

Pistachio Allergy-Prevalence and *In vitro* Cross-Reactivity with Other Nuts

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ABSTRACT

Background: Tree nut allergy is characterized by a high frequency of life-threatening reactions and is typically lifelong persistent. Some people with a pistachio nut allergy, which is common in the pistachio rich area of Iran, develop a hypersensitivity to other tree nuts as well. The aim of this study was to investigate the prevalence of pistachio nut allergy in Iran, the major pistachio cultivation region in the world. The study also addressed the presence of allergenic cross-reactivity between pistachio and other nuts, including almond, peanut, and cashew in pistachio allergic patients.

Methods: A survey was conducted to determine whether the prevalence of pistachio allergy is affected by exposure to this nut in pistachio cultivation regions, as well as possible cross-reactivity between pistachio and other nuts including cashew, almond, and peanut. Inhibition Western blot and inhibition ELISA studies were conducted to assess the presence of allergenic cross-reactivity between pistachio and the other tree nuts.

Results: Our results revealed that the prevalence of pistachio allergy is twice as much in pistachio cultivation regions than other areas. Western blotting and inhibition ELISA presented high percentages of inhibition with pistachio and cashew, followed by almond and, to some degree, peanut which indicates different levels of allergenic cross-reactivity.

Conclusions: The results indicate that exposure of people to pistachio significantly affects the prevalence of its allergic reactions. In addition, it was observed that, among pistachio allergic subjects, such exposure may affect the co-sensitivities with other nuts, including cashew and almond. The plant taxonomic classification of pistachio and other tree nuts does appear to predict allergenic cross-reactivity.

KEY WORDS

almond, cashew, cross-reactivity, peanut, pistachio (*Pistacia vera*)

INTRODUCTION

Tree nut allergies are one of the most common, severe and long lasting food allergies among children and adults. Recently, in addition to peanut allergy, allergy to other tree nuts, such as cashew, walnuts, almond, pistachio, hazel nuts, and pecans have gained much attention in the United States and Europe due to their high risk of inducing severe anaphylactic reactions.¹ Similar to other tree nuts, pistachio are also known to be a source of food allergens.^{2,4} Most identified tree nut allergens are seed storage proteins which accumulate during seed development. They

belong to major protein super-families such as vicilins (7S trimeric globulins composed of ~50 kDa subunits), legumins (11-13S hexameric globulins with subunits of 30 to 40 kDa acidic and 17 to 20 kDa basic peptides), and 2S albumins (~15 kDa) with ~9 and ~5 kDa subunits.⁵ The composition of proteins isolated from pistachio nut indicates that 66% of total proteins were globulins while albumins, glutelins, and prolamins made up 25%, 7%, and 2% of total proteins, respectively.⁶ Albumins and globulins are the major seed storage proteins of angiosperms and also important cross-reactive food allergens.⁷ So far, the major allergens of pistachio nut have been identified

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and characterized as Pis v1, a 2S albumin; Pis v 2, a 11S globulin subunit, Pis v 3, a vicillin, Pis v 4, a manganese superoxide dismutase, and Pis v 5, a 11S globulin subunit.²⁻⁴ Among these pistachio allergens, three out of five have been characterized as globulins, and one is an albumin storage protein which is common among other tree nuts. This study, aimed to investigate *in vitro* IgE cross-reactivity between the allergenic proteins of pistachio nut and the homologous proteins present in almond, cashew, and peanut by using the sera from a group of patients who had a history of allergy to pistachio and the other tree nuts.

METHODS

SURVEY METHOD

A structured questionnaire was employed to obtain demographic information and details about allergic reactions to pistachio nut and three other tree nuts including cashew, almond, and peanut. Sampling was done randomly. Two different populations were approached to study the influence of the exposure to pistachio on the prevalence of allergic reactions to pistachio and the other nuts. One of the groups resided in Kerman Province, known to be the largest pistachio cultivation site in the world. The other group inhabited regions outside this cultivation area. Volunteers completed the questionnaire themselves or as guardians for children under the age of 18. In total 1724 questionnaires from the target populations were used. Among the 564 responses from the cities within the cultivation region, 40% were men and 60% were women. The average age was 31.35 ± 13.6 yr. For the population outside the pistachio cultivation regions, there were 1160 responses, of which 33% were men and 67% women, with an average age of 37 ± 10 yr.

The data were entered into the Access database for further analysis. In the questionnaires, respondents described their reactions to nuts by selecting among a variety of symptoms, including skin (urticaria, erythema, edema), gastrointestinal tract (vomiting, diarrhea, abdominal pain), respiratory tract (breathing difficulties, wheezing, throat tightness, coughing, nasal congestion), and cardiovascular symptoms (drop in blood pressure, loss of consciousness), as well as other symptoms (itchy throat and conjunctivitis). The first occurrence of allergic reaction/s and probable allergy to other food/s and/or pollen/s were also asked. The percentage of each nut allergy was calculated on the basis of the total number of respondents.

PISTACHIO PROTEIN EXTRACT

De-fatting of pistachio powder was performed using cold hexane (1/15 w/v) and shaking for 16 hours. Hexane was removed by suction and then phosphate-buffered saline (PBS, pH: 7.4) was added to the samples (1/10 w/v) and shaken for 8 hours. After a cen-

trifugation at 9000 g for 30 minutes, the supernatant of the mixture was dialyzed against PBS for 24 hours. The whole extraction procedure was performed at 4°C. Protein concentration was measured using the Bradford protein assay, with BSA as the standard protein.⁸

PATIENTS AND SKIN PRICK TEST (SPT)

The diagnosis of pistachio allergy was based on clinical history, positive skin prick test (SPT), positive specific IgE assay (Table 1), and the results of Western blotting. To study the IgE cross-reactivities of pistachio with the other nuts, three of pistachio allergic patients were selected who had a history of clinical allergic reactions, Western blot results, and specific IgE reactions to cashew, almond, and peanut protein extracts, as well (Table 2). Two subjects who showed negative SPT responses and no specific IgE to pistachio extract were considered as negative controls. SPT with a wheal diameter >3 mm were considered positive compared with the results obtained with negative and positive controls. The sera were stored at -30°C until use. The study protocol was approved by the Human Ethics Committee of the Avicenna Research Institute.

The clinical features of the three patients selected are as follows:

Patient 1

The first case was a nine-year-old boy with a history of vomiting, cough, gastrointestinal symptoms, and skin itching after the ingestion of pistachio. He had developed an anaphylactic reaction following the consumption of cashew and almond, and also suffered from coughing and immediate lip itching after eating peanut.

Patient 2

The second case was a 31-year-old man with a history of oral itching with angioedema of the lips and oral mucosa immediately after eating pistachio. He also suffered from coughing and oral itching following ingestion of almond and peanut, in addition to oral itching with angioedema of the lips after cashew consumption.

Patient 3

The third case was a 32-year-old woman with a history of seasonal rhino-conjunctivitis who had suffered from an oral allergy syndrome, vomiting, and skin rash after the ingestion of pistachio. She also had experienced itchy throat and angioedema of the lips immediately after eating almond and peanut, and oral itching upon consuming cashew. She stated that she had consumed cashew only once in her life.

IgE ELISA

Specific ELISA was performed as described previ-

Table 1 Clinical characteristics, specific IgE-reactivity and skin prick test responses of the selected patients with allergy to pistachio nut

Patient No.	Age (years)/sex	Clinical characteristics †	Pistachio nut extract	
			Specific IgE (OD) ‡	Skin prick test (mm)
1	22/M	E, SI	1.1	ND
2	20/M	OAS, SI, C	1.2	3
3	21/F	OAS, SI	0.99	4
4	36/F	SI	0.80	5
5	36/F	OAS, SI	0.74	4
6	9/M	V, C, G, SI	0.65	3
7	31/M	OAS, SI	0.66	5
8	24/M	G, V, R	0.56	-
9	50/F	SI, G	0.43	ND
10	34/F	R, SI	0.61	6
11	40/F	SI, C	0.66	5
12	44/M	R, SI	0.45	4
13	35/M	R, SI	0.57	ND
14	24/F	OAS	0.32	ND
15	19/F	OAS, SI, C	0.81	6
16	53/F	G, V, C	0.3	3
17	50/M	G, V	0.3	-
18	20/F	SI, OAS	0.80	3
19	24/M	OAS, R, SI	0.48	4
20	32/F	SI, OAS, V, R	0.81	6
21	25/M	E, R, C	0.59	10

† C, Cough; E, Eczema; R, Rhinitis; G, Gastrointestinal symptoms; SI, Skin itching; OAS, Oral allergy syndrome (OAS; defined as the onset of immediate oral itching with or without angioedema of the lips and oral mucosa); V, Vomiting. ND, Not determined.

‡ OD, Optical density at 450 nm.

Table 2 Specific IgE to pistachio, almond, peanut, and cashew of three patients with pistachio nut allergy

Patient No.	1	2	3
	Specific IgE (OD)		
Pistachio	0.65	0.66	0.81
Almond	0.72	0.68	0.74
Peanut	0.87	0.87	0.76
Cashew	0.47	0.76	0.57

OD, Optical density at 450 nm.

ously⁹ with minor modifications. Briefly, polystyrene plates (Nunc Maxisorb, Roskilde, Denmark) were coated with 0.1 ml/well of a coating buffer (15 mM Na₂CO₃ and 35 mM NaHCO₃, pH 9.6) containing 10 µg/ml of different nuts protein at 4°C for 16 h. The amount of antigen to be used for plate coating was determined on the basis of preliminary titration experiments. The plates were washed with PBS pH 7.2 containing 0.05% (v/v) Tween 20 (PBS-T). Remaining reactive sites on the solid phase were saturated with 200 µl 2% BSA (overnight at 4°C). After washing, prediluted 1 : 5 human sera were added to the wells and the plate was incubated overnight at room tempera-

ture. The plate was then washed with PBS-T and then incubated with biotinylated anti-human IgE (KPL, Gaithersburg, MD, USA) (1 : 2000 v/v in PBS) for two hours at room temperature. The unbound antibodies were removed from the wells by washing, which was followed by incubation with HRP-linked streptavidin (BD Biosciences, MD, USA) (1 : 20000 v/v in PBS) for 45 minutes at room temperature. After visualization of the enzymatic activity with tetramethylbenzidine (TMB) as a substrate at 37°C for 15 minutes, the reaction was stopped by the addition of 100 µl of 3N HCl, and absorption was measured at 450 nm. The assays were performed in duplicate.

WESTERN BLOTTING ASSAY AND INHIBITION WESTERN BLOT

Total extract of pistachio nut from different samples were subjected to a reducing 12% (w/v) SDS-PAGE.¹⁰ Separated protein bands by electrophoresis were electro-transferred to Polyvinylidene difluoride (PVDF) membranes (Immobilon P, Millipore Corp., Bedford, MA, USA).¹¹ After blocking with 2% BSA for 16 hours at 4°C, the blots were incubated for 16 hours at 4°C with human sera, diluted 1 : 6 in PBS. The blots were then incubated with anti-human IgE biotin-conjugated goat antibody (KPL) (1 : 1000 diluted in

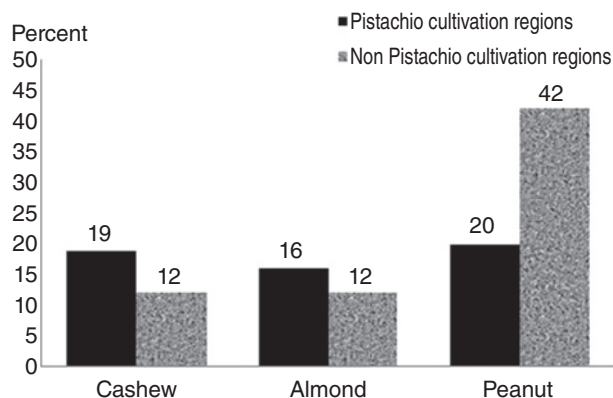


Fig. 1 The comparison between percentage of allergy to cashew, almond, and peanut among pistachio allergic subjects in pistachio and non pistachio cultivation regions in Iran.

BSA 1%) for 2 hours at room temperature. After each step, washing was done 3 times with PBS with shaking followed by one hour of incubation with horseradish peroxidase streptavidin (BD Biosciences) (1 : 40000 diluted). Blots were incubated with Supersignal West Pico Chemiluminescent Substrate Kit (Pierce, USA) for 5 min, and binding antibodies were then visualized by chemiluminescence using G-Box gel documentation system (Syngene, Cambridge, UK). For the inhibition experiment, 150 μ l of individual serum with IgE reactivity to pistachio nut extract was mixed with 150 μ l of total extract of pistachio extract (containing 70 μ g protein) and shaken at room temperature for three hours. Incubated serum was used to assess the reactivity on PVDF membrane blotted with raw extract of different nuts as described above.

COMPETITIVE INHIBITION ELISA

A pooled serum from the three patients was prepared. Ninety-six-well ELISA microplates (Nunc Maxiisorb, Roskilde, Denmark) were coated with raw pistachio nut extract (10 μ g/well in 0.1M sodium bicarbonate, pH 9.6) and incubated overnight at 4°C. Plates were then washed four times with PBS-T and blocked with 2% BSA for one hour at 37°C. After washing, 100 μ l of pooled sera from the three patients were pre-incubated for two hours at room temperature with 0.01, 0.1, 1, 10, 100, and 1000 μ g of cashew, almond, and peanut protein extract as inhibitors or with BSA as a negative control. Incubated sera were added to each well and the plates were incubated overnight at room temperature. Plates were washed again and then incubated with biotinylated anti-human IgE (KPL) (1 : 1000 v/v in 1% BSA) for two hours at room temperature. The unbound antibodies were removed from the wells by washing, which was then followed by incubation with 1 : 20000 v/v in

BSA1% HRP-linked streptavidin (BD Biosciences) for 45 minutes at room temperature. After visualization of the enzymatic activity with tetramethylbenzidine (TMB) as a substrate at 37°C for 15 minutes, the reaction was stopped by the addition of 100 μ l of 3N HCl, and absorption was measured at 450 nm. The assays were performed in duplicate.

RESULTS

According to the questionnaires, pistachio allergy was diagnosed based on the allergy symptoms which were declared by respondents. The results revealed that pistachio allergy was prevalent among 0.65% of the surveyed subjects who lived in cities within the pistachio cultivation regions and 0.3% for those residing in regions outside the pistachio cultivation area. This two-fold difference could prove that being exposed to pistachio may significantly affect the prevalence of allergenicity among inhabitants of pistachio cultivation areas. On the other hand, pistachio allergic patients in pistachio rich area reported more allergic reactions to cashew and almond compared to the other group which lived in regions outside the cultivation area (Fig. 1).

The results of the Western blot for twenty-one individuals with pistachio allergy (Table 1), which were confirmed by SPTs and documented histories of allergic reactions, revealed the presence of at least eleven bands according to their molecular weights (Fig. 2). The prevalence of the IgE reactivity to each individual allergen of pistachio among the twenty-one allergic patients is illustrated in Figure 3. Sera from 90.5% of pistachio allergic patients reacted with a protein with an apparent molecular weight (MW) of 36 kDa which may correspond to Pis v5, an 11S globulin subunit.^{2,3} Another protein band with an apparent MW of 32 kDa and that corresponded to an 11S globulin, was also recognized by the 42.8% of the patients' sera. Furthermore, 47.6% of allergic sera detected a protein band with the approximate MW of 55 kDa which could be related to vicillin. Considering the apparent MW of manganese superoxide dismutase, it could be assumed this allergen was probed by 47.6 percent of allergic patients' sera. So far, manganese superoxide dismutase has not been detected in any other nuts.¹²

To study the possible cross-reactivity between pistachio and three other nuts including cashew, peanut, and almond, the protein patterns of these nuts were first analyzed. Figure 4 presents similarities among apparent molecular weights of pistachio nut protein bands as well as for the proteins of the other three nuts. In the second step, inhibition Western blot and inhibition ELISA were carried out to determine the IgE reactivity of pistachio allergic patients' sera towards cashew, almond, and peanut extracts. Inhibition Western blot, using sera from three pistachio allergic subjects and pre-incubating with crude pista-

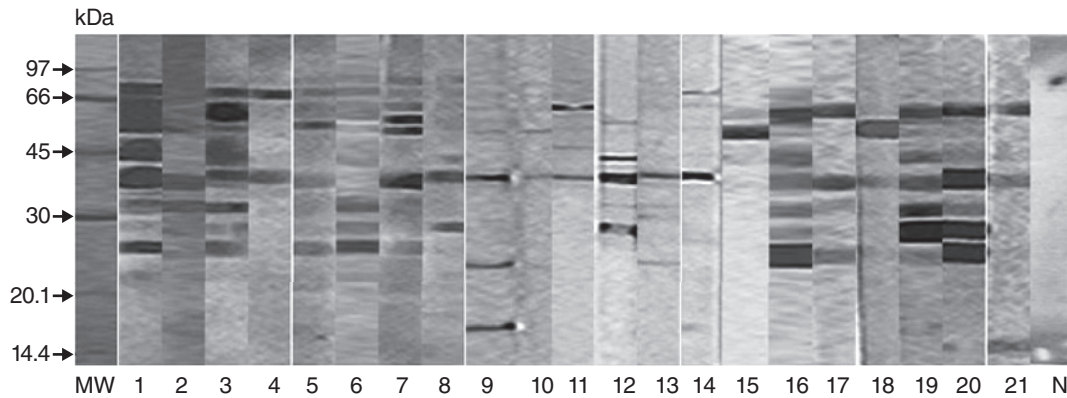


Fig. 2 IgE Immunoblot of pistachio extracts using twenty one allergic patients' sera. Lane MW, low molecular weight (Amersham, Buckinghamshire, UK); lanes 1-21, probed with individual patients' sera; lane N, negative control.

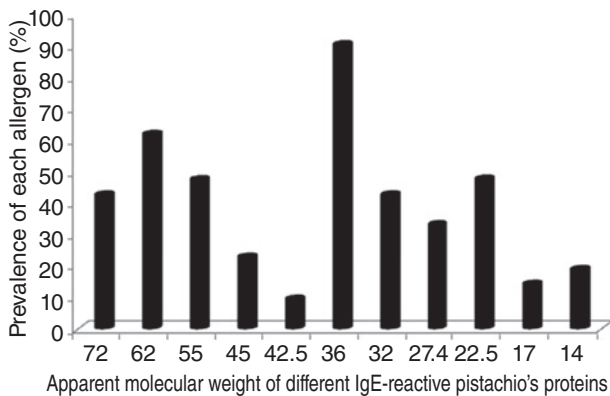


Fig. 3 Prevalence of different IgE-reactive pistachio's protein using twenty one pistachio allergic patients' sera.

chio extract showed that IgE binding to cashew protein blotted strips was significantly abrogated. This suggests cross-reactivity between these two nuts. For the almond blotted strips, the majority of IgE binding proteins were inhibited by pistachio extract except for an allergen of approximately 20 kDa (Fig. 5). A limited inhibition with pistachio extract was also proven when the strips were blotted with peanut.

The results of inhibition ELISA presented the IgE reactivity of pistachio allergic patients' sera towards cashew, almond, and peanut extracts. The pre-incubation of pooled sera with cashew extract resulted in a significant decrease in IgE reactivity, followed by almond extract. Pre-incubation sera with peanut extract did not show minimal inhibition (IC₅₀) at the maximum inhibitor concentration of 1000 µg/mL, indicating the lack of sufficient cross-reactive allergens in this extract (Fig. 6).

DISCUSSION

The data from our study's survey revealed that the prevalence of pistachio allergy is twice as much in

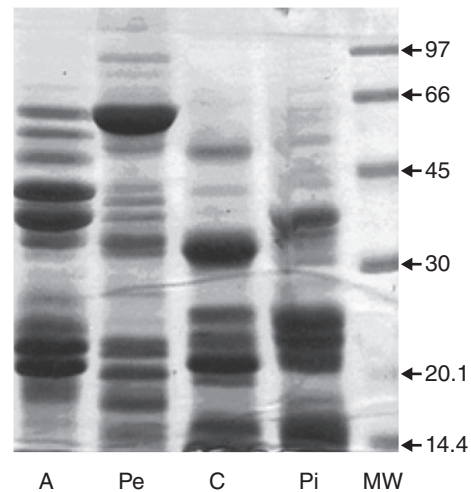


Fig. 4 SDS-PAGE (12%) of protein extracts from different nuts. Almond (A); Peanut (Pe); Cashew (C); Pistachio (Pi); and Low molecular weight marker (Amersham) (MW).

pistachio cultivation regions than other areas. It has previously been reported that since allergic people avoid the consumption of those allergenic foods, allergic reactions mostly occur due to hidden or undeclared allergens in products.¹³ To the best of our knowledge, pistachio is the most commonly consumed nut in the pistachio cultivation regions in Iran. It is also predictable that allergic people in these cultivation regions are more exposed to pistachio allergens in the course of their lives. Spanjersberg *et al.* (2007) suggested a probabilistic model for the relationship between the exposure to allergenic materials and the frequency of allergic reactions.¹³

In this study, based on the Western blot results of twenty one pistachio allergic subjects, at least eleven allergens were found to exist in pistachio. The most frequent allergen (90.5) was 36 kDa protein followed

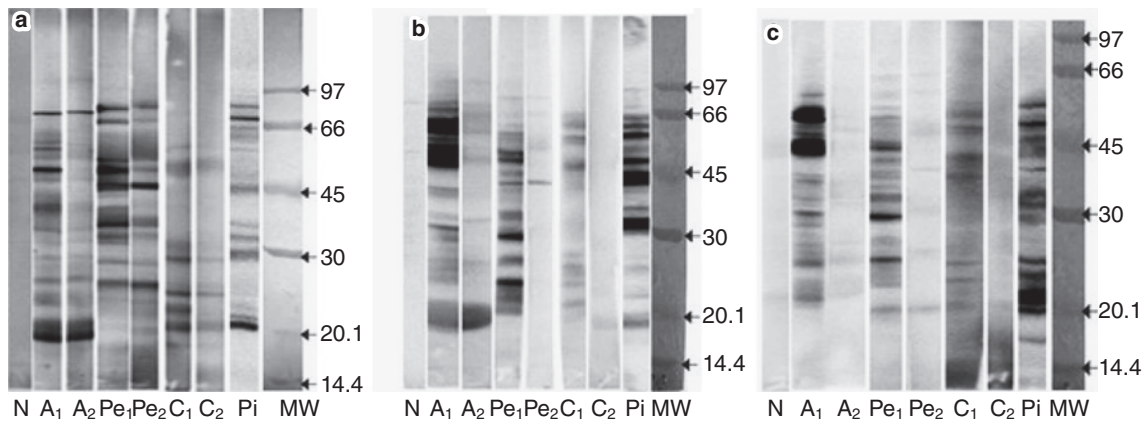


Fig. 5 Western blot result of IgE binding to different immobilized nuts along with their pistachio nut inhibited extracts, probed using three individual patients' sera (**a**, **b**, and **c**). Negative control (N); A₁ (Almond); A₂ (immobilized almond inhibited with pistachio); Pe₁ (Peanut); Pe₂ (immobilized peanut inhibited with pistachio); C₁ (Cashew); C₂ (immobilized cashew inhibited with pistachio); Pi (pistachio); Low molecular weight marker (Amersham) (MW).

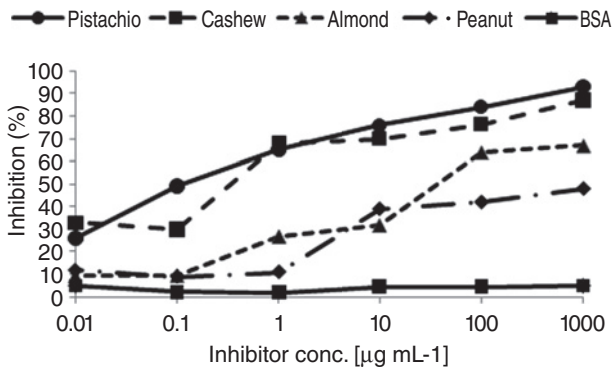


Fig. 6 Competitive inhibition ELISA of IgE binding to immobilized raw pistachio nut extract by increasing concentrations of pistachio, cashew, almond, and peanut extracts using patients' pooled sera.

by 62, 55, 22.5, 32, 72, 27, 45, 14, 17, and 42.5 kDa proteins respectively. Malet *et al.* (1994) detected five allergenic fractions in pistachio by the means of SDS-PAGE immunoblotting; the 57.1 and 74.0 kDa allergenic fractions were those that most frequently bound IgE to the studied sera.¹⁴ E. Funes *et al.* (1999) demonstrated that the most common allergens were around 14, 30, 40, and 55 kDa.¹⁵ It means there are more pistachio allergens that need to be identified. In addition, the considerable variability reported in the IgE recognition of pistachio proteins could be a source of different cross-reactivities between pistachio and the other nuts in different population groups. On the other hand, SDS-PAGE results of pistachio, cashew, almond, and peanut proteins revealed that the similarity in their apparent molecular weight may provide clues to the correspondence

among their proteins.

The existence of cross-reactivity among different nuts has also been extensively investigated.¹⁶⁻²² Data from the Food Allergy and Anaphylaxis Network's Peanut and Tree Nut Registry (FAAN), shows the following percentages of self-reported reactions among allergic patients: 34% to walnuts, 20% to cashew, 15% to almond, 9% to pecans, 7% to pistachio, and less than 5% each to hazelnuts, Brazil nuts, pine nuts, macadamia nuts, and hickory nuts.¹⁶ In studies on children with tree nut allergies, 92% of 111 patients with peanut allergy, tree nut allergy, or both showed elevated IgE levels to more than one tree nut, while 37% of 54 patients had experienced convincing reactions and showed specific IgE levels to more than one nut.¹⁹ In our study, allergy symptoms to cashew, almond, and peanut were investigated among the pistachio allergic populations. Considering the fact that, in the main pistachio cultivation regions of Iran, the inhabitants traditionally consume pistachio as a part of their usual diet, the results revealed more reported allergic reactions to cashew and almond than those in non-pistachio cultivation regions. The lower number of peanut's allergic reactions in the pistachio rich area might be due to little consumption of this nut or the limited IgE cross-reactivity between pistachio and peanut. Several studies suggested that there may be some common allergens among different tree nuts. For instance, a study on 142 peanut allergic patients indicated 50% positive skin tests to almond, 40% to cashew, 30% to pistachio, 26% to Brazil nuts, and 21% to hazelnuts.²³ It has also been reported that multiple tree nut sensitivities observed in allergic subjects may be due to cross-reactive IgE epitopes present in homologous tree nut allergens.²⁴ The CAP-inhibition studies revealed a significant cross-reactivity between

pistachio and cashew nuts both belonging to the Anacardiaceae family and also between pistachio and other dried fruits belonging to taxonomically unrelated botanical families.²⁵ It was shown that peanut is serologically IgE cross-reactive with pistachio. It has also been reported that hazelnuts, cashew, Brazil nuts, pistachio, and almond make up a group of moderately cross-reactive tree nuts.²⁰ The results of this study also suggest that the cross-reactivity among the tree nuts is related to botanical family associations: pistachio and cashew belong to the same botanical family, Anacardiaceae, followed by pistachio/almond, and pistachio/peanut in the same botanical subclass, Rosaceae. However, this finding is in contrast to a past study which reported the observed level of cross reactivity between peanut, almond, Brazil nuts, cashew and hazelnuts did not correlate with the plant taxonomic relationship.²¹ In the present study, the cross-reactivity between pistachio/cashew and pistachio/almond were proven by an inhibition ELISA and also an inhibition Western blot. The inhibition ELISA did not demonstrate minimal inhibition at the maximum inhibitor concentration of 1000 µg/mL, thus indicating an insufficient cross-reactivity between pistachio and peanut. These data suggest that in the pistachio rich regions, pistachio are highly consumed by local inhabitants; hence, the immune system is exposed to several B cell and T cell epitopes from different pistachio antigens. These epitopes might be the cause of the high occurrence of type one food allergy reactions and consequently, an increased number of cross-reactive responses to other nuts. The results of this study's survey also confirmed that exposure to pistachio positively affected the incidence of cashew and almond allergies. In conclusion, although the overall consumption of cashew, almond, and peanut is low in the pistachio rich regions of Iran, in comparison with pistachio, we deem to point out that cashew, almond, and, to some extent, peanut, may represent allergic sensitization in pistachio allergic patients due to cross-reactivity. More studies with purified allergens or families of allergens are needed to clarify the allergens, the involved epitopes, and the homology of the proteins responsible for the cross-reactivity between pistachio and other nuts.

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CONFLICT OF INTEREST

No potential conflict of interest was disclosed.

REFERENCES

- Hasegawa M, Inomata N, Yamazaki H, Morita A, Kirino M, Ikezawa Z. Clinical features of four cases with cashew nut allergy and cross-reactivity between cashew nut and pistachio. *Allergol Int* 2009;**58**:209-15.
- Ahn K, Bardina L, Grishina G, Beyer K, Sampson HA. Identification of two pistachio allergens, Pis v 1 and Pis v 2, belonging to the 2S albumin and 11S globulin family. *Clin Exp Allergy* 2009;**39**:926-34.
- Ayuso R, Grishina G, Ahn K *et al*. Identification of a MnSOD-like protein as a new major pistachio allergen. *J Allergy Clin Immunol* 2007;**119**:s115.
- Willison LN, Tawde P, Robotham JM *et al*. Pistachio vicilin, Pis v 3, is immunoglobulin E-reactive and cross-reacts with the homologous cashew allergen, Ana o 1. *Clin Exp Allergy* 2008;**38**:1229-38.
- Roux KH, Teuber SS, Sathe SK. Tree nut allergens. *Int Arch Allergy Immunol* 2003;**131**:234-44.
- Shokraii EH, Esen A. Composition, solubility, and electrophoretic patterns of proteins isolated from Kerman pistachio nuts (*Pistacia vera* L.). *J Agric Food Chem* 1988;**36**:425-9.
- Ferreira F, Hawranek T, Gruber P, Wopfner N, Mari A. Allergic cross-reactivity: from gene to the clinic. *Allergy* 2004;**59**:243-67.
- Bradford M. Rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye-binding. *Anal Biochem* 1976;**72**:248-54.
- Sankian M, Varasteh A, Pazouki N, Mahmoudi M. Sequence homology: a poor predictive value for profilins cross-reactivity. *Clin Mol Allergy* 2005;**3**:13.
- Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;**227**:680-5.
- Towbin H, Staehlin I, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci U S A* 2009;**776**:4350-4.
- Noorbakhsh R, Mortazavi SA, Sankian M, Shahidi F, As-sarehzadegan MA, Varasteh AR. Cloning, expression, characterization, and computational approach for cross-reactivity prediction of manganese superoxide dismutase allergen from pistachio nut. *Allergol Int* 2010;**59**:295-304.
- Spanjersberg MQI, Kruizinga AG, Rennen MAJ, Houben GF. Risk assessment and food allergy: the probabilistic model applied to allergens. *Food Chem Toxicol* 2007;**45**:49-54.
- Malet A, Amat P, Lluch M, Serra E, Valero A. Hipersensibilidad al pistacho: estudio de las fracciones alergénicas. *Rev Esp Alergol Inmunol Clin* 1994;**9**(Suppl 11):69.
- Funes E, Milán JM, Pagan JA *et al*. Alergia a anacardiáceas. Identificación de alérgenos. *Allergol Inmunol Clin* 1999;**14**:82-9.
- Sicherer SH, Furlong TJ, Muñoz-Furlong A, Burks AW, Sampson HA. A voluntary registry for peanut and tree nut allergy: characteristics of the first 5,149 registrants. *J Allergy Clin Immunol* 2001;**108**:128-32.
- Hourihane JO'B, Kilburn SA, Dean P, Warner JO. Clinical characteristics of peanut allergy. *Clin Exp Allergy* 1997;**27**:634-9.
- Ewan PW. Clinical study of peanut and nut allergy in 62 consecutive patients: new features and associations. *BMJ* 1996;**312**:1074-8.
- Sicherer SH, Burks AW, Sampson HA. Clinical features of

- acute allergic reactions to peanut and tree nuts in children. *Pediatrics* 1998;**102**:e6.
- 20.** David WG, Bonnie AW, Andrew DG. Cross-reactivity among edible nuts: double immunodiffusion, crossed immunoelectrophoresis, and human specific IgE serologic surveys. *Ann Allergy Asthma Immunol* 2005;**95**:45-52.
- 21.** de Leon MP, Glaspole IN, Drew AC, Rolland JM, O'Hehir RE. Immunological analysis of allergenic cross-reactivity between peanut and tree nuts. *Clin Exp Allergy* 2003;**33**:1273-80.
- 22.** Fernández C, Fiandor A, Martínez-Garate A, Martínez Quesada J. Allergy to pistachio: crossreactivity between pistachio nut and other Anacardiaceae. *Clin Exp Allergy* 1995;**25**:1254-9.
- 23.** Moneret-Vautrin DA, Rance F, Kanny G *et al.* Food allergy to peanut in France-evaluation of 142 observations. *Clin Exp Allergy* 1998;**28**:1113-9.
- 24.** Wallowitza ML, Teubera S, Sampsonc HA *et al.* Cross-reactivity of walnut, cashew, and hazelnut legumin proteins in tree nut allergic patients. *J Allergy Clin Immunol* 2004;**113**:S156.
- 25.** Jansen A, de Lijster de Raadt J, van Toorenenbergen AW, van Wijk RG. Allergy to pistachio nuts. *Allergy Proc* 1992;**13**:255-8.