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Letter to the Editor

Diagnosis of IgE-mediated hypersensitivity to sesame seeds supplemented with lipid body proteins

Dear Editor,

The increasing consumption of sesame (*Sesamum indicum*) seeds in Western countries has conducted to an increased prevalence of sesame allergy in both children and adults.¹ Sesame seeds provoke severe IgE-mediated immediate reactions induced by proteins. Diagnosis is usually fulfilled based on a clinically convincing history after consumption of this seed, or a positive oral food challenge (OFC). Nevertheless, OFC is potentially dangerous and besides, skin-prick-test (SPT) and sesame-specific IgE are negative in most of the patients.^{1,2} On the other hand, although skin-prick-prick (SPP) to sesame paste or oil is an easy and available diagnosis method,³ it is possible to display false-negative results in severe sesame-induced allergic reactions.⁴ Out of the eight allergens of sesame seed described so far, *Ses i 4* and *Ses i 5* are oleosins.⁵ Oleosins are associated with more severe systemic reactions and negative SPT.² The aim of this study was to evaluate the utility of oleosins for diagnosing patients with severe reactions to sesame and a negative allergy work-up.

A total of 10 patients who visited our outpatients clinic and had described a convincing history of reaction to sesame were recruited from July 2009 to April 2014, namely, 8 anaphylaxis, 1 urticaria-angioedema, and 1 rhinitis-asthma. An allergological study was performed: SPT, SPP, serum total IgE, serum specific IgE to sesame and to lipid transfer protein (LTP, *rPru p 3*), and serum baseline tryptase. All *in vitro* tests were measured by the ImmunoCAP System (ThermoFisher, Massachusetts, USA). Of note, the commercially available sesame solution for SPT (Diater Laboratories, Madrid, Spain) did not contain lipid antigens due to this protein extract obtained from sesame seeds was defatted and solubilized in phosphate buffered saline and only contained hydro-soluble proteins.² The SPP of this study was obtained from sesame seeds and contained the lipid fraction (Supplementary Fig. 1, SDS-PAGE, lane L). Total and specific IgE to aeroallergens and different food allergens (sesame included) were determined with the fluoroenzymatic method (ThermoFisher ImmunoCAP System, Massachusetts, USA). A SDS-PAGE followed by immunoblotting with the patients' sera was also carried out. Proteins from the hydro-soluble and lipid-soluble fraction were transferred to a PVDF membrane to analyze them with the above-mentioned analytical technique. The proteins were

obtained by sesame seeds as follows. *S. indicum* seeds (Ducros™, McCormick, Spain) were weighed, crushed and suspended at 0.25 g/mL in phosphate buffered saline (PBS) [1.37 mM NaCl, 14.7 mM KH₂PO₄, 78.1 mM Na₂HPO₄, 26.8 mM KCl] pH 7.4 and 25% glycerol (v/v) and then homogenized using a mortar and pestle at 5 ± 3 °C and maintained homogenized until the greatest possible content was released. The homogenate was agitated magnetically for 30 min at 5 ± 3 °C. The sample was sieved to remove the remains of seeds and the extract was centrifuged at 10,000 g for 15 min at 5 ± 3 °C. The oil body (lipid-soluble) fraction was separated from the aqueous (hydro-soluble) fraction. The fractions were treated independently to obtain the aqueous and oil body extracts. The aqueous extract was obtained by filtering the soluble fraction through an AP type 20 glass fiber filter (Merck-Millipore™, Darmstadt, Germany), comprising a glass fiber prefilter and a 0.8–8 µm membrane (AP membrane 2009000, Merck-Millipore™, Darmstadt, Germany), and dialyzed against de-ionized water with membranes with a molecular cut-off of 3500 Da (Visking, Iberlabo) for 16 h at 5 ± 3 °C. The oil body extract was obtained by re-suspending the aqueous fraction in PBS pH 7.4 and 50% glycerol at 5 ± 3 °C, and then centrifuging at 10,000 g for 5 min. The process was performed twice. The precipitate containing the oil body extract was re-suspended in a chloroform: methanol 2:1 ratio (v/v) and centrifuged at 3000 g for 10 min. The organic fraction was collected and acetone at 5 ± 3 °C was added at a fraction of 2:1 (v/v) and left for 15 min at –20 °C, and then centrifuged at 10,000 g for 15 min and then stabilized at –20 °C.⁶

The results of all SPT, SPP, serum total IgE, serum specific IgE to sesame and LTP, and serum baseline tryptase from our 10 patients are showed in the Table 1. The maximum SPP with sesame paste measured was 25 × 15 mm (Fig. 1A). Of note, patient #1 suffered from anaphylaxis but showed a negative result to sesame paste. Besides, patients #4 and 10 reproduced the same symptoms they had showed after sesame ingestion after performing SPP. In order to further evaluate the patient #8, we performed a cautious oral challenge which resulted in a new episode of urticaria-angioedema after accumulating 6 g of sesame seeds. Immunoblotting with the patients' sera revealed different IgE-binding bands with molecular weights of 15 and 17 kDa, which are oleosins, detected in the lipid-soluble fraction isolated from sesame seed. To check that the oleosins reacted with our patients'

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Table 1
Data from the 10 patients with different clinical reactions to sesame seeds.

Patient	1	2	3	4	5	6	7	8	9	10
Gender/Age	M/72	M/54	M/56	F/53	M/41	F/25	M/20	F/74	M/56	F/40
Atopy	None	None	Pollen rhinitis	Pollen rhinitis and asthma; OAS by peach, melon, nuts, celery, bean, and soy	Pollen rhinitis	OAS by nuts, fruits, and tomato	Anaphylaxis by nuts and peach	Urticaria and angioedema by mustard	Pollen rhinitis; Anaphylaxis by fishes	Pollen rhinitis; OAS by plant-derived foods
Sesame reaction	Palm, sole and scalp pruritus, generalized urticaria, eyelid angioedema, and wheezing	Palm, scalp and genital pruritus, urticaria, eyelid angioedema, malaise, and wheezing	Oral pruritus, tongue angioedema, throat discomfort, malaise, generalized urticaria, vomiting, and diarrhea	Rhinitis and wheezing	Dry cough, malaise, abdominal pain, vomiting, dizziness, and paleness	Palm and scalp pruritus, generalized urticaria, and vomiting	Generalized urticaria and angioedema, malaise, dysphonia, and abdominal pain	Urticaria and angioedema	Palm, sole, scalp, oral, and genital pruritus, generalized urticaria, facial angioedema, sudden dyspnea, and loss of consciousness	Generalized urticaria, rhinitis, and wheezing
Time of onset (minutes)	5–10	10–15	1–2	15–20	30	5–10	20–30	10–15	2–3	20–30
Number of episodes	8	11	1	1	2	1	1	1	6	1
Sesame SPT (mm)	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Sesame SPP (mm)	Negative	4 × 4	6 × 4	7 × 6	25 × 15	8 × 5	5 × 4	6 × 6	8 × 6	7 × 5
Total IgE (U/ml)	175	49	430	176	103	268	170	<15.30	53	241
Sesame IgE (kU/L)	0.09	0.05	0.33	1.92	<0.10	0.66	0.28	0.07	0.04	<0.10
LTP IgE (kU/L)	<0.10	<0.10	<0.10	<0.10	2.48	<0.10	12.40	<0.10	<0.10	<0.10
Trypsinase (μg/L)	2.90	4.20	4.50	3.20	4.80	1.10	6.80	5.70	3.60	3.20

M, male; F, female; OAS, oral allergy syndrome; SPT, skin-prick-testing; SPP, skin-prick-prick; LTP, lipid transfer protein. Atopy is defined as a clinical condition to suffer an allergic reaction respiratory and/or cutaneous, as well as a food allergy after that an allergen has been inhaled or ingested by a predisposed individual. Oral allergy syndrome involves itching or swelling of the mouth, face, lip, tongue, and throat which usually appear immediately after eating raw fruits or vegetables. Anaphylaxis is defined as a severe, rapidly progressive and life-threatening allergic reaction, which was suffered by nearly all our patients (except patients #4 and 8). Of note, four patients (#1, 2, 5, and 9) displayed several episodes, and two patients (#4 and 10) reproduced their clinical picture after performing SPT.

sera, we performed a combined search of the peptide fingerprint and fragmented peptides in NCBI, restricting the taxonomy to *Viridiplantae*, of the 17 kDa oil body protein separated by 15% SDS-PAGE gel. The resulting sequence corresponded with the sesame seed oleosin called *Ses i 4*. An IgE-binding band of 20 kDa was also detected in both the hydro-soluble and lipid-soluble fraction in the majority of sera from our patients (Fig. 1B). We cannot exclude that this hydro-soluble protein was actually polymers of lower molecular weight or even a protein with its own identity.

In this study, we report a series of patients who suffered from distinct clinical pictures after the ingestion of sesame seeds, 8 out of 10 with anaphylaxis, and 4 out of these 8 having several episodes. All of them showed a negative result using commercial sesame SPT and only two patients displayed a positive result by means of specific IgE to sesame. We performed SPP to reveal such a sensitization, although it was not positive always. The explanation we found is that most allergens are obtained through hydrosoluble methods that remove the lipid derivatives from the allergenic extract.⁷ Therefore, a negative SPT to sesame may be explained by the current extraction procedures used in order to obtain a standardized extract, which include de-fatting steps.² It is well known that lipoproteins, and concretely oleosins, are biologically active and recognized by patient's IgE antibodies.^{2,7} Most abundant oil body-associated proteins belong to the oleosin family and much of the oil body surface may be covered by oleosins.⁶ Oleosins are alkaline constituting proteins of 15–30 kDa and pI±10, that constitute major proteins in oil bodies (lipoproteins).² Their functional role is to stabilize triacylglycerol containing oil bodies in the aqueous cytoplasm due to their highly conserved hydrophobic central domain, hydrophilic α -helical N-terminus, and amphipathic α -helical C-terminus.² Currently, further investigation is being carried out in order to enlighten the real implication of oleosins in these severe reactions.⁸ Moreover, an accurate SPT and a specific IgE to soluble recombinant oleosin reagents from other seeds are suitable and recommended for this kind of patients.⁸

Our patients displayed a sensitization to lipoproteins, which could explain the anaphylactic reactions caused by the intake of different sesame products, as previously reported for olive fruit.⁷ Therefore, the lipoproteins of sesame have allergenic properties, which are as relevant as hydrosoluble proteins, as also was reported for hazelnut.^{2,7} A potential limitation of our study was the absence of OFC except for the patient #8. The reason was that all our patients presented a truly convincing history and besides, 8 of them suffered from anaphylaxis. Moreover, we had to treat two patients (#4 and 10) due to the reappearance of symptoms after performing their sesame SPP. On the other hand, it has been demonstrated that a sesame prick-test response of ≥ 8 mm of diameter has 95% positive predictive value for challenge-proved sesame allergy,⁹ and the mean papule showed in our patients suits the former statement (Fig. 1A), except the abovementioned patient #1. Furthermore, oleosins could be a risk factor for severe sesame allergy, independent from non-pollen related allergens, such as seed storage proteins and LTP, as previously reported for hazelnut and peanut.² In fact, in our study, patients #5 and 7 showed sensitization to LTP manifested as an asymptomatic sensitization to cereal grains tolerating distinct kinds of bread, and anaphylaxis to nuts and peach, respectively.

In conclusion, we state that the lipid-soluble protein fraction could be used for diagnostic purposes, such as recombinant oleosin reagents, in those patients with different reactions to sesame, mainly anaphylaxis, which had negative results to standard extracts in both cutaneous tests and specific IgE measurements.

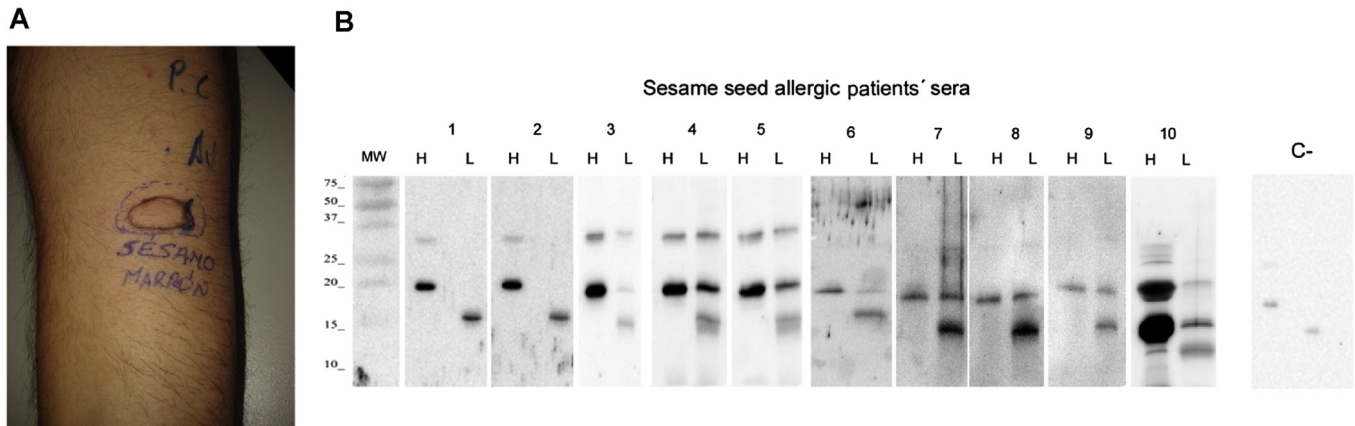


Fig. 1. A) Skin-prick-prick to brown sesame (“sésamo marrón”) paste with a positive response (25 × 15 mm, patient #5). B) Western blot with the 10 patients' sera showing IgE-binding bands with molecular weights (MW) of 15 and 17 kDa in the lipid-soluble fraction (lane L), and of 20 kDa in the hydro-soluble fraction (lane H).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.alit.2015.07.001>

Conflict of interest

FP is an employee of Diater Laboratories. The rest of the authors have no conflict of interest.

José Barbarroja-Escudero ^{a,*}, María-Jose Sanchez-Gonzalez ^a, Dario Antolin-Amerigo ^a, Mercedes Rodriguez-Rodriguez ^a, Fernando Pineda ^b, Melchor Alvarez-Mon ^a

^a Servicio de Enfermedades del Sistema Inmune–Alergia, Hospital Universitario Príncipe de Asturias, Departamento de Medicina y Especialidades Médicas, Universidad de Alcalá, Alcalá de Henares, Madrid, Spain

^b Diater Laboratories, Madrid, Spain

* Corresponding author. Servicio de Enfermedades del Sistema Inmune–Alergia, Hospital Universitario Príncipe de Asturias, Departamento de Medicina y

Especialidades Médicas, Universidad de Alcalá, Carretera Madrid-Barcelona Km 33.600, 28805 Alcalá de Henares, Madrid, Spain.

E-mail addresses: jose.barbarroja@gmail.com, jose.barbarroja@uah.es (J. Barbarroja-Escudero).

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