cat epithelium, *Cladosporium*, and *Alternaria*. They found that the CAP system was more sensitive than the RAST. In another study by Bousquet et al (17), the sensitivity and the specificity of CAP were found better than RAST except for orchard-grass pollen. Boccagni et al (18) compared four techniques (CAP System, Kallested Allercoat System, Neo Abello, Hamlet-IgE) for specific IgE detection and they showed that the CAP system offers the highest sensitivity. Pastorello et al (19) found high sensitivity (95.5%) and specificity (98.1%) for the CAP system.

TABLE I - THE PERCENTAGE OF ASSOCIATED ALLERGIC DISORDERS

	Seasonal allergic conjunctivitis	Perennial allergic conjunctivitis	Vernal conjunctivitis
Rhinitis	28.2%	17.6%	20%
Asthma	12.2%	11.7%	20%
Dermatitis	4%	11.7%	10%

SAC symptoms become more frequent and severe with increasing pollen counts (20-23). Mimura et al (24) evaluated serum allergen specific IgE levels against frequent allergens in Japan (five animal epithelia, house dust, *Dermatophagoides pteronyssinus*, *Acarus*, moth, *Candida*, *Alternaria tenius*, Japanese cypress pollen, ragweed, mugwort, and orchard grass) using UNICAP. In SAC group, specific IgE positivity against cedar pollen, cypress pollen, dp, and animal epithelia were 52.5%, 37.5%, 30%, and 5%, respectively (24). In another study, Mimura et al (25) found IgE positivity against house dust and dp in 51.9% and 48.1%, respectively, in autumn group and cedar (68.8%) and cypress pollen (59.4%) in the spring group. There were studies from Turkey about pollen types from different regions (26-29). Arboreal pollen types are predominant in the atmosphere of Bursa, Balikesir, and Ankara. In their study, Pinar et al (27) found high pollen concentration in June and *Pinaceae, Cupressaceae/Taxaceae, Gramineae, Platanus, Populus, Moraceae, Chenopodiaceae/Amaranthaceae, Acer, Quercus, Betula, Salix, Rumex,* and *Plantago* were found to be the dominant pollen types in Ankara, Turkey.

Grass pollens are known as the major allergens worldwide, especially in the temperate zone (20, 30). The most important pollens are grasses and olive trees in Spain from April to June (31), grass pollens in Greece from March to June (32), and grass pollens in summer in Poland (30). In this study, we evaluated specific IgE positivity against mixed grass pollen (gx1) in allergic subjects in a period between May and September. In our SAC group, positive IgE against grass pollens was observed in

TABLE II - SERUM-SPECIFIC IGE LEVELS AGAINST VARIOUS ANTIGENS

	SAC			PAC			VC		
	dp, df	Grass	Animal epithelia	dp, df	Grass	Animal epithelia	dp, df	Grass	Animal epithelia
Class I	0	1	1	0	0	0	0	0	0
Class II	0	1	1	1	0	0	3	0	1
Class III	0	5	0	0	0	0	0	2	0
Class IV	0	3	0	0	0	0	0	1	0
Class V	0	0	0	0	0	0	0	0	0

40% of the subjects, against animal epithelia in 8% of the subjects, whereas none of the SAC subjects had measurable specific IgE against dp and df. The percentage of SAC patients with positive serum specific IgE was low compared to the literature. We measured only IgE levels against grass pollens; however, some of these patients might be allergic to arboreal pollens. Another limitation of this study was that the diagnosis of the allergic subjects was made according to their clinical symptoms and signs. We did not perform prick tests or conjunctival cytology to all of them. Only patients with associated allergic diseases (asthma, atopic dermatitis) were evaluated by the allergologists and prick tests were performed to these subjects.

Fujishima et al (12) found house dust positivity in 60%, house dust mite positivity in 80%, and cat epithelia in 60% of the 10 subjects with VC. Tuft et al (13) evaluated 120 patients with VC and found that serum specific IgE levels against grass pollen were 90% in the limbal group and 94% in the palpebral and mixed group, which were higher than the control group (29%). The positivity against cat and mite ranged from 82–100% in VC and 14–29% in the control group. Kitazawa et al (33) found IgE positivity against Japanese cedar pollen as 34.3% in SAC group, against house dust mites as 81.8% in VC, and 40% in PAC groups. In our VC group, specific IgE positivity against house dust mite, mixed grass pollen, and animal epithelia were 30%, 30%, and 10%, respectively, which were lower than the previous studies. The number of subjects was very low and we have to enlarge our sample size to confirm these results in VC group.

Gradman et al (34) found allergic conjunctivitis in 42% of the subjects with allergic rhinitis, 24% of the subjects with asthma, and 30% of the subjects with dermatitis. Sensitization to house dust mites was more frequent in chronic allergic conjunctivitis than in acute allergic conjunctivitis (95% vs 53%) and sensitization to grass pollen was more frequent in children with acute allergic conjunctivitis (34). Leonardi et al (35) found associated allergic diseases in 46% of the VC cases and the most frequent disease was rhinitis (30.1%), followed by eczema (16.3%) and asthma (14.6%). In a case series of 109 patients with VC from Nigeria (36), a history of atopic diseases was present in only 4.5% of the subjects. In our study, allergic rhinitis was the most common concomitant allergic disease in VC (20%) and SAC (28%). As ocular allergy might be a part of a systemic allergic disorder such as rhinitis, asthma, and/or dermatitis, these subjects should be managed in collaboration with an allergologist.

Ocular allergic diseases might occur as a result of sensitization to various antigens, such as pollens, house dust mites, and animal epithelia, which might be detected simply by measuring specific IgE levels using UNICAP system, a rapid and easy technology. The allergens should better be selected according to the region of the country and the type of the ocular allergic disorder. Specific IgE measurement to only limited antigens is not always a sensitive diagnostic test in ocular allergic diseases, since it might be negative in almost half of the subjects. However, as shown in our study, these tests might be helpful to determine the extent of sensitization of the subjects to selected antigens and might aid in the comprehensive management of the problem.

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