Skin Prick Test Reactivity to Aeroallergens among Egyptian Patients with Isolated Allergic Conjunctival Disease

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Allergic conjunctival disease (ACD) is a type of ocular allergy, which includes seasonal allergic conjunctivitis (SAC), perennial allergic conjunctivitis (PAC), and vernal keratoconjunctivitis (VKC). Little is known about the pattern of sensitization or prevalent aeroallergens among patients with isolated ACD in Egypt. We aimed to evaluate the prevalence of skin prick test positivity to common aeroallergens among Egyptian patients with isolated allergic conjunctival disease. The study included 75 patients with isolated ACD recruited from a tertiary Egyptian outpatient clinic. Skin prick test (SPT) was performed for all patients with common aeroallergens. Total serum immunoglobulin E (IgE) was measured by ELISA. A positive SPT reaction was present among 32 patients (42.7%). The most prevalent aeroallergens among all patients were mites and pollens (12% respectively), followed by grass (8%) and hay dust (6.7%). Eight patients (10.7%) had SAC, 19 patients (25.3%) had PAC, and 48 patients (64%) had VKC. Prevalence of SPT positivity to indoor allergens was significantly more common among PAC (52.6%) than among SAC (25%) and VKC (16.7%), *P*= 0.011. Outdoor allergen sensitization did not differ significantly between the 3 subgroups, *P*= 0.614. Elevated IgE levels were observed among 62.5%, 73.7% and 66.7% of patients with SAC, PAC and VKC, respectively, with no statistically significant difference between them, *P*= 0.806. In conclusion aeroallergen sensitization is common among Egyptian patients with isolated ACD. Accordingly, SPT should be included in the diagnostic workup of these patients.

Ocular allergy may include seasonal (SAC) and perennial allergic conjunctivitis (PAC), both of which involve an immunoglobulin E (IgE)-mediated allergic reaction, and are characterized by the presence of itching, which may be associated with tearing, conjunctival redness, and small papillary hypertrophy of the tarsal conjunctiva. Less frequently, chronic severe forms, as vernal keratoconjunctivitis (VKC) and atopic keratoconjunctivitis (AKC) may be observed. These forms show more severe symptoms and exacerbations, and may be complicated by corneal involvement (Leonardi *et al.,* 2008). The increase in prevalence of allergic conjunctivitis over the last few years is possibly attributed to an increased use of contact lenses, exposure to environmental factors such as smoke and pollution, and an increase in associated atopic disorders (Mantelli *et al.,* 2011). Acute allergic conjunctivitis is an immunoglobulin E (IgE)-mediated response, whereas chronic disease is a mixed cell response (McGill *et al.,* 1998).

Allergic conjunctivitis is frequently associated with allergic rhinitis; however, it may be observed as the only or prevalent allergic sensitization (Leonardi *et al.,* 2008). To date, there are inadequate data to assess the prevalence of ocular allergies, and only the epidemiology of allergic rhino-conjunctivitis has been well-studied (Leonardi *et al.,* 2012). The diagnosis of allergic conjunctivitis is mainly clinical and depends on a concordant case history. Patients with

isolated allergic conjunctivitis will probably seek the medical consultation of an ophthalmologist, with treatment options including various topical agents as antihistamines, mast cell stabilizers and topical steroids. Frequent long-term use of local steroids may lead to many complications such as cataract and glaucoma (del Cuvillo *et al.,* 2009). In addition, these patients may not be directed to perform allergy testing which helps to identify the causative allergen in order to adopt appropriate preventive and treatment measures against it. Therefore, there is a great need to study the role of skin allergy testing in isolated allergic conjunctivitis.

To our knowledge, the causative allergens in the Egyptian environment are unknown. We therefore aimed to evaluate the prevalence of skin prick test positivity to common aeroallergens among a cohort population of Egyptian patients with isolated allergic conjunctival disease.

Materials and Methods

Methods

The present cross-sectional study included 75 patients with isolated allergic conjunctivitis. They were recruited from the Ophthalmology and Allergy outpatient clinics at Ain Shams University Hospital, Cairo. Allergic conjunctivitis was diagnosed on the basis of past history, clinical symptoms such as ocular itching, redness, tearing, or ocular pain, and slit-lamp examinations showing filamentous (mucous) discharge, chemosis, hyperemia, or papillae of the palpebral conjunctiva (Fujishima *et al.,* 1996; Takamura *et al.,* 2011). Exclusion criteria included patients with other atopic disorders such as rhinitis, asthma, food allergy, and drug allergy, patients with major system failure, autoimmune diseases, and patients who had received steroids or cytotoxic drugs within one month before recruitment. Further exclusion criteria included patients who had received antihistamines and steroids within 7 days before recruitment. An informed consent was obtained from all participants, and the study was approved by the Research Ethics Committee of Ain Shams University.

Skin Prick Test (SPT)

Atopic status was determined by SPT reaction to a large panel. The allergens were selected by the allergy laboratory of Ain Shams University Hospitals to include the most common locally encountered aeroallergens in the Egyptian environment. These included house dust mites, animal danders (cat, dog and horse), molds, pollens, grass, wool, hay dust, straw, cotton dust, tobacco, pigeon feather, Candida, and American cockroach (Periplaneta Americana). Histamine (0.1%) in phosphate-buffered saline and physiologic saline were used as positive and negative controls, respectively.

The extracts have been prepared for SPT panel by the allergy laboratory of Ain Shams University Hospitals. Extracts were prepared as glycerinated solution, using weight/volume (wt/vol) unit.

The wt/vol unit indicates how the extract was produced. A potency of 1:100 indicates that 1 g of dry allergen was added to 100 cc of a buffer (phenol saline) for extraction. The allergen was eluted for a time, and then the solid material was filtered out, leaving an aqueous solution (Li *et al.,* 2003).

After sterilizing the skin of the anterior surface of the forearm, drops of each allergen extract were applied approximately 3 cm apart, followed by careful pricking of the epidermis through each drop. After 20 minutes, the immediate response was evaluated, and the mean wheal diameter was calculated by adding the largest diameter to the diameter perpendicular to it, and dividing the result by two. The SPT was considered valid if the difference in mean wheal diameter between the positive and negative controls was at least 1 mm. A mean wheal diameter of at least 3 mm greater than the negative control was considered positive (Arbes *et al.,* 2007).

Serum Total Immunoglobulin E (IgE) assay

Serum total IgE levels were measured for all recruited patients by the Immuno-CAP system (Pharmacia Diagnostics, Uppsala, Sweden).

Blood was collected from each patient by withdrawing venous blood by a single puncture technique of the antecubital vein. Serum was prepared by centrifugation for 10 minutes at 3000 rpm at room temperature, aliquoted and stored after labeling at -20°C until analysis. At the time of assay, samples were thawed, mixed and allowed to stand at room temperature for at least 15 minutes.

One kit was used for monoclonal antibody specific for total IgE. The monoclonal antibody was pre-coated

into the microplates. Standards and samples were pipetted into the wells, and any total IgE present in standards or samples was bound by immobilized antibody. After washing away any unbound substances, enzyme-linked monoclonal antibodies specific for total IgE was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and a color developed in proportion to the amount of total IgE bound in the initial step. The reaction was stopped and the intensity of color was measured. The normal limit of total IgE was 100 IU/mL.

Statistical Analysis

Analysis of data was performed using the SPSS program, version 12. Data were expressed as mean ± standard deviation (SD) for parametric data, and as median and interquartile range (IQR) for non-parametric data, respectively. For comparison of the three groups, one-way analysis of variance (ANOVA) test was used for parametric data, and Kruskal-Wallis test was used for non-parametric data. Chi-square test was used to compare categorical data. A p-value of less than 0.05 was considered significant.

Results

This cross-sectional study included 75 patients with isolated allergic conjunctivitis who were recruited from a tertiary Egyptian outpatient clinic. The majority of patients [48 (64%)] had vernal conjunctivitis, 19 (25.3%) had perennial conjunctivitis and 8 (10.7%) had seasonal conjunctivitis. Age, disease duration and family history of atopy were all comparable between the three groups. Patients with perennial and vernal conjunctivitis were predominantly males. In each of the three groups, the majority of patients had elevated levels of total serum IgE. There was no statistically significant difference in the presence of elevated IgE levels between the three groups (Table 1).

In total, 32 patients had positive skin test reactivity to one or more common aeroallergens. The most prevalent aeroallergens were mites, pollens, and grass, followed by hay dust, molds, and tobacco (Figure 1).

Table 1. Characteristics of the studied groups of Egyptian patients with Allergic conjunctival disease

	Seasonal, n= 8	Perennial, n= 19	Vernal, n= 48	*P* value
Age (y)	15.0 ± 7.45	18.50 ± 10.55	15.11 ± 6.67	NS
Sex, n (%)				
Male	3 (37.5)	17 (89.5)	41 (85.4)	0.003
Female	5 (62.5)	2 (10.5)	7 (14.6)	
Disease duration (y)	4.0 (2.0, 13.25)	4.0 (2.0, 7.0)	5.0 (3.0, 7.75)	NS
Family history of atopy				
Positive, n (%)	2 (25.0)	9 (47.4)	24 (50.0)	NS
Negative, n (%)	6 (75.0)	10 (52.6)	24 (50.0)	
Total serum IgE				
Elevated, n (%)	5 (62.5)	14 (73.7)	32 (66.7)	NS
Normal, n (%)	3 (37.5)	5 (26.3)	16 (33.3)	

Age is presented as mean ± standard deviation; disease duration is presented as median (interquartile range); IgE, immunoglobulin E

P>0.05 in non significant (NS)

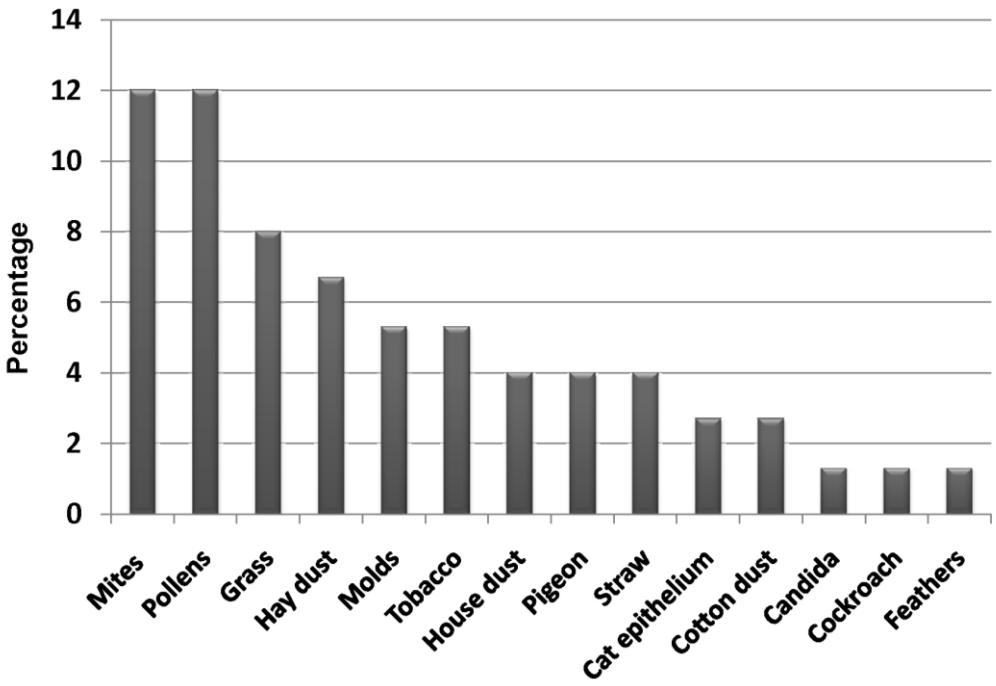

Figure 1. Common aeroallergens by skin prick test among the study population. According to the skin prick test, the most prevalent aeroallergens among the study population were mites, pollens, and grass, followed by hay dust, molds, and tobacco.

The majority of patients with seasonal and vernal conjunctivitis had negative skin test reactivity, whereas half the patients with perennial conjunctivitis had positive skin test reactivity (Figure 2). Results of skin test reactivity to common aeroallergens among the three groups are displayed in table 2. Pollens and grass were the most prevalent aeroallergens among patients with vernal conjunctivitis, whereas patients with perennial conjunctivitis had a positive skin test result predominantly for mites, tobacco, and house dust.

Skin test positivity for indoor allergens was more common among patients with perennial conjunctivitis (52.6%) than among patients with seasonal (25%) and vernal (16.7%) conjunctivitis, *P*=0.011. Outdoor allergen sensitization did not differ significantly between the three groups, *P*= 0.614 (Figure 3).

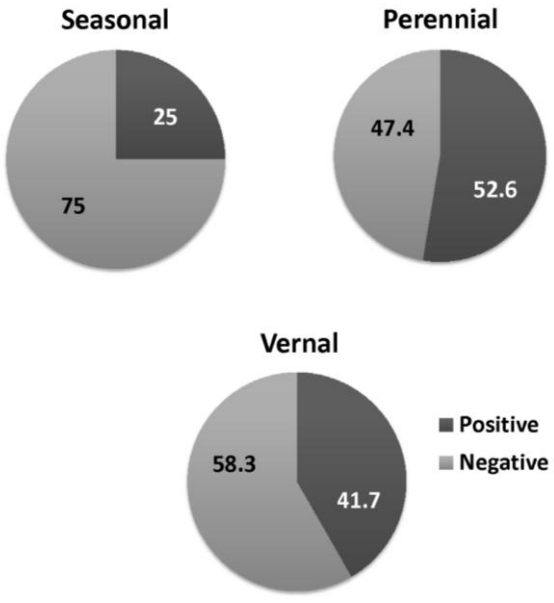

Figure 2. Skin prick test reaction among the 3 study groups. 75% of patients with seasonal conjunctivitis and 58.3% of patients with vernal conjunctivitis had negative skin test reactivity, whereas half the patients with perennial conjunctivitis had positive skin test reactivity (52.6%)

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Table 2. Results of skin test reactivity among the studied groups of Egyptian patients with Allergic conjunctival disease

Allergen, n (%)	Seasonal, n= 8	Perennial, n= 19	Vernal, n= 48	Total, n= 75
Mites	0	6 (31.6)	3 (6.3)	9 (12)
Pollens	0	1 (5.3)	8 (16.7)	9 (12)
Grass	0	0	6 (12.5)	6 (8)
Hay dust	1 (12.5)	1 (5.3)	3 (6.3)	5 (6.7)
Molds	0	1 (5.3)	3 (6.3)	4 (5.3)
Tobacco	1 (12.5)	3 (15.8)	0	4 (5.3)
House dust	1 (12.5)	2 (10.5)	0	3 (4)
Pigeon	1 (12.5)	1 (5.3)	1 (2.1)	3 (4)
Straw	1 (12.5)	0	2 (4.2)	3 (4)
Cat epithelium	0	1 (5.3)	1 (2.1)	2 (2.7)
Cotton dust	0	1 (5.3)	1 (2.1)	2 (2.7)
Candida	0	0	1 (2.1)	1 (1.3)
Cockroach	0	0	1 (2.1)	1 (1.3)
Feathers	0	1 (5.3)	0	1 (1.3)

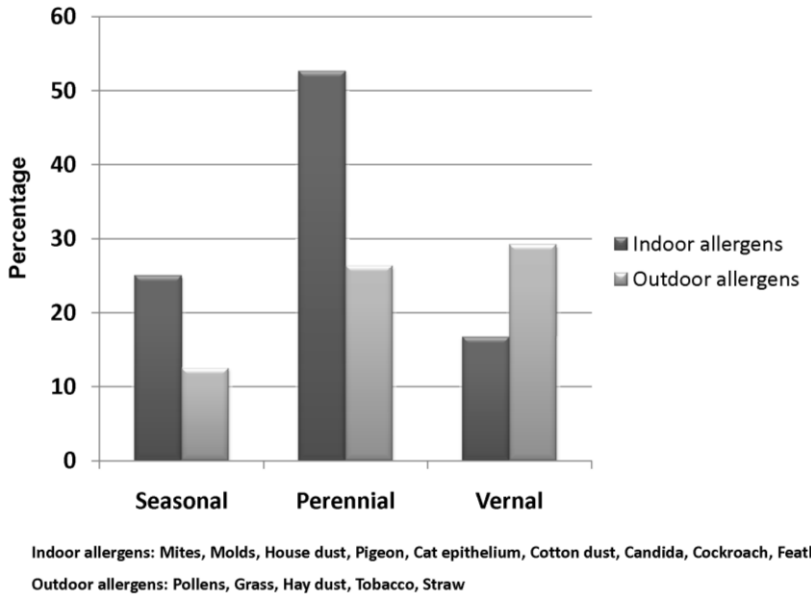

Figure 3. Skin prick test positivity to indoor and outdoor allergens among the 3 study groups. Skin test positivity for indoor allergens was more common among patients with perennial conjunctivitis (52.6%) than among patients with seasonal (25%) and vernal (16.7%) conjunctivitis. Outdoor allergen sensitization did not differ significantly between the three groups.

Discussion

The present study was conducted on 75 patients with allergic conjunctivitis, and showed that SAC, PAC and VKC accounted for 10.7%, 25.3 %, and 64 % of cases, respectively. Patients with PAC and VKC were predominantly males. In a study from a referral clinic at a University Hospital in Brazil, VKC accounted for 46% versus 8% for PAC in a consecutive case series (Belfort *et al.,* 2009).

Conversely, in a Japanese study on patients with ocular allergies, SAC was the most predominant form (81.2%), followed by perennial allergic conjunctivitis (10.6%) and VKC (3.8%) (Uchio *et al.,* 2008). The warm climate in Egypt may explain the relatively higher percentage of cases with VKC in our study, as VKC is known to be more prevalent in warm climates among male children (Leonardi *et al.,* 2012) (Ono & Abelson, 2005).

Skin testing is an important tool in identifying allergens that can cause allergic diseases. In the present study, skin prick test was positive in only 42.7% of patients. The majority of patients with SAC and VKC had negative skin test reactivity, whereas half the patients with PAC had positive skin test reactivity. An Italian study conducted on patients with VKC demonstrated a positive skin prick test result among 40% of patients (Leonardi *et al.,* 2008). Additionally, Ridolo *et al.* conducted a study on Italian patients suffering from allergic rhinoconjunctivitis, and demonstrated that 63.6% of patients had positive SPT reactivity to inhalant allergens (Ridolo *et al.,* 2007).

In the present study, the most prevalent aeroallergens among patients with allergic conjunctivitis were mites and pollens (12% respectively), followed by grass (8%) and hay dust (6.7%). A Brazilian study on 20 patients with VKC demonstrated hypersensitivity to at least one of the allergens in the majority of cases; all of them were sensitive to house dust mites, 60% to allergens derived from cat, 40% to allergens derived from dog, 13.3% to allergens derived from fungus, and 6.7% to allergens derived from feather (Augusto de Oliveira *et al.,* 2007).

The prevalence of allergic conjunctivitis was assessed in the third National Health and Nutrition Examination Survey (NHANES III), where all seasonal and perennial aeroallergens were examined. The population with ocular symptoms revealed a greater percentage of positive wheal reactivity to weeds (24.2%), 3 times the amount compared with grass (8.1%), mold, and indoor allergens (8.4%) (Singh *et al.,* 2010). Similarly, a recent Egyptian study showed that 62% of patients with VKC were sensitive to pollens, 19% to house dust, and 19% to more than one allergen (Mahdy *et al.,* 2012).

In the present study, the prevalence of SPT positivity to indoor allergens was significantly more common among PAC (52.6%) than among SAC (25%) and VKC (16.7%). Mite, as an indoor allergen, was the most frequently implicated aeroallergen for ocular allergy among patients with PAC (31.6%). On the other hand, outdoor allergen sensitization did not differ significantly between the 3 subgroups.

Elevated total serum IgE levels were observed among 62.5%, 73.7% and 66.7% of patients with SAC, PAC and VKC, respectively, with no statistically significant difference between them. Leonardi *et al.,* 2008 stated that although skin prick testing may be useful in detecting relevant allergens, it may be negative in a significant number of patients presenting with SAC. Furthermore, a study has demonstrated a poor correlation between systemic sensitization (as assessed by serum IgE) and ocular sensitization (as

assessed by IgE from the tear film), and showed that 35% of patients with VKC presented specific IgE only in tear samples. The authors suggested that the conjunctiva may be the only target tissue in some patients with allergic conjunctival disease (Leonardi *et al.*, 1990).

We conclude that aeroallergen sensitization is common in Egyptian patients with isolated allergic conjunctivitis. Skin prick test with aeroallergens should be included in the diagnostic workup of Egyptian patients with isolated allergic conjunctivitis as an easy, rapid and cheap tool to detect the common etiological aeroallergens, and so that these patients could be directed to avoid the implicated allergens. Future large-scale studies are required to assess the possible role of specific allergen immunotherapy as an adjuvant therapy in those patients to avoid the side effects and costs of pharmacotherapy commonly used in these conditions.

References

1. Arbes SJ Jr, Gergen PJ, Vaughn B, Zeldin DC (2007). Asthma cases attributable to atopy: results from the Third National Health and Nutrition Examination Survey. J Allergy Clin Immunol.; 120: 1139-1145.
2. Augusto de Oliveira L, Mallozi MC, Sole D, Freitas Dd, Sousa LB, Mannis MJ (2007). Are cutaneous hypersensitivity tests to inhalant allergens a severity marker for vernal keratoconjunctivitis? Arq Bras Oftalmol.; 70: 991-995.
3. Belfort R, Marbeck P, Hsu CC, Freitas D (2000). Epidemiological study of 134 subjects with allergic conjunctivitis. Acta Ophthalmol Scand. Suppl; (230): 38-40.
4. del Cuvillo A, Sastre J, Montoro J (2009). Allergic conjunctivitis and H1 antihistamines. J Investig Allergol Clin Immunol.; 19 Suppl 1: 11-18.
5. Fujishima H, Shimazaki J, Yang HY, Toda I, Tsubota K (1996). Retrospective survey of a link between cat and dog antigens and allergic conjuctivitis. Ophthalmologica.; 210: 115-118.
6. Leonardi A, Fregona IA, Gismondi M, Daniotti E, Carniel G, Secchi AG (1990). Correlation between conjunctival provocation test (CPT) and systemic allergometric tests in allergic conjunctivitis. Eye (Lond).; 4: 760-764.
7. Leonardi A, Motterle L, Bortolotti M (2008). Allergy and the eye. Clin Exp Immunol.; 153 Suppl 1: 17-21.
8. Leonardi A, Bogacka E, Fauquert JL (2012). Ocular allergy: recognizing and diagnosing hypersensitivity disorders of the ocular surface. Allergy.; 67: 1327-1337.
9. Li JT, Lockey RF, Bernstein IL, Portnoy JM, Nicklas RA (2003). Allergen immunotherapy: A practice parameters. Ann Allergy Asthma Immunol; 90: S1-40.
10. Mahdy RA, Nada WM, Marei AA (2012). Subcutaneous allergen-specific immunotherapy versus topical treatment in vernal keratoconjunctivitis. Cornea.; 31: 525-528.
11. Mantelli F, Lambiase A, Bonini S, Bonini S (2011). Clinical trials in allergic conjunctivits: a systematic review. Allergy; 66: 919-924.
12. McGill JI, Holgate ST, Church MK, Anderson DF, Bacon A (1998). Allergic eye disease mechanisms. Br J Ophthalmol.; 82: 1203-1214.
13. Ono SJ, Abelson MB (2005). Allergic conjunctivitis: update on pathophysiology and prospects for future treatment. J Allergy Clin Immunol.; 115: 118-122.
14. Ridolo E, Albertini R, Giordano D, Soliani L, Usberti I, Dall'Aglio PP (2007). Airborne pollen concentrations and the incidence of allergic asthma and rhinoconjunctivitis in northern Italy from 1992 to 2003. Int Arch Allergy Immunol.; 142: 151-157.
15. Singh K, Axelrod S, Bielory L (2010). The epidemiology of ocular and nasal allergy in the United States, 1988-1994. J Allergy Clin Immunol.; 126: 778-783.e6.
16. Takamura E, Uchio E, Ebihara N (2011). Japanese guideline for allergic conjunctival diseases. Allergol Int.; 60: 191-203.
17. Uchio E, Kimura R, Migita H, Kozawa M, Kadonosono K (2008). Demographic aspects of allergic ocular diseases and evaluation of new criteria for clinical assessment of ocular allergy. Graefes Arch Clin Exp Ophthalmol.; 246: 291-296.