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The pattern of allergic sensitization by the skin prick test and immunoblotting method among patients with atopic dermatitis in 2018

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Abstract

Background: Atopic dermatitis (AD) is a common allergic disorder. Detection of responsible pathogenic allergens in AD patients by reliable methods has a fundamental role in the prevention, management, and treatment of AD. This study was conducted to determine the most common allergens by the skin prick test (SPT) and immunoblotting among AD patients referring to an allergy clinic in Birjand City, Iran.

Methods: The presence of AD was confirmed by an expert allergist. Serum levels of total and specific immunoglobulin E (sIgE) against 30 food and inhalant allergens were evaluated by a commercial immunoblotting kit (AlleisaScreen).

Results: The skin prick test was performed by a battery of 17 allergens. In total, 34 AD patients (mean age, 28.76 ± 17.36 years; range, 1-60 years; F/M ratio: 0.88) were enrolled in this study. The sensitization rates to at least 1 fungus, pollen, food, or indoor allergen by the immunoblotting method were 32.35%, 61.76%, 52.94%, and 47.05%, respectively. The most prevalent allergens were ragweed (52.94), Olive tree (41.16), Eucalyptus (35.29), date palm (35.29), and grass mix (32.28).

Conclusion: The study found that 85.29% of the studied population were sensitized to at least 1 allergen. Pollens and date palms were the most common allergens among AD patients, but the pattern of sensitization in SPT and immunoblotting was not exactly similar. Detection of allergens to which patients are sensitized and avoidance can help in the management of the disease and its symptoms.

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Introduction

Atopic dermatitis (AD) is a chronic inflammatory disease of the skin with substantial morbidity and quality of life impairment (1). It can affect up to 30% of children and 10% of adolescents. Its incidence is still rising, particularly in developing countries (2-4).

Exposure to environmental allergens is considered a predisposing factor that can contribute to the sensitization of the immune system and the production of specific immunoglobulin E (sIgE) (5). Therefore, identifying the relevant allergens that trigger symptoms is essential for proper prevention, diagnosis, and management of the disease, particularly in the case of immunotherapy (6).

Considering the prevalence of AD and the side effects of medication used for the treatment of AD, as well as the identification of causative allergens in the management of AD, the purpose of this study was to determine the most common allergens by the skin prick test (SPT) and immunoblotting among a group of patients with AD in Birjand City, Iran.

Methods

The study population was selected from AD patients who were referred to an allergy clinic in Birjand City, Iran, in 2018. Demographic data were collected by a questionnaire, and the presence of AD was confirmed by an expert allergist based on history and physical examination. Patients with pregnancy and serious health problems (such as acute infections, autoimmune disorders, malignancies, or addiction) were excluded from the study. The study was approved by the Ethics Committee of Birjand University of Medical Sciences (Ir.bums.REC.1396.229), and written informed consent was obtained from all participants or their parents.

The skin prick test was performed by an expert allergist according to a standard method (7) with a battery of 17 allergens (Greer Labs Inc, Lenoir, NC, USA; Table 1), as well as histamine and glycerol-saline as positive and negative controls, respectively. The mean wheal size was documented after 15 minutes, and SPT was considered positive if the mean wheal size was 3 mm larger than the negative control (8).

After performing the SPT, 5 mL of venous blood was taken immediately, and serum was separated by centrifugation and stored at -20 until analysis. Serum total IgE was measured by a commercial ELISA kit (Euroimmun AG, Lubeck, Germany). According to the kits' manual, IgE higher than 100 IU/mL in adults and higher than 200 IU/mL in children is considered a high IgE level. The level of serum sIgE was measured by a commercial immunoblotting kit (AlleisaScreen, Mediwiss Analytic GmbH, Moers, Germany) for 30 different inhalants and food allergens according to the kit's protocol (Table 1). After completion of the test procedure, the strip was scanned, and the kit's software revealed the quantitation of sIgE for each allergen based on the density of the individual allergen band. The results classified as follows: 0 (< 0.35 kU/L), 1 (0.35-0.7 kU/L), 2 (0.7-3.5 kU/L), 3 (3.5-17.5 kU/L), 4 (17.5-50 kU/L), 5 (50-100 kU/L), and 6 (> 100 kU/L). Subjects were considered positive if the class of sIgE was 2 or higher.

SPSS version 18 (SPSS Inc., Chicago, USA) was used for data analyses. Comparison of sensitization to allergens between the methods was done using the chi-square test and Fisher's exact test. Agreement between AlleisaScreen and SPT was assessed by kappa (κ) statistics. P values less than 0.05 are considered statistically significant.

Results

A total of 34 AD patients (mean age, 28.76 ± 17.36 years; range, 1-60 years; F/M ratio: 0.88) were enrolled in this study.

The mean serum total IgE was 292.2 ± 322.1 IU/mL, and 67.6% of patients had a high level of total IgE. The mean of total IgE levels was lower in men than in women (272.6 ± 187.7 and 309.5 ± 411.8), but the difference was not significant.

In the immunoblotting method, 85.29% of the cases were sensitized to at least 1 allergen. The total rate of sensitization to fungi, pollen, food, or indoor allergens (house dust mite [HDM], cockroach, cat, dog, and latex) was 32.35%, 61.76%, 52.94%, and 47.05%, respectively. In the case of SPT, 67.64% of the study population were sensitized to at least 1 allergen. Table 1 shows the frequency of sensitivity to individual allergens by immunoblotting and SPT methods.

Table 2 shows the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and kappa value of immunoblotting in comparison with SPT. The sensitivity of the immunoblotting was slightly higher for aeroallergens (71.5%) than food allergens (66.7%), while the specificity was higher for food allergens (95.6%) than aeroallergens (85.7%).

Discussion

In this study, 85.29% of the study population were sensitized to at least 1 allergen by the immunoblotting method. In the study conducted by Moghtaderi et al. in Shiraz, Iran, the sensitivity rate to local allergens was approximately 74.5%, which is consistent with our findings (9). In another research by Bonyadi et al., which was conducted on individuals with AD in the northwestern part of Iran, 91% of patients had positive sIgE to at least 1 allergen (10). Consistent with these studies, the prevalence of sensitization to at least 1 inhalant allergens in children with AD was 64.7% in the Sybilski study (11). The sensitivity rate in another

study conducted by Moghtaderi et al. in Shiraz (which evaluated food allergens by the immunoblotting method) was 51%; the rate was lower in comparison with previously mentioned studies because this study evaluated only food allergens and did not assess aeroallergens (12). In general, these results indicate the high rate of sensitization to allergens in AD patients, and the difference in the frequency of allergens sensitization could be due to the differences in the type, number, and availability of allergens studied, as well as the type and severity of the disease.

In the current study, a high percentage of patients were sensitized to inhalant allergens, including pollen, fungi, and indoor allergens, proposing the potential role of inhaled aeroallergens in pathogenesis and exacerbation of AD-related symptoms. In the case of outdoor inhalant allergens, grass pollens, and molds had the highest and the lowest rates of sensitization, respectively, in both immunoblotting and SPT methods. Consistent with our result, Ghaderi reported grasses as the most prevalent allergens among AD patients in Birjand, Iran, using the immunoblotting method but with a lower rate (13). The lower prevalence in that study might be due to the patient's selection criteria or because different kits were used. Bonyadi and colleagues reported that about half of their AD patients from northwest Iran were sensitized to grasses by the immunoblotting method (14). The high rate of sensitization to pollen in our study is possibly due to differences in geo-climate factors, as Birjand City is located in an arid area with low humidity and an abundance of windy days, increasing the concentration of grass and weed pollen in the atmosphere. Reports from other countries also confirmed the high rate of sensitization to pollen among patients with AD as Alqahtani carried out an investigation in Saudi Arabia on 212 children and young adults with AD by SPT; their results revealed that *Cladosporium* (14.6%) and Bermuda grass (14.1%) were the most prevalent allergens in the mentioned study (15).

In the case of food allergens, our results showed a high rate of sensitization to date palm (35.29%), citrus mix (29.41), tomato (26.46%), and peanut (23.52%). While in a similar study carried out in Birjand using the immunoblotting method, potatoes (45.2%), apples (32.2%), and cow milk (29%) had the highest rate of sensitization (13). In a report from the northwest part of Iran by immunoblotting method, potato (11.3%), hazelnut, and soybean (10%) were the most common food allergens among AD patients (14), and the same author reported potato (11.33%), egg white, and cow milk (8.7%) as the most prevalent food allergens identified using SPT (10). In another study, Al-Tamemi reported cow milk, wheat (49.1%), and chicken egg (42.6%) as the most prevalent food allergens in Omani AD patients (16).

There are several reasons for this discrepancy between the results of different studies in the case of food allergens. First, age is a confounding factor as the prevalence of food allergens in children is higher than in adults, and the rate of sensitization to food decreases with age (17). In our study, 82% (n = 28) of subjects were adults (>15), which explains the difference in reported frequencies. The second reason is the difference in the methodology of studies as the sensitivity and number of allergens in each method are different as Owczarek et al. showed that the method used would make the difference in the prevalence of allergens (18); thus, the exact comparison is not possible. Finally, racial and cultural differences may influence the results as well (19).

According to Benedict et al.'s study (19), the most important inhaled allergens are HDM, cat, grass pollen, and Alternaria, which is inconsistent with our study. In the current study, cat, dog, and HDM had a low frequency, which might be because of different cultures, lifestyles, or religious roles, and in the case of HDM, due to the dry climate, which is an unfavorable condition for mite growth. In this regard, we found that food allergy to date palm is the most frequently observed allergen in the food allergens group in our study, which is inconsistent with other studies (20, 21). There are 2 possible explanations for that. The first idea is that the rate of date palm consumption is high in Iran, especially in the southeastern part of the country. In fact, Iran is the second country with the most global date fruit production. The second possibility is the possible cross-reaction between date palm polypeptides and other foods (22).

In this study, we used both SPT and immunoblotting methods, which are widely used worldwide to confirm the presence of causative allergens (23). Although the results of the 2 methods were generally similar for most allergens, there were some instances of inconsistency. This finding is consistent with findings from other studies (24-26). Inconsistency between skin tests and sIgE may be caused by different factors, such as patient-related factors, SPT method, quality, quantity, source, and nature of allergens used in each method. The differences observed between sIgE levels and SPT results, as indicated in our study, are consistent with previous reports that have also noted discrepancies between sIgE and SPT. The rates of disagreements varied according to the type of allergens and patient factors (25, 27, 28). Jiang et al. (29) reported the sensitivity and specificity of Allergyscreen (Mediwiss Analytic GmbH, Moers, Germany) compared with SPT, as 78%, and 86.2%, respectively, which is in concordance with our results. de Vos et al. investigated the agreement between SPT for 7 common aeroallergens (grass pollen, ragweed pollen, dust mite, cockroach, mouse, cat, and dog) and sIgE testing using Immulite 2000 (Siemens AG, Munich, Germany) in 40 atopic children. The study showed that most allergens had a fair but not good correlation (kappa: 0.04-0.50) (25). In addition, Asha'ari et al. (30) recruited patients with different allergic conditions. They also used a different method for sIgE testing (chemo-luminescent), while we used immunoblotting in our study. The sensitivity, specificity, PPV, and NPV of the sIgE method vs SPT in their study were 73.27%, 56.03%, 23.12%, and 91.67%, respectively.

Conclusion

A large percentage of AD patients were sensitized to pollen allergens, indicating that avoiding exposure to these allergens could potentially alleviate symptoms. Among food allergens, date palm, tomato, and peanut were found to be the most commonly implicated allergens in this study. Although SPT and immunoblotting methods had a good concordance for most allergens, there was some discrepancy, especially in the case of food allergens; therefore, specialists should consider the advantages and limitations of each method to identify causative allergens in patients. Further studies are also needed to reveal the role of aeroallergens in the pathogenesis and development of AD.

Table 1. Frequency of skin sensitivity and positive specific IgE to different allergens

Allergen	Prevalence n(%)	Allergen	Prevalence n(%)	Allergen	Prevalence n(%)	Allergen	Prevalence n(%)	Allergen	Prevalence n(%)
Immunoblotting method						SPT			
Ragweed pollen	18 (52.94)	Peanut	8 (23.52)	Cypress	3 (8.82)	Grass mix****	11 (32.34)	Egg yolk	3 (8.82)
Olive tree pollen	14 (41.16)	Cockroach	8 (23.52)	Dog epithelia	3 (8.82)	Tomato	9 (26.46)	Der p	3 (8.82)
Eucalyptus pollen	12 (35.29)	Nut mix***	7 (20.58)	Cat epithelia	3 (8.82)	Peanut	8 (23.52)	Shrimp	2 (5.88)
Date palm	12 (35.29)	Banana	6 (17.64)	Cypress pollen	3 (8.82)	Soybean	8 (23.52)	Milk	2 (5.88)
Grass mix*	11 (32.28)	Cladosporium herbarum	6 (17.64)	Aspergillus fumigatus	2 (5.88)	Olive pollen	7 (20.58)	Der f	2 (5.88)
Citrus mix**	10 (29.41)	Der f	5 (14.7)	Egg white	2 (5.88)	Banana	7 (20.58)	Tuna fish	1 (2.94)
Acacia pollen	9 (26.47)	Cedar pollen	5 (14.7)	Shrimp	2 (5.88)	Latex	7 (20.58)	Aspergillus fumigatus	1 (2.94)
Parietaria pollen	9 (26.47)	Camel hair	5 (14.7)	Tuna fish	2 (5.88)	Cockroach	6 (17.64)		
Tomato	9 (26.46)	Der p	5 (14.7)	Cow's milk	1 (2.94)	Cat epithelia	4 (11.76)		
Latex	9 (26.46)	Soy	5 (14.7)	Egg yolk	0 (0)	Egg white	3 (8.82)		

*** Unknown**
**** Sour Lime, Lemon, Orange**
***** Hazelnut, Walnut, Almond**
****** Kentucky bluegrass, Ryegrass, Bermuda grass, Orchard grass, Timothy grass**
Abbreviation: SPT, Skin Prick Test.

Table 2. The sensitivity, specificity, positive predictive value, negative predictive value, and kappa value of immunoblotting in comparison with skin prick test

Allergen	Method	Number of positive tests	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Kappa value	P- value
Aeroallergen	SPT Alleisa Screen	41 63	71.5	85.7	44.5	95.07	0.43	0.11
Food allergens	SPT Alleisa Screen	42 39	66.7	95.6	63.13	94.58	0.58	0.06
Total	SPT Alleisa Screen	83 102	69.18	91.01	53.81	94.81	0.50	0.08

Abbreviations: PPV, Positive Predictive Value; NPV, Negative Predictive Value.

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Ethical statement

The study was approved by the Ethics Committee of Birjand University of Medical Sciences (Ir.BUMS.REC.1396.229)

Conflicts of interest

The authors have no conflicts of interest to disclose.

Author contributions

Author 1 collected the data, performed the analysis, and wrote the paper. Authors 2 and 3 support grants and equipment. Author 4 collected the data. Author 5 designed the study and gave final approval for the version to be published.

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