Sensitization to Aeroallergens in Patients with Respiratory Allergies Based on Skin-Prick Test Results

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Abstract

**Background:** The aim of this study was to identify the most common aeroallergens in patients with asthma and rhinitis.

**Methods:** The study enrolled 102 participants including 64 patients with respiratory allergies (among them 15 were clinically diagnosed as asthma patients, 41 with rhinitis, 8 were both) and 38 healthy controls. All of participants were subject of skin prick tests (SPT) with series of common allergenic extracts. Sera from all participants were tested for total IgE and eosinophil count. To measure airflow limitation and reversibility in asthma patients the pulmonary function testing were carried out.

**Results:** M/F ratio was 1:1.6 in patients and 1:0.7 in control group with mean age 28.88 year (SD 13.16; range 6 – 55 year) and 20.47 respectively (SD 1.16; range 19-23 year). The most common risk factors in these patients were total IgE more than 100 IU/ml, eosinophils above 4% and positive family history of atopy. Skin prick testing results showed prevalence rates for allergen groups in this manner: house dust mites 81.3 %, pollens 57.8 %, animal dandruff 12.5% and moulds 4.9%. Polysensitization was common in 51.6% of all sensitized patients being positive to more than one group of allergens.

**Conclusion:** House dust mites are the main sensitizing allergens among our allergic patients as well as healthy controls. Next in importance, in all participants, are grasses. This pattern of prevalence was expected based on herbal geography, climate and specially lifestyle. It was also compatible with the results from studies carried out in places with the same habitat.

**Keywords:** Aeroallergens, Asthma, Eosinophil, Immunoglobulin E, Rhinitis, Skin prick test

Introduction

“Half of the European Union’s population will suffer from an allergic disease within eight years, according to experts” (1). Clinical manifestation of allergic diseases is extremely broad and involves a number of systems. Respiratory manifestations, as allergic asthma and rhinitis, are most commonly encountered, but frequently associated with them is conjunctivitis. In overall they represent a raising global health problem of epidemic proportions (2-4).

There are to blame several factors for these disorders, including genetic predisposition and environmental factors (5). Studies of twins, molecular studies of genome, genetic linkage of β-2 adrenergic therapy response along with “hygiene hypothesis” are the best predictors of clinical manife-
station of allergic symptoms (6-10). As an allergic response begins with sensitization to an antigen, in atopic individuals, allergen exposure results in binding to IgE, activation of mast cells and releasing of preformed and newly synthesized mediators (11-13). These effects, along with neuronal parasympathetic reflexes result in the clinical syndrome of immediate and late phase allergic response, which includes well known symptoms of coughing, wheezing, nasal itching, sneezing and discharge (14). Although, upper and lower, respiratory symptoms develop for other reasons, allergic etiologies are strongly related to inhaled allergens (15). Dust mites, pollens, moulds and animal dandruff are well known elicitors of immune-mediated response. When will someone experience these symptoms depends on genetic background, sensitization, family size, climate, allergen burden and lifestyle (16-18). In industrialized countries with a Western lifestyle, sensitization to perennial aeroallergens is strongly associated with asthma, whereas sensitization to seasonal aeroallergens is closely related to allergic rhinitis (19). Epidemiological, pathophysiological and clinical studies conducted in the last decade have contributed to the new widely accepted united airways concept (1).

The objective of this study was to investigate the association between skin test reactivity to aeroallergens and asthma or rhinitis in favour of better preventive strategies.

Material and Methods

We included only patients with physician-diagnosed asthma and rhinitis who reacted positive to skin prick tests. They were all referred to the Allergy & Clinical Immunology Department, outpatient service, UCC Pristina, between July 2005-2006. We excluded subjects younger than 5 and older than 56 years (because of decreased reactivity of the skin to histamine, in infants and elderly patients), those with dermatographism, patients unable to discontinue antihistamines and corticosteroids as well as pregnant women. Together with allergic patients we tested 38 healthy volunteers, Faculty of Medicine students, as control group (they were selected according to answers as not having, nor ever had symptoms of respiratory allergies: coughing, sneezing, itching and nose discharge).

Procedures

Protocol for the research project has been approved by the Faculty of Medicine-Teaching-Science Council and was conform to the provisions of the Declaration of Helsinki (paragraph 11, 13, 15, 16, 20). Informed written consent was obtained from all participants who were than included in research.

To each subject was handed over a study questionnaire requesting demographic data, family history of atopy and respiratory symptoms. In addition, data about CBC results, eosinophilic count, total IgE level, response to SPT reaction, spirometry and radiographic images (chest, head and neck for evaluating possible structural abnormalities or to help detect complications or co morbid conditions, such as sinusitis or adenoid hypertrophy) were appended. CBC results were measured by standard methods.

Eosinophil count

Eosinophils were counted from peripheral blood sample stained with May Grünewald and Giemsa, and examined under light microscope (normal range 1-4%).

Measurement of serum total immunoglobulin E levels

Serum total IgE levels were determined by IRMA (Immunotech product France) in Endocrinology laboratory, Institute of Physiology, UCC Pristina and values more than 100 IU/ml were considered raised.

Allergy skin testing

All participants were subjected to SPT with test kit G (Allergopharma product, Reinbeck Germany); allergenic extracts included 4 groups of allergens: pollens, house dust mites, animal dander and moulds, along with positive control-histamine (1mg/ml) and negative-saline. The allergenic ex-
tract was placed on to the volar surface of forearm and introduced into the epidermis with sterile lancet (1 mm depth), new for each allergen. 15 minutes later, for each subject, both diameters of skin reaction were recorded and SPT was considered positive if diameter were ≥3 mm compared with control.

**Spirometry**
The pulmonary function tests, were performed with Flow screen version 2.10d (Erich Jaeger GmbH, Wurzburg Germany), in asthma patients, in seated position with nose clips (to prove allergic component of obstruction). As many as three attempts were made by each participant to obtain the best effort FEV$_1$/FVC and the highest FEV$_1$ value. Spirometry was repeated 10 minutes after subjects inhaled 100 µg (two puffs) of salbutamol using a spacer device. The reversibility of airflow obstruction was measured when >12% improvement in FEV$_1$ (or FVC) and absolute improvement of >0.2 L, according to the ATS criteria.

**Statistical analysis**
All data were presented in tables and graphics analyzed with many statistical parameters, including structure index, arithmetic MEAN, SD, t-test of MEAN, t-test of proportion, chi-test, relative risk and fi-test of correlation.

**Results**
This study included 102 participants: 64 patients with respiratory allergies, who reacted to at least one allergen, and 38 healthy volunteers, Faculty of Medicine students, as control group. Among patients 15 (23.4%) were asthmatics, 41 (61%) with rhinitis and 8 (12.5%) were both. M/F ratio was 1/ 1.6 in patient and 1/ 0.7 in control group, with mean age 25.88 ±13.16 (range 6-55 years) and 20.47 ±1.16 respectively (range 19-23 years) (Table 1).

**Table 1:** Subject characteristics of allergic patients vs. controls according to age and disease (n = 102)

<table>
<thead>
<tr>
<th>Age group (yr)</th>
<th>Asthma (n = 15)</th>
<th>Asthma/ Rhinitis (n = 8)</th>
<th>Rhinitis (n = 41)</th>
<th>Allergic patients (n = 64)</th>
<th>Controls (n = 38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-15</td>
<td>6 (40%)</td>
<td>3 (37.5%)</td>
<td>5 (12.2%)</td>
<td>14 (21.9%)</td>
<td>-</td>
</tr>
<tr>
<td>16-35</td>
<td>6 (40%)</td>
<td>1 (12.5%)</td>
<td>26 (63.4%)</td>
<td>33 (51.6%)</td>
<td>38 (100%)</td>
</tr>
<tr>
<td>36-55</td>
<td>3 (20%)</td>
<td>4 (50%)</td>
<td>10 (24%)</td>
<td>17 (26.6%)</td>
<td>-</td>
</tr>
<tr>
<td>*</td>
<td>21.07</td>
<td>29.75</td>
<td>26.88</td>
<td>25.88</td>
<td>20.47</td>
</tr>
<tr>
<td>**</td>
<td>14.90</td>
<td>20.03</td>
<td>10.06</td>
<td>13.16</td>
<td>1.16</td>
</tr>
<tr>
<td>Range (years)</td>
<td>6-55</td>
<td>6-53</td>
<td>7-46</td>
<td>6-55</td>
<td>19.23</td>
</tr>
</tbody>
</table>

*MEAN, ** SD

Elevated serum total IgE levels were measured in 80% of asthma, 63.4% of rhinitis and 62.5% of asthma rhinitis patients, in contrast to control group 21.1%. Patients with asthma and asthma rhinitis had eosinophil count more than 4%, comparing to rhinitis and controls as well (73.3% vs.62.5% vs. 34.1% vs. 7.9%) (Table 2). When evaluating positive family history of atopy we found 78.2% of patients and 21% of controls to have relative risk of developing allergies as two fold as much (Chi-test 10.77; P<0.001; RR 2.128; CI 95% 1.292-3.505). After performing SPT to all participants, we found reaction to allergens in this manner: 81.3% of patients reacted to house dust mites (86% of asthma, 82.9% of rhinitis, 62.5% of asthma rhinitis patients and 10% of controls).
Table 2: Comparison of IgE level and eosinophils in allergic and control group

<table>
<thead>
<tr>
<th>Allergic subjects</th>
<th>Asthma (n = 64) (%)</th>
<th>Asthma/rhinitis (n = 8) (%)</th>
<th>Rhinitis (n = 41) (%)</th>
<th>Controls (n = 38) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgE level (IU/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 100 IU/ml</td>
<td>3 (20.0)</td>
<td>37. 40*</td>
<td>25. 75**</td>
<td>28. 78**</td>
</tr>
<tr>
<td></td>
<td>12 (80.0)</td>
<td>5 (62.5)</td>
<td>26 (63.4)</td>
<td>8 (21.1)</td>
</tr>
<tr>
<td>&gt; 100 IU/ml</td>
<td>383. 25*</td>
<td>148. 88**</td>
<td>137. 38**</td>
<td>44. 86**</td>
</tr>
<tr>
<td></td>
<td>4 (26. 7)</td>
<td>3 (37. 5)</td>
<td>27. (65. 9)</td>
<td>35 (92. 1)</td>
</tr>
<tr>
<td>Eosinophyls (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 4 %</td>
<td>3. 50*</td>
<td>2. 33*</td>
<td>2. 41*</td>
<td>1. 57*</td>
</tr>
<tr>
<td></td>
<td>1. 00**</td>
<td>1. 53**</td>
<td>1. 08**</td>
<td>1. 04**</td>
</tr>
<tr>
<td>&gt; 4 %</td>
<td>11 (73, 3)</td>
<td>5 (62.5)</td>
<td>14 (34. 1)</td>
<td>3 (7, 9)</td>
</tr>
<tr>
<td></td>
<td>7. 73*</td>
<td>7. 20*</td>
<td>8. 36*</td>
<td>7. 33*</td>
</tr>
<tr>
<td></td>
<td>2. 87**</td>
<td>3. 83**</td>
<td>3. 56**</td>
<td>1. 53**</td>
</tr>
</tbody>
</table>

*MEAN, ** SD.

No differences between genders were seen but slight decrease in positive reaction to mites with growing ages. Positive reaction to pollen allergens were recorded in 57.8% of patients and 1.6% of controls, with grasses representing the main cause of sensitization (46.7%, 37.5% and 51.2% respectively) (Fig. 1). We found no differences between genders but positive reaction to pollens with growing ages (6-15 years 42.9% vs. 16-35 years 60.6% vs. 36-55 years 76.5%). To animal dander reacted 12.5% of patients and 7.9% of controls with golden hamster representing main allergen in asthma patients (20%) but cat and dog in rhinitis patients (7.3%). We also found M/F differences (8.0% vs. 15.4%, t-test = 6.54, P<0.01) with positive reaction in age group 36-55 years. Sensitization to moulds is recorded in rhinitis patients only (4.9%); they were females and age group 35-55 years. Polisensitization was common in 51.6% of patients reacting to more than one group of allergens. Based on duration of symptoms we found seasonal pattern in 19.5%, perennial pattern in 24.4% and 56.1% had perennial pattern with seasonal exacerbation.

Fig. 1: Patients’ assessments on sensitization to pollen (grasses, weeds and trees) according to disease group (rhinitis, asthma/rhinitis and asthma only)

**Discussion**

According to International Study of Asthma and Allergies in Childhood (ISAC) there is a worldwide prevalence of allergic rhinitis and asthma
(20), it varies throughout the world and is associated with the 'westernized' lifestyle (1, 21). On the other hand, scientific studies conducted in the last decade, have contributed to the concept of one air way-one disease (21). In Western Europe, 10-15% of the general population has asthma symptoms (80-90% of allergic asthma have concomitant rhinitis symptoms), while around 20% of people suffer from allergic rhinitis (40% of them have lower airways involvement) (1, 21). Nevertheless, studies suggest that seasonal rhinitis (hay fever) occurs in 20% of the population, perennial in 40% and mixed in 40% (22). In our study 56.1% of AR patients had perennial symptoms with seasonal exacerbation. Our findings are also in agreement with the results from most studies of adults showing that rhinitis is more prevalent in women than in men (70.7%). As for asthma symptoms they were in 33.3% seasonal, 26.7% perennial and 40% were both. There is a statement that excessive amounts of the antibody immunoglobulin E (IgE) and positive family history of atopy are the risk factors for development of respiratory allergies (23). In our study 80% of asthma and 62.5% of asthma rhinitis and rhinitis patients had raised total serum IgE levels (MEAN=316.68 vs. 200.71 vs. 194.59 IU/ml) in respect to control group (MEAN=62.2 IU/ml) with statistical significance (P< 0.001). In overall 67% of patients had total serum IgE above 100 IU/ml and is compatible with similar results reported in Teheran from Farhoudi et al. (71%) (24). We found positive family history of atopy in 78.2 % of patients, in contrast to control group (47%), which provides the relative risk for developing allergies as two fold as much, and this also comply with the results found in Iran (24). We found raised eosinophil count to be concomitant of asthma in respect to rhinitis patients and controls as well (MEAN= 6.60 vs. 4.44 vs. 2.03; P<0.001). Sensitization to allergen has been shown to be one of the strongest determinants of asthma and rhinitis, and individuals with a predisposition for atopy are at higher risk (25).

Of all known common allergens, the house dust mite is known to be strongly implicated as a potential cause of asthma (26, 27). Among our asthma patients 86.7% reacted to D. pter and this comply with the results reported from Puerto-Rico (61.6%) (28), California (53%) (29), Serbia and Montenegro (21%) (30) and one in China (31). Our results are not compatible with similar studies from Ezhampuzhe in Kuvajti (32) and Farhoudi in Iran (33) because of dry climate and herbal geography. Pollens are well known elicitors of allergic rhinitis in Ankara (88.8%) (34), Shiraz and Karaj city (24, 35), but in our study and only 63.4% of rhinitis patients reacted to pollens and 82.9% reacted to house dust mites (D. pter. 80.5%), which is compatible with the similar studies in Thailand (36), Singapore (37) and Mexico city (38), also expected because of lifestyle and climate. According to positive skin prick-test reactions we found D. pter. as main cause of respiratory allergies in our patients with 80.5% of cases; grasses/cereals among pollen allergens with 48.4%; golden hamster with 20% and cladospor/aspergillus with 4.9%.

We found no differences between genders but raised reaction to pollens with growing ages (6-15 years 42.9% vs. 16-35 years 60.6% vs. 36-55 years 76.5%) and slight reduction in reaction to house dust mites (6-15 years 85.7%, 16-35 years 81.8%, 35-55 years 76.5%). Polysensitization was common with 51.5% reacting to more than one group of allergens with combination house dust mites/pollens as most abundant. These results certify that mites are the main cause of sensitization and pollens of polysensitization (39). Perennial pattern with seasonal exacerbation was seen in 53.1% of all patients.

**Conclusion**

Our results indicate a high frequency of sensitization to house dust mites in both patients (asthma and AR) and healthy controls, followed by grasses, as major pollen allergen in seasonal exacerbations. Physical measures and changing lifestyle to reduce dust mite allergen levels may improve respiratory symptoms in our patients with respiratory allergies. Providing a pollen counter may result in symptomatic improvement in polysensitized patients.
Ethical Considerations

Ethical issues (including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgment

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References


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