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Detection of 20 kDa and 32 kDa IgE-binding proteins as the major allergens in Italian sesame seed allergic patients

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Key words

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Summary

Background and objective. *Sesame seed allergy, a potentially very severe food allergy, seems on the rise worldwide but is still uncommon in Italy. The aim of the present study was to investigate the allergenic profile of Italian sesame seed-allergic patients.* ***Methods.*** *Patients with genuine sesame seed allergy diagnosed over one year in a large number of allergy centers scattered through Italy were considered for the study. Their IgE reactivity to sesame seed allergens was characterized by immunoblot analysis.* ***Results.*** *Eleven sesame seed allergic patients were detected and studied. 10/10 patients showed IgE reactivity against a sesame allergen at about 20 kDa, and 7/10 showed an extremely strong reactivity at about 32 kDa. The same 7 sera reacted also against a 28 kDa allergen, although such reactivity was significantly weaker in 6/7 cases. Eight patients showed IgE reactivity at about 48 kDa, and 5 sera reacted against higher m.w. proteins at about 67 kDa. Two sera showed IgE reactivity at about 43 kDa as well. Only one serum appeared to react to 2S-albumin.* ***Conclusions.*** *Italian sesame seed-allergic patients react mostly against allergens other than those described so far as major ones. A large number of recombinant sesame allergens will be needed for a comprehensive component-resolved diagnosis of allergy to this food.*

Introduction

Sesamum indicum (*Pedaliaceae* family) is widely cultivated in many countries in the Middle East, Asia, Latin America and also in the USA. Probably due to its widespread use in international fast food restaurants, in bakery products and in snacks and salad dressings, a global increase in sesame seed allergy has been recorded (1). Following some isolated case reports, the first case series was reported from Switzerland in 1993 (2). Subsequently, reports of sesame seed allergy in both adults and children from Israel, many European countries, USA, Canada, and Australia have appeared in the literature (3). In certain geographic areas where large amounts of sesame are traditionally present in the common diet, this type of food allergy may occur very early in life (4). To date, 8 allergens have been identified in sesame seeds: Ses i 1 and Ses i 2 (2S-albumin; m.w. 7-9 kDa), Ses i 3 (7S vicilin; m.w. 45 kDa), Ses i 4 and Ses i 5 (oleosins; m.w. 15-17 kDa), Ses i 6 and Ses i 7 (11S globulin, legumin; 52-57 kDa) (5-10) and, although not yet an official IUIS allergen, Ses i 8 (profilin, m.w. 14 kDa) (11). Sesame seed allergy does not seem

very common in Italy. In a recent survey on more than 1000 food allergic adults, only 4 were allergic to sesame seed (12). Notably, sesame seed allergic patients show a high prevalence of severe systemic reactions following the ingestion of foods containing the offending allergen (12,13). A recent Italian in-vitro study found a high prevalence of reactivity to the 11S globulins (14), whereas previous studies reported a prevalent reactivity to 2S-albumins (6). In the present study, sesame seed allergic subjects were sought over one year and their IgE was characterized by immunoblot analysis in order to assess the major sesame allergens in Italy.

Patients and methods

Patients

The study was carried out on outpatients diagnosed as having sesame seed allergy referred to 33 Italian allergy departments from January 1st to December 31st, 2011. The diagnosis of sesame seed allergy had to be based on a clear-cut clinical history of oral allergy syndrome, asthma, urticaria/angioedema, and/or anaphylaxis following the ingestion of sesame seed under any form (raw, cooked, roasted, ground, etc.) except in one case (see beyond) with an unequivocally positive SPT with fresh sesame seed and/or commercial sesame seed extract. Since the objective of this study was to investigate the IgE reactivity to specific sesame seed proteins, patients sensitized to cross-reacting plant-food allergens such as PR-10, profilin, and LTP were excluded. Admitted patients were thoroughly interviewed to detect their clinical reactivity to foods other than sesame seed, particularly walnut, hazelnut, almond, peanut, pine nut, Brazil nut, and sunflower seed. The study was carried out as a part of the routine clinical activity of all participating centers, hence no formal approval by an Ethical Committee was required. All study patients gave an informed consent to the serological analyses. Sesame seed hypersensitivity was detected by SPT with fresh seeds using the prick-prick technique. In some centers where commercial sesame seed extracts were available (Lofarma SpA, Milan, Italy; ALK-Abellò, Madrid, Spain), SPT with such extracts were used as well. Hypersensitivity to PR-10, profilin and LTP was excluded on the basis of negative SPT with commercial birch pollen extract, date palm pollen profilin (Pho d 2, 50 µg protein/ml; ALK-Abellò, Madrid, Spain) and commercial peach allergen (30 µg LTP/ml; ALK-Abellò). All skin tests, either skin prick tests or prick-prick tests, were carried out and read following established criteria (15). Only wheals showing a mean diameter exceeding 3 mm at 15 min were considered as a positive response.

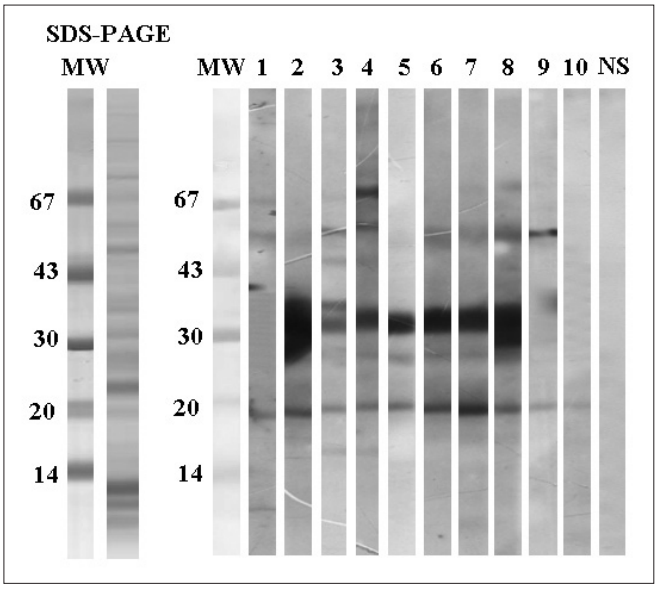
Immunoblot analysis

Eight grams of sesame seeds defatted with hexane were extracted for 1 hour in 100 ml of 0.9 M NaCl, at 4 °C under stirring. After centrifuging, the supernatant was harvested and dialyzed against the same buffer. The protein content, measured by Bradford's method (16), was 1.6 mg/ml. The electrophoresis of sesame seed extract (25 µg per lane) was carried out in a 10% polyacrylamide precast Nupage Bis-Tris gel according to manufacturer's instructions (Invitrogen, Milan, Italy) at 180 mA for 1 h under both reducing and non-reducing conditions. The resolved proteins were transferred onto a nitrocellulose membrane (Protran BA 85, Schleicher & Schuell, Milan, Italy) according to Towbin (17). The membrane was saturated in TBS (tris buffered saline) buffer containing 5% defatted dry milk (saturating buffer) and incubated with patient's serum or normal serum diluted 1:5 in saturating buffer. Specific IgE bound was detected by adding peroxidase-conjugated anti-human IgE from goat (diluted 1:8000, BioSpacific, Emeryville, CA, USA) and ECL western blotting kit (Amersham, Milan, Italy) as substrate.

Results

Eleven patients (aged 4-51; M/F ratio 7/4) diagnosed at 7 allergy centers fulfilled the admission criteria and were included in the study (**table 1**). Eight patients had a history of sesame seed allergy only, one had a clinical history of allergy to Brazil nut and another one to sunflower seed too, and one had a history of clinical allergy to multiple nuts and seeds, including walnut, hazelnut, almond, pine nut and Brazil nut. Sera from 10 pa-

Figure 1 *- Molecular weight markers, SDS-PAGE of white sesame seed extract, and immunoblot analysis. Lanes 1-10: sera from sesame-allergic patients, lane numbers correspond to patients' numbers in* **table 1**. *NS: normal serum*



tients (all but the 4 year old child, patient # 11 in **table 1**) were available for in-vitro testing. Immunoblot analysis results are shown in **figure 1**. All patients showed IgE reactivity against a sesame allergen at about 20 kDa, and 7/10 showed an extremely strong reactivity at about 32 kDa. The same 7 sera reacted also against a 28 kDa allergen, although such reactivity was significantly weaker in 6/7 cases. Eight patients showed IgE reactivity at about 48 kDa, and 5 sera reacted against higher m.w. proteins at about 67 kDa. Two sera showed IgE reactivity at about 43 kDa as well. Surprisingly, only one serum recognized (even if weakly) a zone corresponding to that of 2S-albumin. In order to rule out the possibility that reducing conditions used in SDS-PAGE might have destroyed IgE-binding epitopes of 2S-albumin, making them no more recognizable by sera in immunoblots, SDS-PAGE was also performed in non-reducing conditions, but IB profile against 2S-albumins profile (data not shown) did not change.

Table 1 *- Clinical features of study patients*

No.	Age	Sex	Other offending foods	Symptoms with s esame	Positive SPT other than sesame
1	22	F	None	Anaphylaxis	h, pn
2	20	M	None	Laryngeal oedema	None
3	58	M	sf	Anaphylaxis	sf, bn
4	39	M	None	Anaphylaxis	None
5	70	M	None	Anaphylaxis	None
6	56	M	None	Anaphylaxis	P
7	53	F	w, h, a, pn, bn	Urticaria	w, h, a, pn, bn
8	16	F	bn	Gastrointestinal	Bn
9	54	F	None	Urticaria	A
10	51	M	None	Urticaria	P
11	4	M	None	Urticaria/ angioedema	None

w = walnut; h = hazelnut; a = almond; pn = pine nut; bn = Brazil nut; sf = sunflower seed; p = peanut

Discussion

Sesame allergy is uncommon in Italy; in fact only 11 patients were diagnosed over one calendar year in 33 allergy clinics scattered throughout the country, where thousands of subjects suspected to be allergic are visited monthly. Not unexpectedly, most patients had a history of severe systemic allergic reactions following sesame intake. Surprisingly, few sera from study patients showed an IgE reactivity at molecular weights corresponding to those of sesame allergens described so far. All patients' sera recognized a protein at about 20 kDa and most of them showed an extremely strong IgE reactivity at about 32 kDa. Along with a protein at about 50 kDa (possibly 7S vicilin, Ses i 3 or 11S globulin-legumin, Ses i 7) these proteins seem to be the major allergens in this population. The specificity of recognition was confirmed by the lack of any IgE reactivity by a normal control serum (figure 1). Only one serum showed IgE response at the m.w. of the 2S-albumin (serum 1, **figure 1**), whereas 7 S vicilin recognition seemed weak and uncommon. A pattern of IgE recognition similar to that of our patients has been reported by Fremont et al. (18), whose patients reacted mainly to proteins at about 13 kDa and about 30 kDa. Similarly, in their study Beyer and co-workers found IgE reactivity against a number of proteins at different m.w. including 20 kDa, 32 kDa and 34 kDa (5). Since we did not carry out the N-terminal sequencing of the major allergens recognized by our patients' sera nor inhibition studies, we cannot exclude that the proteins recognized are polymers of lower m.w. allergens (e.g., 2S-albumin) or fragments of allergen proteins showing a higher molecular weight, although in previous studies (5,6) 2S-albumins showed a molecular weight of 7-9 kDa and vicilins a molecular weight of 45 kDa, respectively. We can exclude, however, that our study population was sensitized to PR-10 homologous proteins, profilin, and lipid transfer protein, the latter being the major food allergen in Mediterranean area even among tree nuts and seed-allergic subjects. This study suggests that sesame allergy shows some geographic variability, as is the case for other food allergies, and that probably our understanding of this type of food allergy is still incomplete. In the future, a large number of recombinant sesame allergens will be needed for a comprehensive component-resolved diagnosis of allergy to this food.

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