Cross-reactivity patterns between Timothy grass pollen and peanut, melon and tomato allergens: Bosnia and Herzegovina experience

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Abstract

Aim: People who suffer from allergy to pollen after a certain time may develop allergic reactions to certain food. This cross-reactivity in allergy occurs due to the similarity of the pollen and certain food proteins. The most common cross-reactions that cause crossed allergies are grasses' pollen in combination with melon, watermelon, wheat flour, kiwi, peach, plum, or cherry. Timothy grass pollen (Phl p 1) shows similarity with proteins that are found naturally in fruits from Cucurbitaceae, Fabaceae and Solanaceae families. The main aim of this research was to determine the correlation between the presence of allergy to *Phleum pratense* (timothy grass) and allergy to peanuts, melon and tomato.

Methods: This study was conducted in Sarajevo, Bosnia and Herzegovina. Examinees from 20 to 24 years old (N=30) were administered the test. The forearm was disinfected with chlorhexidine-digluconate and a drop of aqueous allergen solution of *Phleum pratense* was placed on the forearm and pricked with lancet. A prick-to-prick test was used to analyze the allergic reaction on peanuts, melon and tomato.

Results: A total of 15 individuals had extremely positive timothy grass allergy (diameter of hive >15 mm), whereas 10 examinees had hive diameter between 10 mm and 15 mm, which highly correlated with melon, tomato and peanut allergies (R=0.673, R= -0.342 and R=0.309, respectively).

Conclusion: These results suggest the existence of common antigenic epitopes in melon and timothy grass pollen firstly. This type of study is the first one in the Bosnian and Herzegovinian population. Further investigations should be obtained in terms of IgE analysis in individuals allergic to grass pollen and certain foods.

Keywords: Bosnia and Herzegovina, cross-reactivity, melon, peanut, Timothy grass pollen, tomato.

Introduction

The immune system of allergic persons is identified during the first contact with a food allergen. As a consequence, B-cell IgE antibodies production is increased to fight against these allergens. The first contact with a foreign allergen sensitizes the immune system and usually passes without symptoms. However, when a person prone to allergies re-consumes a food that causes an allergy during a second contact, allergens contained in it are recognized by the antibody and allergy reaction happens. Food allergy is estimated to affect between 1.5% and 10% of the population worldwide (1), while respiratory allergies affect between 10% and 30% of people at a global scale (2).

The cause of crossed allergic reaction is the chemical similarity of proteins found in various foods or allergy triggers. Contemporary research has provided enough information to predict cross-linked allergic reactions. Cross-reactivity is important for various reasons, such as its immunologic basis and for the identification of the patterns of cross-reactivity, because they often, but not always, may reflect the pattern of clinical sensitivities (3). Panallergens are cross-reactive allergens which belong to proteins and are able to trigger IgE antibody binding. Panallergens are responsible for IgE cross-reactivity to a wide variety of related and unrelated allergenic sources (4). The main proteins responsible for cross reaction in allergies are profilins. Profilins are highly cross-reactive allergen components which bind IgE antibodies. As the immune system recognises similar proteins as the same, a positive skin test or blood test (serum IgE) can result for a food, yet the patient may be actually allergic to a substance that is cross-reactive to that food. The individual may not have allergic symptoms from a food that is cross-reactive with another food or pollen to which the individual is allergic. Symptoms of oral allergy syndrome (cross reactivity) include itchy mouth, scratchy throat and sometimes swelling of mouth, tongue and throat.

Around eleven groups of grass pollen allergens eliciting specific IgE response in individuals have been identified (4). Allergens found in various Poaceae exhibit high homology in terms of their amino acid sequence composition, which translates in significant cross-reactivity in terms of antibodies and T-cell response. Grass pollen is cross-reactive with some foods in patients with oral allergy syndrome.

Research has found that Bermuda grass *Cynodon dactylon* pollen profilin has significant cross-reactivity with profilins from tomato *Solanum lycopersicum* and melon *Cucumis melo* (1). Cross-reaction between cereal grains and grass pollen is generally considered clinically insignificant. Beta-expansin 11 is major allergen of timothy grass (*Phleum pratense*) pollen and it may have high cross-reactive potential in patients who suffer from both food allergy and pollinosis (5).

In addition, in vivo cross-reactivity between cashew, walnut and peanut was assessed in mice (6). The presence of IgE cross-reactivity between peanut allergens and allergens from other legumes grass and tree nuts has been demonstrated in some studies, but the identification of the involved allergens is still limited (7).

Seventeen allergens in peanut have been included in the official allergen nomenclature database (http://www.allergen.org/). They belong to the cupin, the prolamin, the profilin, the glycosyl transferase GT-C and the scorpion toxin-like knottin super-families (7). Understanding the relationship between pollen and food allergens is important in order to maintain normal functioning of the organism and reduce the chance of exposure to allergic reaction.

The main aim of this research was to determine the potential cross-reactivity between grass pollen (*Phleum pratense*) allergy presence and allergy to peanuts, melon and tomato in individuals 20 to 24 years old.

Methods

The study, conducted during the period March-May 2018, included 30 examinees who had symptoms of

allergic rhinitis with previously identified allergy. Before the experimental section, an informed consent was obtained from examinees. The procedures were in accordance with the ethical standards and with the Helsinki Declaration of 1975, as revised in 1983. Skin testing was conducted at the Department of Biology, Faculty of Science, University of Sarajevo, Bosnia and Herzegovina.

The analysis was performed by skin prick-test (SPT) with 0.9 mm lancets (Heinz Herenz Medizinalbedarf, Germany). First, the forearm was disinfected with chlorhexidine-digluconate (Hibibos, Bosnalijek d.d., Sarajevo, Bosnia and Herzegovina) and a drop of aqueous allergen solution of *Phleum pratense* ("Torlak" Institute, Belgrade, Serbia) was placed on the forearm and pricked with lancet.

A prick-to-prick test was used to analyze the allergic reaction on peanuts, melon and tomato. The test included a direct fruit prick with lancets and its transfer to hypodermis of the skin. The distance between the tested allergens was at least two cm.

The size of the hives was determined as the average of the longest and the shortest diameter. If dimensions of hive are greater than negative control, then it indicates significant hypersensitivity. Detailed measurement of hives was done by using manual microscope Kanavano digital magnifier (20-1000x magnification).

Statistical analysis was done by using IBM SPSS Statistics 20.0 and Microsoft Excel 2010.

Results

Table 1 presents the results of prick-tests, including number of examinees that had urticaria (significant diameter of hives). Results were presented only for examinees with positive prick-tests.

Table 1. Results of prick-test analysis on Timothy grass, peanut, melon and tomato

Diameter of hive	**Old „+" scale**	Number of positive prick-test (n)	Timothy grass pollen (*Phleum pratense*)	Peanut (*Arachis hypogaea*)	Melon (*Cucumis melo*)	Tomato (*Solanum lycopersicum*)
<4 mm	-	N	2	10	8	11
5-10 mm	+		3	5	2	6
10-15 mm	++		10	4	11	6
>15 mm	+++		15	11	9	7

Fifteen individuals had a diameter of hive greater than 15 mm, and two less than 4 mm when it comes to Timothy grass pollen (*Phleum pratense*). Hive greater than 15 mm was observed in eleven individuals, while ten of them had a diameter of hive less than 4 mm for peanut (*Arachis hypogaea*). Melon-induced hive greater than 15 mm was observed in nine patients, while eight of them had a diameter less than 4 mm. When it comes to tomato (*Solanum lycopersicum*), seven of examinees had a hive diameter greater than 15 mm, while eleven of them had a diameter less than 4 mm.

Table 2 presents the correlation between grass pollen (Timothy grass pollen) and nutritional allergens which were tested (peanut, melon and tomato).

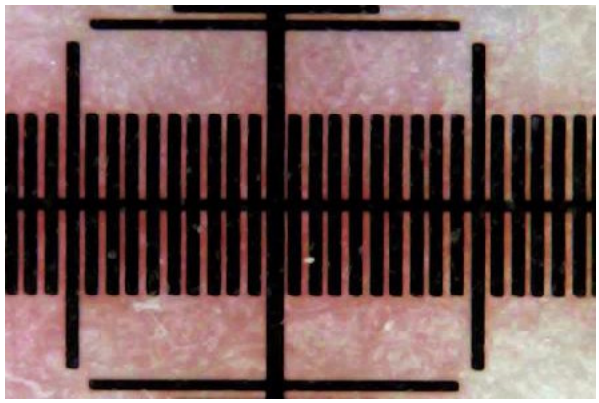
Table 2. Spearman's correlation coefficients between grass pollen and food allergens

	Timothy grass pollen (*Phleum pratense*)	**Peanut (*Arachis hypogaea*)**	**Melon (*Cucumis melo*)**
Peanut (*Arachis hypogaea*)	0.309		
Melon (*Cucumis melo*)	0.673	0.196	
Tomato (*Solanum lycopersicum*)	-0.342	0.637	0.144

The largest correlation was observed between hive diameters (R=0.673) of Timothy grass pollen (*Phleum pratense*) and melon (*Cucumis melo*), while the presence of negative correlation was observed between Timothy grass pollen (*Phleum pratense*) and tomato (*Solanum lycopersicum*) (R= -0.342), based on statistical significance at P<0.05.

Figure 1 presents melon-induced allergy on an individual that was previously allergic to *Phleum pratense* pollen. Hive diameter was greater than 15 mm on the left picture. Hive diameter magnified 40x with Kanavano digital magnifier is presented on the right picture.

Figure 1. Melon induced hive (>15 mm) (left); hive magnified by Kanavano digital magnifier 40x (right)



Discussion

Allergy is an overactive response to a substance such as pollen, animal hair or some foods. Among allergic diseases, the most common are seasonal allergic rhinitis (pollen fever) and allergic rhinitis during all year (caused by mites, house dust and spore mold). Frequently, allergic reactions to various food ingredients (food supplements, additives, preservatives, dietary colors) are most commonly seen by skin symptoms, such as urticaria or allergic dematitis (neurodermitis). Cross-reactivity is observed in many people who suffer from allergic diseases. The reason for this cross-reaction is proteins, which are structurally similar among different plants and foods, so the immune system cannot recognize the differences between them. Antibodies that react to the "standard" allergen (pollen) react to completely different (food allergen) causing specific symptoms of allergy.

Timothy grass is native to Europe, North Africa and Northern Asia, and has been introduced and widely cultivated as a hay and pasture grass. *Phleum pratense* contains at least 28 antigens, of which 15 have been shown to bind to IgE. A number of major allergens have been detected (8). Most of the patients allergic to Timothy grass pollen could be diagnosed with a combination of recombinant Phl p 1, Phl p 2, Phl p 5, and profilin allergens isolated from *Phleum pratense* as stated by Laffer (9). In our research, 15 out of 30 patients had diameter of hive greater than 15 mm after skin prick test on *Phleum pratense* which indicates high prevalence of grass pollen allergies in examinees. As confirmed by Yman (10), extensive cross-reactivity among the different individual species could be expected, as well as to a certain degree among members of the family Poaceae, in particular grasses belonging to the subfamily Pooideae: Rye grass, Canary grass, Meadow grass, Cocksfoot, Meadow Fescue, Velvet grass, Redtop, Meadow Foxtail and Wild Rye grass. Phl p 4 protein of Timothy grass cross-reacts with Amb a 1, the major allergen of ragweed as

confirmed by study (11).

Our study showed that nine examinees had extremely high sensitivity, while eleven of them had moderate sensitivity on melon allergens. Similar research of cross-reactivity between grass pollen and melon allergy were studied in 262 patients sensitized to pollen. Forty-four patients (16.7%) showed some allergic symptoms after testing with fruits and vegetables, melon being the food most frequently involved (24 patients) as confirmed by Ortiz study (12). Profilins from melon are strong sensitizers, but their biologic activity is often poor.

Eleven and four (out of 30) examinees in our study showed extremely high and moderate sensitivity to peanut allergens after prick to prick test, respectively. Significant cross-reactivity between the pollen of plane tree, hazelnut and banana fruit, and an intermediate cross-reactivity with celery and peanut is observed in previous studies (13,14). It was observed that Ara h 8, a homolog of the major birch pollen allergen Bet v 1, and Ara h 5, a profilin, are mostly involved in pollen-associated food allergy, while the peanut nsLTP Ara h 9 is involved in the so-called nsLTP-syndrome due to the cross-reactivity with their homologs in birch and/or grass pollen or in fruits and seeds, respectively (15-17). IgE reactivity to Ara h 5 coincided with profilins Phl p 12, confirming cross-reactivity in one study (18). The simultaneous presence of IgE reactivity to tomato fruit and grass pollen allergens is evident in many patients with allergy and may be caused by cross-reactivity. IgE reactivity against both extracts was demonstrated in eight serum samples in research of Petersen and colleagues (19). However, epitopes exclusive to grass pollen and tomato have not been detected yet. It was observed that 38% of Indian patients had sensitization to both grass pollen and tomato fruit, of which 92% were sensitized to tomato profilin (20). This leads to the conclusion that tomato profilin is an important cross-sensitizing panallergen in respiratory allergic patients in India. A considerably high frequency of positive reactions to tomato (39.2%), peanut (22.5%), green pea (13.7%), and wheat (11.7%) was observed in children with allergy to grass pollen according to one study (21). It was found that tomato allergy had been detected in 33% of subjects with plant food allergy, and was significantly associated with profilin hypersensitivity ($P<0.001$) (22). These results showed some differences in comparison to our research, where 46% of examinees with grass pollen allergies showed extremely high sensitivity to tomato allergens.

Conclusion

Understanding the spectrum of cross-reactive pollen and food allergies in different populations is required for a proper diagnosis and treatment. The association between grass pollen sensitization and allergy to tomato, melon and/or peanut fruit is known from studies in Europe, USA and Japan. However, there are no reports of such studies in the Bosnian and Herzegovinian population. Allergic cross-reactivity between foods from Cucurbitacae, Fabaceae and Solanaceae families and grass pollens can have serious consequences, ranging from oral allergy syndrome to anaphylaxis. Recognition and assessment of these syndromes is essential. The present study describes a preliminary investigation based on allergy simptoms and skin prick test in a study group of 30 pollen-induced allergic examinees in Sarajevo, Bosnia and Herzegovina. It may be advantageous to incorporate purified tomato profilins and other proteins specific to peanuts and melon in the routine SPT procedures for patients with respiratory allergy.

Conflicts of interest: None declared.

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