

Identification of allergens in a selected group of asthmatics in Lebanon

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Abstract. Anti-allergen antibodies were searched for by an Enzyme Immunoassay in the sera obtained from 60 patients with a clinical diagnosis of asthma. Allergic rhinitis was also present in 28 patients. The diagnosis of asthma was based on clinical criteria that include history and clinical evidence of airflow obstruction. Ten potential allergens (Bermuda grass, Olive tree, Parietaria, Alternaria, Cat hair dander, Dog hair dander, Mite DPT, Mugwort, Birch tree and Timothy grass) common to the Mediterranean area, were utilized. Twenty-five of 60 specimens were seropositive. Mite DPT was the allergen identified in 16 of the 25 seropositive specimens. Six of the 25 seropositive specimens reacted with more than one allergen. This may be due to the existence of similar antigenic deter-

minant groups in the allergens used. There was no correlation between the occurrence of rhinitis in addition to asthma on one hand and the identified allergen on the other. Sixteen of the 25 seropositive patients were female. This observation is believed to be related to their occupation. Dog hair dander was identified as the allergen in 1 of the 25 seropositive patients. The low figure obtained was expected because keeping house pets is not a common practice in Lebanon. The 35 seronegative patients may belong to the intrinsic asthma group or the causative allergen in each case was not one of the 10 allergens used in this study. These results indicate that the house dust mite appears to be the most common allergen in the Lebanese asthmatic group studied.

Key words: Animal dander, ELISA, Miten, Pollen

Introduction

Asthma or Hyperactive airway disease is characterized by bronchial hyper-responsiveness to specific or non specific stimuli. Such stimuli include allergens, tobacco smoke, cold air, exercise and a variety of chemicals. Two types of asthma have been described: extrinsic and intrinsic. The majority of asthmatics belong to the extrinsic group which occurs in children and adults, and is related to allergen exposure, IgE and genetic factors. Intrinsic asthma on the other hand, is less common, it is seen mainly in adults and appears to be IgE independent [1, 2].

A number of allergens have been implicated as causative agents of allergy in genetically predisposed individuals [3]. Respiratory allergens include mainly a number of wind-borne pollens which vary in geographic location and are shed at different periods of time, and in-door allergens such as house dust mite, animal danders and molds. Because of their size (17–85 µm), wind-borne pollens usually localize mainly in the upper airways and are more likely to cause rhinitis rather than asthma. On the other hand, a number of in-door allergens have a smaller size and are consid-

ered to be important causes of allergic asthma. The allergens of the house dust mite which are mainly proteases found in mite feces represents the most clinically important allergen in countries where causative allergens of asthma were identified [3–5].

To the best of our knowledge no study hitherto has been done to identify the causative allergen(s) in Lebanese asthmatics. In this study we attempted to do so using a panel of 10 allergens which included various pollens and in-door allergens.

Materials and methods

Subjects

Sixty patients with a diagnosis of allergic asthma. They included 29 males and 31 females, age ranged between 12 to 83 years, the mean age was 45.2 years. Most of the patients had in-door jobs (Table 1). Inclusion of subjects in this study was based on a clinical diagnosis of asthma irrespective of age and sex. The clinical criteria included history and evidence of airflow obstruction. Spirometry was done in 12, all of which showed evidence of airflow obstruction. The

Table 1. Occupation of seropositive and seronegative patients

Occupation	Number of seropositive		Number of seronegative	
	Males	Females	Males	Females
Housewife	–	13	–	8
Airline stewardess	–	2	–	–
Carpet salesman	1	–	2	–
Secretary-clerk	2	1	2	1
Student	3	–	7	4
Restaurant employee	1	–	1	–
Tobacconest	1	–	–	–
Farmer	1	–	–	–
Painter	–	–	1	–
Ballet dancer	–	–	–	1
Tailor	–	–	1	1
Retired	–	–	6	–
Total	9	16	20	15

other 48 had wheezing detected on physical examination with a reduction in peak flow measurement. All patients who had a substantial smoking history were excluded. None had evidence of chronic bronchitis, emphysema, bronchiectasis or cystic fibrosis. All patients had shown symptomatic response to inhaled bronchodilator therapy, on which they were maintained. Rhinitis, in addition to asthma was diagnosed in 28 of the 60 patients.

Serum specimens

Blood from patients was collected in plain vacutainers, allowed to clot, and centrifuged to separate the serum. The serum was used in the assay.

Allergy screen

The Enzyme Immunoassay, CIS Biointernational was used and the manufacturers procedure was followed. Briefly, 10 different allergen-bound paper pads and one control pad which were attached to a plastic dipstick was used. The allergens were Bermuda grass, Olive tree, Parietaria, Alternaria, Cat hair dander, Dog hair dander, Mite DPT, Mugwort, Birch tree and Timothy grass. The dipstick containing the paper pads was inserted in 1 ml of serum to be tested and incubated for 14 hours to allow binding of allergen-specific IgE. The dipstick was then washed with tap water for 30 seconds and placed in a tube containing 1 ml of enzyme labeled antibody for 30 minutes. The dipstick was washed again with tap water and placed in a test tube containing the substrate solution for 30 minutes. The dipstick was blot dried and the reflectance of each pad was determined using a Quidel Reflectance Analyser.

The results were graded as follows:

Classification	Reflectance	Level of specific IgE
0	0–29	Very low
1	30–50	Low
2	51–100	Moderate
3	101–150	High
4	> 151	Very high

Mite, Alternaria, Bermuda grass, Timothy grass, Parietaria and Olive tree are native to Lebanon. Plants related to Mugwort and Birch tree, namely, *Artemesia monosperma* and *Alnus Orientalis*, respectively, are native to Lebanon [6, 7]. Some homes in Lebanon keep pets such as dogs and cats but it is not a common practice.

Results

The results obtained are summarized in Table 2. Twenty five of 60 specimens (41.7%) were seropositive. The age range of the seropositive individuals was 12–83 years (mean 44 years, sd = 19.6). The majority of the seropositive serum specimens reacted with Mite DPT (16 of 25 specimens). The reaction was graded (reflectance) as very high in 3, high in 6, medium in 4 and low in 3. In as much as the reactions to other allergens are concerned, 2 specimens reacted with Parietaria, 1 with Dog hair dander, 1 with Mugwort, one with Bermuda grass and one with Birch tree. Serum specimens reacting with more than 1 allergen were also observed. One specimen reacted with Bermuda and Timothy grass, 2 reacted with Mugwort and

Table 2. Reflectances and specific IgE levels of seropositive specimens

Patient No.	Reactive allergen	IgE reflectance units	Level of specific IgE ^a	Sex	Age (year)
0	None	—	—		
1	Mite DPT	33	Low	F	67
2	Mite DPT	132	High	F	39
3	Mite DPT	60	Medium	M	39
4	Mite DPT	63	Medium	M	25
5	Mite DPT	35	Low	F	37
6	Mite DPT	199	Very high	M	12
7	Mite DPT	105	High	M	17
8	Mite DPT	108	High	F	63
9	Mite DPT	126	High	M	30
10	Mite DPT	110	High	F	83
11	Mite DPT	97	Medium	F	23
12	Mite DPT	33	Low	F	26
13	Mite DPT	153	Very high	F	33
14	Bermuda grass	61	Medium	F	66
	Timothy grass	59	Medium		
15	Dog hair	49	Low	F	22
16	Mugwort	37	Low	M	35
17	Mugwort	45	Low	F	72
	Birch tree	42	Low		
18	Mugwort	35	Low	F	73
	Birch tree	41	Low		
19	Bermuda grass	44	Low	M	34
20	Birch tree	64	Medium	M	59
21	Parietaria	29	Low	F	64
22	Parietaria	76	Medium	F	49
23	Mite DPT	118	High	F	30
	Birch tree	30	Low		
24	Mite DPT	197	Very high	F	61
	Olive tree	34	Low		
	Bermuda grass	37	Low		
25	Mite DPT	54	Medium	M	40
	Timothy grass	35	Low		

^a Refer to Materials and methods for the interpretation of very high, high, medium and low.

F = female, M = male. 16 females and 9 males were seropositive.

Age range of seropositive females: 22–83 years (mean = 50.2, s.d. = 19.8, n = 16).

Age range of seropositive males: 12–59 years (mean = 32.9, s.d. = 13.1, n = 9).

Age range of seronegative females: 17–82 years (mean = 48.4, s.d. = 19.9, n = 15).

Age range of seronegative males: 20–82 years (mean = 43.5, s.d. = 22.9, n = 20).

Birch tree, 1 with Birch tree and Mite DPT, 1 with Mite DPT and Timothy grass and 1 with Mite DPT, Olive tree and Bermuda grass. It can be noted from Table 2 that in these specimens the reflectance was highest with Mite DPT suggesting that it was the major allergen in these patients.

The results obtained in patients with asthma alone and those with asthma and rhinitis are given in Table 3. It can be noted that there is no correlation between the type of allergen giving a positive reaction and the clinical condition of the patient. However, those reacting with Mite DPT in the asthmatic group were more

than those in the Asthmatic-rhinitis group (11/15 versus 5/10). In both groups there was reactivity to plant allergens. Sixteen of 25 seropositive patients were female. Their age range was 22–83 years (mean = 50.2 years, sd = 19.8). Ten of which the allergen identified was Mite DPT and 1 dog hair dander. In the 9 seropositive males, the allergen identified in 6 was Mite DPT and in the other 3 it was a plant allergen. Their age range was 12–59 years (mean = 32.9 years, sd = 13.1).

Table 3. Number of seropositives in asthmatics alone and asthmatics with rhinitis

Allergen	Number of patients with		Total
	Asthma	Asthma + Rhinitis	
Mite DPT	9	4	13
Mite DPT + Birch tree	–	1	1
Mite DPT + Timothy grass	1	–	1
Mite DPT + Olive tree + Bermuda grass	1	–	1
Birch tree	–	1	1
Bermuda grass	–	1	1
Parietaria	–	2	2
Mugwort	–	1	1
Mugwort + Birch tree	2	–	2
Bermuda grass + Timothy grass	1	–	1
Dog hair dander	1	–	1
Total	15	10	25

– There were 32 asthmatics and 28 who had rhinitis in addition to asthma.

– Alternaria and cat hair were non-reactive.

Discussion

Type I allergic reactions are caused by the production of allergen specific IgE antibodies which bind to Fc receptors on mast cells and basophils. The allergen binds to the cell-bound IgE causing the cross linking of receptors which leads to intracellular signals that results in degranulation and liberation of preformed and newly synthesized vasoactive substances. These substances cause smooth muscle contraction, increased vascular permeability and edema. The signs and symptoms that result depend on the anatomical site of the reaction. Reactions occurring in the upper airways result in rhinitis and those in the lower airways, asthma.

Out of the patients that were seropositive, the identified allergen in the majority of cases was Mite DPT. This result concurs with those reported elsewhere [4, 5]. Dust mite were first implicated in asthma by Dekker in 1928 [8]. They are small sightless arthropods. They grow best at a relative humidity of 70–80% and a temperature of 21 °C. Beirut, the capital of Lebanon is at sea level and fulfils the growth requirements of mite. Moreover, home furniture usually include carpets, mattresses, pillows and blankets which are usually made of cotton or wool, all of which accumulate dust, mites and mite remnants. It is interesting to note that an old tradition in Lebanon was to send asthmatics to dry areas in mount Lebanon where they would get symptomatic relief. It is postulated, but not scientifically established that such areas have a low prevalence of mite. In support of this view is a French study where it was demonstrated that there was a lower prevalence of asthma in individuals living at high altitudes as compared to those at low altitudes [9].

Some of the seropositive patients were plant allergen-positive. Although pollen size is an obstacle for localization in the lower airways, it is plausible to assume that pollen can be broken into smaller particles in the upper airways leading to their localization in the lower airways. This may be the case in those patients who have both rhinitis and asthma and are reactive with plant allergen. Moreover, although the pollen grain is believed to be the primary source of allergen, some reports indicated that allergens may be associated with submicronic particles [3].

Reactions with more than one allergen were observed in 6 patients. Mite DPT appeared to be the major allergen in 3 of the 6 patients. It is known that allergens have several different antigenic determinant groups and IgE class antibodies to each may be produced. Cross reactivity among phylogenically related and unrelated species will occur if they have common antigenic determinant groups.

To be of significance in causing allergy, an allergen must occur over a prolonged period of time. The majority of the patients in whom the allergen was identified were female. In most countries allergic sensitization tends to be less common in older ages. However, younger age in this study did not seem to influence the female preference. The mean age of the seropositive females was 50.2 years and that of the seropositive males was 32.9 years. It may be related to the fact that the females in this study spend most of their time indoors where they are in contact with allergens for prolonged periods. The seropositive males with the exception of one had in-door jobs.

Dog hair dander was identified as the allergen in only one of the seropositive patients. This is because keeping pets such as dogs and cats is not a common

practice in Lebanon. In the USA dogs are present in nearly one third of all homes [2]. In Los Alamos, NM, nearly 75% of the families have in-door pets, and dog and cat sensitization correlate strongly with asthma [10].

In 35 of 60 patients (58.3%) the allergen was not identified. This could be because the causative allergen was missing from the panel used or some of the patients belonged to the intrinsic asthmatic group which is believed to be IgE independent. Information concerning the prevalence of IgE sensitization in the Lebanese population is not available. However, the prevalence of asthma symptoms among 13–14 year old school children in Beirut, based on wheezing and whistling during the last 12 months, as determined through the International Study of Asthma and Allergic Disorders in Children (ISAAC) was 14.8% (Ramadan, unpublished data). The prevalence of allergic rhinitis in the group studied was 35%.

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