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Clinical and Laboratory Investigation of Oral Allergy Syndrome to Grape

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ABSTRACT

Oral allergy syndrome (OAS) is occasionally observed following consumption of raw fruits in allergic adults. Since this phenomenon was commonly reported in Khorasan province of Iran; we intended to check if common diagnostic tests could be applied for differential diagnosis of OAS to grapes.

IgE reactivity of 84 patients with OAS to grape and 34 patients with OAS to other fruits were analyzed by *in vivo* and *in vitro* methods, and the results were compared with those of controls. The patients underwent skin prick test (SPT) with common allergic pollen extracts as well as grape extract. The specific IgE level to grape proteins was determined by an indirect ELISA. The correlation of SPT results with ELISA and western blotting patterns was checked by statistical methods.

The results showed a significant correlation of grape SPT diameters with grape specific IgE levels. Furthermore, a significant association of grape SPT results with IgE immunoreactivity of a 10 kDa grape protein, probably lipid transfer protein (LTP) was prominent. Immunoreactivity of other proteins was linked with mild clinical symptoms.

The study showed a significant correlation of grape SPT results with grape total extract, as well as its 10 kDa component's IgE reactivity. The results suggested that OAS to grape should not be considered as a main criterion in diagnosis of grape allergy and a combination of grape SPT results with evaluation of IgE reactivity to grape 10 kDa allergen should be considered to achieve a more reliable grape allergy diagnosis.

Keywords: Food hypersensitivity; Grape (*Vitis vinifera*); Lipid transfer protein; Oral allergy syndrome; Skin prick test; Specific IgE; Western blotting

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INTRODUCTION

More than thirty percent of the American and European populations claim to have food allergies and they give a history of adverse reactions to some sort of foods. However, the clinical and laboratory findings showed that most of those symptoms are not immune mediated and should not be categorized as allergic reactions.¹⁻⁴ Fruit allergy is a subtype of food allergy commonly observed in adults and is defined as sensitivity to edible fleshy ripened ovary of a plant. Although fruit allergy is a common complaint in general population, it usually causes mild symptoms.^{5,6} The most frequently noted clinical manifestation of IgE-mediated fruit allergy is oral allergy syndrome (OAS); which is a cluster of symptoms such as itching and swelling of the lips, mouth and throat as well as sneezing and also a runny nose. However, in some cases the symptoms could be more severe, and may involve other target organs and result in facial angioedema, asthma and even anaphylactic reactions.^{7,8}

Among fruits causing IgE mediated allergic reactions; a small number of botanical families namely Rosaceae and Cucurbitaceae are of great importance. Therefore, most of the basic and clinical studies in the field of fruit allergology have been focused on these fruits. In details, several major allergens of apple, cherry, peach, apricot, pear and plum from Rosaceae family as well as melon, watermelon, pumpkin, zucchini from Cucurbitaceae family have been characterized. Moreover, clinical and laboratory assessment of OAS patients, revealed significant cross reactivity of pollen and food proteins and proved that the primary sensitizer of fruit allergies are usually pollen allergens.⁹⁻¹¹

A questionnaire based study of approximately 2000 individuals about fruit allergy, showed that Persian melon from Cucurbitaceae family and grape from Vitaceae family contributed to 70% and 30% of self-reported OAS cases in Mashhad, northeast of Iran, respectively [Assarezaghegan et al, unpublished data]. Both of these fruits are vastly cultivated and consumed in this area. Other studies have also reported these two fruits as common causes of OAS.¹²⁻¹⁵ Probably allergenic molecules which are common among melon, grape, and several other foods and pollens, called pan-allergens, play a key role in provoking the manifestations of OAS.^{5,16-18} Profilins, a family of actin

binding proteins, and lipid transfer proteins (LTPs), a family of phospholipid transporting molecules, have also been identified as the key pan-allergens responsible for allergic reactions of melons^{13,19,20} and grapes,^{12,21-23} respectively.

Grape (*Vitis vinifera* subsp. *vinifera*) is a domestic species of the Vitaceae family and has ancient historical connections with the development of human society culture. Although many cultivars of this unique horticulture crop is used as table grapes or applied in food industry; several studies showed potential allergenicity of this fruit with a range of manifestations from asymptomatic sensitization up to severe anaphylactic reactions.^{12,21,23,24}

In this study we intended to check whether routine clinical or laboratory allergy tests could differentiate patients with OAS to grapes and other fruits (namely apple, banana, kiwi, peach, tomato, nuts) from non-OAS controls.

MATERIALS AND METHODS

Study Subjects

A total of 118 patients complaining of OAS to grape or other fruits who were referred or attended from 2007-2010 to the outpatient Clinic of Allergy in Ghaem general hospital of Mashhad University of Medical Sciences were enrolled in this study. All of patients had experience of at least one or two episodes of considerable OAS following ingestion of a bunch of fresh grapes or other fruits. We also included 31 individuals without any report of OAS, including seventeen non allergic volunteers (Negative controls) and fourteen subjects with respiratory allergic symptoms without OAS, as controls. All participants were inhabitants of Khorasan province in northeastern of Iran. The age of the subjects ranged from 11 to 54 years old. The mean demographic data regarding age and sex is summarized in table 4. All patients underwent routine allergy diagnosis tests including clinical history, physical examination, and skin prick test (SPT). A detailed questionnaire was filled by each participant, explaining the symptoms. Ten milliliter of blood was drawn from brachial vein of each patient and the separated sera were stored at -20°C until examination. The study was approved by the Ethics Committee of the Medical School at the Mashhad University of Medical Sciences.

Preparation of Crude Extracts

Sultana grape (*Vitis vinifera* subsp. *vitis*) was provided from Golmakan vineyard of Iranian Ministry of Agriculture. Total grape proteins were extracted using the method of Bjorksten with some modifications as previously reported.²⁵ Briefly, 100 grams of grape berries were grounded using a fruit juicer and homogenized with the same amount of cold 0.1 M potassium phosphate buffer pH 7.0 containing 20 mM ethylenediamine tetra acetic acid (EDTA) and 5% (w/v) previously soaked polyvinylpyrrolidone (PVPP). The mixture was shaken at 300 RPM for 6 hour (h) and centrifuged at 9000 g for 20 min and the clear supernatant dialyzed against 10 mM potassium phosphate buffer pH 8.0 and lyophilized. The lyophilized powder were reconstituted in 1:10 volume of initial solution in distilled water and filtered through a disposable 0.22 µm membrane filter. The aliquoted extract was kept at -20°C until use. The protein concentration was determined by Bradford's method using bovine serum albumin as standard and the results were rechecked by Lowry's method.

Skin-Prick Test

Participants underwent routine SPT. The skin of the forearm was pricked with 10 µl of the grape extract at the final concentration of approximately 250µg/ml, as well as with other commercial extracts from different local allergic pollens including *Amaranthus retroflexus*, *Chenopodium album*, *Artemisia douglasiana*, *Kochia scoparia*, *Salsola kali*, *Crocus sativus* (saffron), *Platanus orientalis*, *Platanus occidentalis*; as well as dust mix and food/fruit extracts including melon, peach, cantaloupe, kiwi, banana, tomato, pistachio and walnut (Hollister-Stier Laboratories LLC, Spokane, WA, USA). Histamine dihydrochloride (10 mg/ml) and 50% glycerol/phosphate buffered saline (PBS, pH 7.2) were used as positive and negative controls, respectively. The responses were observed after 15 min and the wheal diameters were recorded. Every week a new batch of freezed aliquots of grape extract was de-freezed and used for SPT.

Enzyme Linked Immunosorbent Assays (ELISAs)

Total serum IgE was measured by a commercial kit (Radim, Pomezia Terme, Italy) according to the manufacturer's instructions. To determine the levels of grape specific IgE, an indirect ELISA was developed.

Briefly, grape crude extract (500 µg/ml) were diluted 1:50 with carbonate buffer (0.1 M, pH 9.6) and 100 µl of it was applied per well of microtiter plates. Plates were incubated overnight at 4°C, then were washed with PBS containing 0.05% tween 20 (PBS-T) and blocked with 300 µl of 2% bovine serum albumin (BSA) for 2 h at room temperature (RT) on an ELISA shaker. Following another washing step, the plates were incubated overnight with 100 µl of 1:5 diluted sera in a duplicate manner at 4°C. Afterwards, the plates were washed and 100 µl of biotinylated goat anti-human IgE (Kirkegaard & Perry Laboratories, MD, USA) (1:2000 in BSA 1%) was added to each well and the plates were incubated for 2 h in RT. After another washing step 100 µl of a HRP conjugated streptavidin (BD Biosciences Pharmingen, USA) (1:30000 in BSA 1%) was added for detection of the bond human IgE and plates were incubated for 45 min in RT. After the final washing step, the bound enzyme was detected using 100 µl of chromogenic substrate (TMB+H₂O₂). After 15 min of incubation in the dark, the reaction was stopped with 100 µl of 3 M HCl and the optical density (OD) was measured at 450 nm.

Western Blotting

Grape total extract proteins were separated by SDS-PAGE on a 15% separating gel under reducing conditions and electro-transferred onto PVDF membranes within 15 min at 300 mA, as previously described.²⁶ Each membrane was then cut into strips. After blocking, the strips were first incubated with 1:5 diluted patients' sera at 4°C for 12 h on a rocker. The membranes were then washed with PBS-T and incubated with biotinylated goat anti-human IgE (1:2000 in BSA 1%) for 2 h at RT. After another washing, strips were incubated with HRP conjugated streptavidin (1:30000 in BSA 1%) for 45 min. After a final vigorous washing, the reactive bands were detected by chemiluminescent method. Briefly, the strips were put in a disposable tray and incubated with supersignal west pico chemiluminescent substrate (Pierce, USA) for 90 sec. The strips immediately covered by a plastic sheet and the signals were captured by G-Box chemi-documentation system (Syngene, Cambridge, UK).

Statistical Analysis

The results were analyzed by SPSS version 12 and the level of statistical significance was set at p < 0.05.

The values of the quantitative variables were checked by Kolmogorov-Smirnov test for normal distribution. Results with normal distribution were analyzed by parametric ANOVA or Student's t-test. Non-parametric Mann-Whitney and Kruskal-Wallis tests were also used for assessment of results with non-normal distributions.

RESULTS

Skin Prick Test Results

The participants underwent SPT with commercial crude extracts from common local trees, grasses, weeds, fruit allergens and also a homemade grape extract. As Table 1 shows, patients with OAS to grapes had a significantly higher wheal diameters of SPT to grape (2.96 ± 2.290 , $N=74$) compared to those without OAS (1.19 ± 1.520 , $N=34$) or with OAS to other fruits (2.06 ± 1.669 , $N=31$) ($p=0.0004$). Likewise, the mean SPT results for some common local pollens including *K. scoparia* (8.43 ± 3.867 vs. 2.90 ± 4.999 , $p=0.011$), *S. kali* (13.31 ± 5.161 vs. 7.95 ± 7.413 , $p=0.002$) and *P. orientalis* (6.58 ± 2.859 vs. 4.58 ± 3.988 , $p=0.035$) was significantly higher in patients with OAS to grapes compared to Non-OAS individuals.

There were significant correlations between SPT to

grape and SPT to common local allergic pollens including *A. retroflexus* ($p=0.010$, $r=0.251$), *C. album* ($p=0.0001$, $r=0.395$), *A. douglasiana* ($p=0.006$, $r=0.307$), *K. scoparia* ($p=0.042$, $r=0.308$), *S. kali* ($p=0.001$, $r=0.331$), *P. orientalis* ($p=0.0001$, $r=0.56$), *P. occidentalis* ($p=0.007$, $r=0.705$), and *C. sativus* ($p=0.049$, $r=0.57$).

Moreover, we found strong correlations between SPT to grape and SPT to other fruits including peach ($p=0.019$, $r=0.94$), melon ($p=0.0001$, $r=0.522$), cantaloupe ($p=0.013$, $r=0.574$), pistachio ($p=0.002$, $r=0.583$), and walnut ($p=0.049$, $r=0.579$). However, the SPT results of kiwi, banana, tomato and dust mix did not show remarkable correlation with grape SPT results.

The results also revealed that about 86 percent of participants with a positive SPT to one of common local weeds (including *A. retroflexus*, *C. album*, *A. douglasiana*, *K. scoparia*, *S. kali*) had a history of self-reported OAS to at least one of allergenic fruits. Moreover about 81% of *P. orientalis* and 92% of grape SPT positive individuals showed the same results. However, all participants with positive SPT to melon, pistachio or walnut complained from previous episodes of OAS to at least one of common allergenic fruits.

Table 1. The results of SPTs and ELISAs for the three groups of participants

Test name	Controls		Patients		P- value
	Non-OAS individuals N=31	OAS to grape N=84	OAS to fruits other than grape N=34		
Total serum IgE (IU)	276.0 ±335.06	323.3±309.37	287.3±335.61		0.349
Grape specific IgE (OD)	0.266±0.224	0.395±0.253	0.322±0.20		0.001 *
SPT to <i>Amaranthusretroflexus</i> Pollen	7.35±6.201	10.20±4.744	10.39±4.153		0.060
SPT to <i>Chenopodium album</i> Pollen	5.28±5.686	7.87±3.847	7.48±3.896		0.105
SPT to <i>Artemisiadouglasiana</i> Pollen	3.00±4.257	5.24±3.750	4.70±3.740		0.153
SPT to <i>Kochiascoparia</i> Pollen	2.90±4.999	8.43±3.867	7.85±5.520		0.011 *
SPT to <i>Salsola kali</i> Pollen	7.95±7.413	13.31±5.161	12.00±4.583		0.002 *
SPT to <i>Platanusorientalis</i> Pollen	4.58±3.988	6.58±2.859	3.80±4.213		0.035 *
SPT to <i>Platanusoccidentalis</i> Pollen	3.50±2.121	5.75±3.775	1.86±1.215		0.073
SPT to Melon	0.00±0.000	1.84±1.675	2.14±2.494		0.180
SPT to Pistachio	1.00±1.732	2.65±2.317	1.60±2.302		0.413
SPT to Walnut	0.67±1.155	2.40±1.140	3.00±1.633		0.115
SPT to Cantaloupe	1.67±2.887	2.00±1.549	1.50±1.291		0.873
SPT to Grape	1.19±1.520	2.96±2.290	2.06±1.669		0.0004 *

P-values was calculated by ANOVA or Kruskal-Wallis tests.

* Statistical significance correlation

SPT results are based on millimeter (mm) of weal diameter

OAS to Grape

Table 2. Comparison of SPT and ELISA results between every two groups of the participants

Test name	Without OAS / OAS to grape		Without OAS / OAS to other fruits		OAS to grape/ OAS to other fruits	
	Number of Patients	P-value	Number of Patients	P-value	Number of Patients	P-value
Total serum IgE (IU)	31/84	0.159	31/34	0.482	84/34	0.504
Grape Specific ELISA (OD)	31/84	0.0001 *	31/34	0.055	84/34	0.107
SPT to <i>Amaranthusretroflexus</i> Pollen	20/54	0.038 *	20/31	0.063	54/31	0.858
SPT to <i>Chenopodium album</i> Pollen	18/39	0.091	18/27	0.163	39/27	0.688
SPT to <i>Artemisiadouglassiana</i> Pollen	17/34	0.061	17/27	0.170	34/27	0.584
SPT to <i>Kochiascoparia</i> Pollen	10/21	0.002 *	10/13	0.038 *	21/13	0.720
SPT to <i>Salsola kali</i> Pollen	21/49	0.005 *	21/29	0.034 *	49/29	0.264
SPT to <i>Platanusorientalis</i> Pollen	12/26	0.087	12/20	0.607	26/20	0.011 *
SPT to <i>Platanusoccidentalis</i> Pollen	2/4	0.492	2/7	0.182	4/7	0.029 *
SPT to dust mix	2/21	0.870	2/7	0.936	21/7	0.661
SPT to Melon	2/43	0.053	2/22	0.103	43/22	0.960
SPT to Pistachio	3/17	0.260	3/5	0.713	17/5	0.384
SPT to Walnut	3/5	0.084	3/4	0.091	5/4	0.536
SPT to Cantaloupe	3/11	0.786	3/4	0.921	11/4	0.576
SPT to Grape	27/74	0.0001 *	27/34	0.024 *	74/34	0.043 *

P-values were calculated by the Student's t-test or the Mann-Whitneytest.

* Significant difference

SPT results are based on millimeter (mm) of weal diameter. The mean Value of each test result is shown in table 1.

Total and Specific IgE Levels

Total and specific IgE levels were determined for all 149 individuals. As summarized in table1, total IgE levels did not show any significant differences among the three groups of subjects and there was a correlation between total IgE concentrations with grape SPT diameters, eliminating its diagnostic value.

When the total extract from grape was coated on microtiter plates, the mean OD of negative controls (NC) was 0.159 with SD=0.0475. The cut-off value was defined as mean NC+3SD. Specimens with specific IgE values higher than NC+3SD (OD>0.302) were considered as positive, and values higher than NC+6SD (OD>0.444) were considered as strong positive.

Table 3. Prevalence of IgE reactivity of each protein band in western blotting

Apparent MW of Reactive Bands	Without OAS N=26	OAS to Grape N=73	OAS to others N=26	P-value
10 kDa	0.0	20.5	7.7	0.020 *
16 kDa	0.0	9.6	3.8	0.192
24 kDa	0.0	4.1	3.8	0.580
28 kDa	9.6	19.1	15.3	0.388
30 kDa	3.8	17.8	9.6	0.128
34 kDa	9.6	8.2	0.0	0.324
38 kDa	0.0	9.6	3.8	0.192
45 kDa	15.4	19.2	19.2	0.905
54 kDa	0.0	0.0	3.8	0.147
60 kDa	15.4	30.1	30.8	0.314

The results were shown in percentage of reactive sera in each group.

P-value for Pearson Chi-Square test, * Significant difference

Table 4. Demographic data of patients and controls

Topics	Non-OAS individuals	Patients with OAS to Grape	Patients with OAS to other fruits	Total
Number	31	84	34	149
Age	28.4±11.4	29.2±9.5	22.9±5.8	27.7±9.6
Sex F/M	5/26	41/43	9/25	55/94

Table 5. Prevalence of specific IgE positivity among the three groups of participants

Group of participants Total=149		Without OAS N=31		OAS to grape N=84		OAS to other fruits N=34	
Number of patients and percentage of reactivity		Number of patients	Percentage of reactivity	Number of patients	Percentage of reactivity	Number of patients	Percentage of reactivity
Grape Specific	Negative	23	74	38	45.2	21	61.8
ELISA Result	Weak Positive	4	13	23	27.4	8	23.5
	Strong Positive	4	13	23	27.4	5	14.7

According to these criteria, from a total of 84 patients with OAS to grapes, 46 patients (55%) showed positive reactivity, and 23 patients (27% of all patients) revealed to be strong positive.

In contrast, 45% of patients with clinical symptoms of grape allergy were determined as negative (Table 5).

Interestingly, the levels of grape specific IgE was significantly correlated with the wheal diameters of SPTs to grape ($p=0.0001$, $r=0.375$), melon ($p=0.036$, $r=0.257$), and saffron ($p=0.034$, $r=0.792$) extracts; which all of them are very common agricultural products of Khorasan province.

As it was shown in Table 2, although the grape specific IgE levels were higher in grape allergic patients than in OAS negative controls (0.395 ± 0.253 versus 0.266 ± 0.224 , $p=0.0001$), this immunoassay was not able to differentiate the grape sensitive patients from those with OAS to other fruits (0.395 ± 0.253 versus 0.322 ± 0.20 , $p=0.107$).

Moreover, this test showed some non-specific results too; since 26 percent of individuals without any clinical criteria of grape allergy (Negative controls) and 38 percent of patients with OAS to other fruits showed a positive reaction in this ELISA assay (Table 5).

IgE-Western Blotting Results

The IgE binding reactivity of patients' sera to grape proteins was checked by western blotting. The results for some selected patients are shown in figure 1. The prominent reactive bands were recorded for 125 individuals. The prevalence of strong IgE reactivity to

the 10 kDa allergen, but not other protein bands, was significantly different among the three groups (OAS to grapes, OAS to other fruits, and non-OAS) (20.5%, 7.7%, and 0.0% respectively, $p=0.020$), proposing significant correlation of reactivity of this protein with OAS to grape (Table 3).

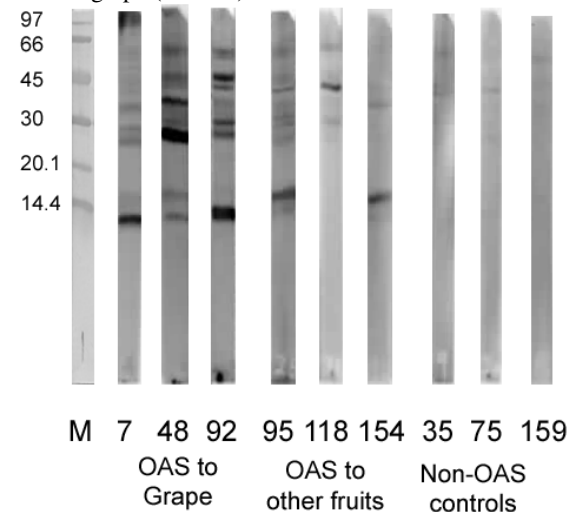


Figure 1. Western blotting of selected patients sera with grape crude extract: M: Amersham low molecular weight marker, 7, 48, 92: blotting from three patients sensitive to grape with outstanding oral allergy syndrome manifestations following ingestion of fresh grapes, 95, 118, 154: blotting from three patients complaining from previous episodes of oral manifestations following consumption of other fruits namely melons or nuts, 35, 75, 159: blotting from three healthy non-allergic controls.

An apparently 60 kDa protein corresponded to the highest percentage of reactivity among all tested subjects; however, these percentages were not significantly different among the three above mentioned groups. Interestingly sera from non-OAS individuals did not show reactivity with 10, 16, 24 and 38 kDa proteins. Moreover, grape sensitive individuals showed more than 2 fold higher prevalence of serum reactivity with the above mentioned protein bands, compared to patients with OAS to other fruits (Table 3).

DISCUSSION

This study showed that in the case of using homemade grape extract; patients with OAS to grapes showed a significantly higher wheal diameter of SPT compared to controls. However, several patients with a clear positive clinical history of OAS to grape did not show positive reaction in SPT. In consistent with this results, Inomata et al studied fruit allergic patients and demonstrated that SPT and Immunocap tests may show less positivity than it may be proposed by patients' clinical history.²⁷ Moreover; the presence of polyamines (including histamine) in fruits such as grapes could result in allergic like symptoms which should be considered in clinical practice. The concentrations of polyamines depend on the variety of fruits and the climate they have been grown in. Grapes infected with *Botrytis cinerea* or *Penicillium* species or grown in drought conditions may contain higher level of histamine which could cause OAS like symptoms following consumption of a large amount of it.²⁸ This could be an explanation for some of patient's clinical history who experienced remarkable episodes of OAS to grapes but did not show any positive diagnostic results in this study. Interestingly according to some patients' declarations they suffered from special grape varieties which reminds probable role of inappropriate or traditional cultivation of grapes in onset of OAS.

Higher SPT diameters for common local aeroallergens²⁹ in OAS positive patients may be indicating the role of pollens in primary sensitization to fruit allergens. Significant correlation of grape and local pollen SPT results is pinpointing to strong cross reactivity of grape proteins with local pollen allergens namely *Platanus* family which have a role in OAS to fruits.³⁰ Correlation of total IgE concentrations with SPT results is additional emphasizing evidence.

Grape specific ELISA was positive in more than half of patients with OAS to grapes; however, this test was not able to differentiate OAS to grape from OAS to other fruits. Moreover this immunoassay was positive in some controls. Significant correlation of grape SPT and specific IgE level in OAS patients showed *in vivo* and *in vitro* reactivity of the prepared grape extract. Similar correlations was shown in some other studies for cherry, apple, peach and celery; albeit, it would not be true for all of fruits or vegetables.^{27,31-33}

In this approach, grape specific IgE levels were in correlation with melon and saffron SPT results too. Notably, Khorasan is the main cultivation area of Persian melon and saffron in Middle East, and these plants are considered as the common allergens of this area. Interestingly most of our patients also showed oral sensitivity to melons, proposing the possible role of cross reactive allergens such as profilins (melon Cuc m 2 and saffron Cor s 2 and grape profilin)^{13,20,34} and pathogenesis related (PR) proteins (melon Cuc m 3 and grape PR1) in onset of oral symptoms.³⁵ These findings showed possible cross reactivity of grape, melon and saffron allergens; hence reducing the desired specificity of the developed ELISA.

A considerable percentage of OAS causing food allergens belong to PR protein families.³⁶ In western blotting, an IgE binding protein with the apparent molecular weight (MW) of 10 kDa, was observed which showed reactivity with sera from patients with OAS to grapes more frequently than two other groups. According to previous reports, this protein is probably LTP, a small allergen belonging to PR14 family, which contributes to the majority of grape anaphylactic reactions.^{21,23} Most of patients with strong reactivity with 10 kDa protein in western blotting, complained from OAS to grape (or alternatively grape and Persian melon), and showed a positive grape specific ELISA and a positive grape SPT, and a rather severe symptoms compared to non-reactive patients. Concerning to other IgE reactive protein bands; we did not find any significant difference among studied groups (Table 3). This approach showed that grape proteins with apparent MWs of 10, 28, 38 and 60 kDa might play the prominent IgE reactive role in western blotting of patients with OAS to grapes.

Regarding to clinical manifestations; most of attended patients had experienced only local symptoms in oral cavity. However, some of them declared hypotension in addition to the typical oral-pharyngeal

symptoms following consumption of grapes; pointing out to some sort of generalized reactions, indicating that grape allergens may participate in true food allergies.³⁷ In this study at least three patients were admitted with OAS to grape who reported nausea and other systematic reactions following consumption of grapes. These participants also showed strong immunoreactivity with 10 kDa protein in western blotting and had a rather high grape specific IgE levels. Moreover, each of these patients showed strong reactivity with 24 or 28 kDa proteins. This finding is consistent with the reports of Vassilopoulou et al who confirmed that LTP is the main allergic protein of the grape, capable of causing generalized allergic symptoms.^{23,37} Other similar studies also confirm our results.^{12,21-23} Densitometry analysis of the SDS PAGE gels with Image J software revealed that a 10 kDa protein composes about 10% of grape proteins. Therefore, the prepared extract contained a reasonable amount of the main allergen and could be a useful material for SPT and ELISA assays.²⁵ The clinical manifestation of grape sensitivity in sixteen percent of participants was merely OAS to grape. Although statistical analysis of western blotting results did not show any significant finding, we found that they showed at least immunoreactivity with 10 or 28 or 60 kDa proteins.

Taking together, this study indicates that grape specific IgE levels are significantly higher in patients with OAS to grapes, but it might have a low sensitivity and specificity in diagnosis of grape allergy. As OAS to grapes is commonly observed in this area; from the clinical point of view, it seems that a positive SPT with grape extract, along with an obvious clinical history (such as OAS) could be suggestive of grape allergy, however regarding to low positive predictive value of SPT in diagnosis of fruit allergy, a western blotting assay, specially a positive immunoreactivity with LTP could help for more precise diagnosis.

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