Original article

Allergy to *Anarcadiaceae.* Identification of allergens

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Background: Pistachio, cashew nut, and mango belong to the *Anacardiaceae* family. The increasing consumption of these fruits in recent years has determined an increase in the frequency of sensitization to these foods. *Methods:* Ten patients (5 women and 5 men) with history of food allergy to *Anacardiaceae* were studied by means of *in vivo* and *in vitro* techniques. The presence of specific IgE against *Anacardiaceae* was determined by prick test and RAST. The allergic profile to pistachio, mango, and cashew nut was assessed by SDS-PAGE followed by immunoblotting. RAST-inhibition test was performed in order to evaluate cross-reactivity among members of the *Anacardiaceae* family. *Results:* Prick tests against pistachio were positive in 8 patients, against cashew nut in 1, and against mango skin in 7, mango pulp in 5 and mango seeds in 3. Proteins of these products were detected by SDS-PAGE: in pistachio the most common proteins were found around 14,30,40, and 55 kDa; in cashew nut around 30 and 50 kDa; in mango skin around 10,12,43, and 45 kDa; and in mango pulp five proteins of 30, 41, 43, 70 and 80 kDa, respectively, were detected. RAST-inhibition test showed different cross-reactivities among members of the *Anacardiaceae* family and it was observed that mango seed extract inhibited all other extracts. *Conclusions:* This study confirms the presence IgE-mediated sensibivity to pistachio, mango, and cashew nut. Different allergens in this family were recognized, as well as cross-reactivity among these allergens.

KEY WORDS: ***Anarcardiaceae allergens*** **/ Cashew nut / Mango / Pistachio / Cross-reactivity.**

Alergia a anacardiáceas. Identificación de alergenos

Fundamento: El pistacho, el anacardo y el mango son miembros de la familia *Anacardiaceae.* El creciente consumo de estos frutos en los últimos años está llevando a un aumento en la frecuencia de la sensibilización frente a ellos. *Métodos:* Se han estudiado 10 pacientes (5 mujeres y 5 varones), con historia de hipersensibilidad alimentaria a anacardiáceas, por medio de técnicas tanto *in vivo* como *in vitro*. Se determinó la presencia de IgE específica frente a anacardiáceas por medio de *prick test* y RAST. Se analizó el perfil alergénico frente a pistacho, mango y anacardo por medio de SDS-PAGE seguido de *immunoblotting.* Se realizó RAST-inhibición con el objeto de valorar la presencia de reactividad cruzada entre los miembros de la familia anacardiácea. *Resultados:* Las pruebas cutáneas frente a pistacho fueron positivas en 8 pacientes, frente a anacardo en uno, frente a piel de mango en 7, frente a pulpa de mango en 5 y frente a semilla de mango en 3. Mediante SDS-PAGE se detectaron proteínas de estos productos: en pistacho, las proteínas más abundantes se encontraron en torno a 14, 30, 40 y 55 kDa; en anacardo, en torno a 30 y 50 kDa; en piel de mango, en torno a 10, 12, 43 y 45 kDa, y en pulpa de mango se detectaron cinco proteínas de 30, 41, 43, 70 y 80 kDa. Por medio de RAST-inhibición se encontraron diferentes reactividades cruzadas entre los miembros de la familia *Anacardiaceae* estudiados y se comprobó que el extracto de semilla de mango inhibía a los otros extractos. *Conclusiones:* Se confirma la sensibilización mediada por IgE a pistacho, mango y anacardo. Se han identificado distintos alergenos de esta familia, así como la reactividad cruzada entre ellos.

PALABRAS CLAVE: **Alergenos anacardiáceas / Anacardo / Mango / Pistacho / Reactividad cruzada.**

INTRODUCTION

Pistachio *(Pistacia vera),* cashew nut *(Anacardium occidentalis)* and mango *(Mangifera indica)* are members of the *Anacardiaceae* family[1]. None of the three is autochthonous in Spain[2]. They are consumed in rather low proportion in comparison to other nuts such as almonds, peanuts and walnuts, which together represent 60% of the total nut consumption in Spain. In our country, pistachio is probably the member of this family which is more frequently consumed (10% of the total)[3]. Its introduction into the Spanish diet is relatively recent. It is consumed both in the form of the roasted or salted nut and as a component in ice cream, sweets and other desserts. The consumption of cashew nut and mango is minimal in Spain; however, their increasing introduction into the market might determine an increase in the frequency of sensitization to these products.

Data on food allergy to members of the *Anacardiaceae* family are scarce. Miell *et al.*[4] in 1988 reported one case of anaphylaxis after the ingestion of mango pulp in a fruit vendor. Other authors have reported clinical cases of allergic reactions to mango[5-9]. Jansen *et al.*[5] studied three patients with adverse reactions to pistachio and mango and demonstrated skin test positivity and specific serum IgE to these fruits. In Spain, Fernández and Fiandor[10, 11] reported two cases of pistachio anaphylaxis and investigated the allergens involved and their cross-reactivity with other *Anacardiaceae.* Parra *et al.*[12] reported (in 1993) three patients with allergy to pistachio and other nuts; they studied the cross-reactivity between these nuts and some pollens, and tried to identify the pistachio allergens. Malet *et al.*[13], in 1994, detected five allergenic fractions in pistachio by means of SDS-PAGE *immunoblotting*; the 57.1 and 74.0 kDa allergenic fractions were those that most frequently bound IgE from the studied sera.

The cashew nut is the seed of a nut produced by an American tropical tree; it is edible, ad is frequently consumed as a snack. Cashew nut anaphylaxis was described by Stricker *et al.* in 1986[14]. Samson *et al.*[15] reported two cases of death after eating cashew nuts in a series of 13 patients with severe anaphylactic reactions induced by

Table I. Results of the skin tests

Case	Pneumoallergens	Trophoallergens
1	*Artemisia, Parietaria, Chenopodium*	Pistachio, almond hazelnut, peanut, walnut
2	*Artemisia, Parietaria, Chenopodium* grasses, olive	Pistachio, pine kernels, sunflowers seeds
3	*Artemisia, Parietaria, Chenopodium* grasses, olive	Pistachio
4	*Artemisia, Parietaria, Chenopodium, Dermatophagoides pteronyssinus,* olive	Pistachio, chard, cabbage, spinach, sunflowers seeds, greens beans, banana
5	*Artemisia, Parietaria, Chenopodium*	Pistachio, celery, hazelnut, peanut, beans, peas, walnut
6	*Artemisia, Parietaria, Chenopodium*	Chestnut, sunflower seeds, grape banana, almonds, peanuts, hazelnuts, walnuts
7	*Parietaria,* grasses, olive	Almond, hazelnut, peanut, grapes peaches
8	*Artemisia, Parietaria, Chenopodium* grases, olive	Pistachio, almond, hazelnut, peanut, chesnut, pine kernels, sunflowers seeds
9	*Artemisia,* grasses	Garlic, almond, hazelnut, chestnut, sunflowers seeds
10	*Artemisia, Parietaria Chenopodium* grasses, olive	Almond, hazelnut, peanut, date, walnut, sunflower seeds, pine kernels

foods. Hernández *et al.*[16] described the coincidence of pollinosis and allergic reactions to fruits and leaf vegetables. Further authors[8, 17, 18] have studied the association of pollinosis and *Anacardiaceae* allergy. There have been studies on allergic sensitization to cashew pollen[19], as well as on the possible cross-reactivity between its antigens and other pollens[20].

Although there are few studies of anaphylactic-type reactions induced by physical exercise in relation to the ingestion of *Anacardiaceae*[21, 22], there can be no doubt that such reactions will be reported in increasing numbers with the passage of time.

Mango is one of the tropical fruits which has been known for the longest time. The green fruits are prepared as preserves and are used in the preparation of jams, jellies and syrups. They are also used as aromas in bakery, ice cream and meat products. An oil is obtained from the seed. Mango is also used as a dentifrice because of its potent enzymatic power, and for the treatment of diarrhoea. It is a fruit with a high protein content which may give it a great allergenic potency.

Besides these three, other plants belonging to this botanical family are poison ivy, poison oak, poison sumac and the Japanese or lacquer sumac, and cell-mediated allergic reactions to these species have been reported.

The aim of the present study was to confirm the presence of IgE-mediated sensitization to pistachio, cashew nut and mango, to identify a number of common allergens, and to demonstrate cross-reactivity between these fruits.

PATIENTS AND METHODS

Patients. Ten patients were studied, five males and five females, with ages ranging between 16 and 40 years, with reported clinical histories suggestive of hypersensitivity reactions to pistachio. All ten patients had a previous history of pollen rhinitis/asthma, in nine cases with positive prick tests with *Parietaria* and in nine with *Artemisia* (ragweed). Nine of the patients had antecedents of fruit or nut sensitisation. As for the clinical symptoms of pistachio hypersensitivity, six patients reported an oral allergy syndrome (OAS), consisting of pruritus and more or less important oedema of the lips and oral mucosa; two patients had had OAS with subsequent urticaria-angioedema, and the other two urticaria-angioedema alone, in one case with glottic involvement. Ten control subjects were included in the study, of which five were atopic and five non-atopic. Table I summarises the positive prick tests with airborne allergens and foodstuffs which the patients presented in their past histories.

Skin tests. The skin tests were performed using the *prick by prick* technique with fresh extracts of mango pulp and skin. Fluid pastes were prepared with pistachio, cashew nut and mango seeds, and the skin tests were performed using the same technique. At the same time, prick tests were performed with a conventional series of inhalant allergens including pollens of five grasses, *Olea europaea, Artemisia vulgaris, Chenopodium* spp. and *Parietaria judaica*, mites (*Dermatophagoides pteronyssinus* and *D. farinae*), moulds (*Alternaria tenuis, Aspergillus* spp. and *Cladosporium*), and epithelia (rabbit, cat and dog), all commercially acquired from Dome/Hollister-Stier. The skin tests were performed on the volar aspect of the forearm with Hollister-Stier lancets (Prick Lancetter). The results were read after 15 minutes. The reactions were compared with those induced by 10 mg/ml histamine hydrochloride. The reactions to the allergens were considered positive when the diameter of the elicited wheal was equal to or greater than 3 mm. In all the skin tests, negative control tests with normal saline were included.

Extract preparation. Extracts were prepared from pistachio, cashew nut and mango seed, epicarpium (skin) and mesocarpium (pulp). Prior to extraction, the pistachio, cashew and mango seeds were defatted with ethyl ether in a Soxhlet apparatus. The Bjorkstén buffer[23] with added enzyme inhibitors was used for the extractions.

The extractions were carried out through homogenisation of the raw materials with the buffer (at 1:1 weight/volume ratio in the case of the mango seed, pulp and skin, and at 1:2 ratio for the pistachio and the cashew nut), followed by centrifugation at 20,000 g for 30 minutes at ambient temperature. The supernatants were filtered through 0.2 µm filters and dialysed for 20 hours at 4ºC against 10 mM phosphate buffer (pH 7.0) with 3 mM NaN_3. Finally, the extracts were again filtered through 0.2 µm filters and then divided into ali-

Table II. Sera pools, according to their specificities

Pool	Sera	Specifity (RAST class ≥2)
A	2,3	Pistachio, cashew
B	4,6,10	Mango skin, mango pulp, pistachio, cashew
C	1,8,9	Mango skin
D	4,10	Mango pulp, mango skin, pistachio, cashew

Pools "B" and "D" differ only in the exclusion of serum No. 6 (RAST class 0 to mango pulp) from the latter, which was used exclusively for the immunodetection of mango pulp extract.

quots and kept at -20°C until the time of use. For some techniques, for which the required protein concentrations were higher than those in the extracts, one aliquot of the extract was dialysed against water and then lyophilised. The quantity of protein present in the extracts was assessed by the method described by Smith *et al.*[24], with results of 19.76 mg/ml for pistachio, 13.14 mg/ml for cashew nut, 0.68 mg/ml for mango skin, 1.28 mg/ml for mango pulp, and 1.06 mg/ml for mango seed.

For the sensitization of cellulose discs, the extracts were brought to a 1 mg/ml concentration with phosphate buffer (PO_4H_2Na 0.2 M, PO_4HNa_2 0.2 M, NaCl 0.15 M; pH 8.0), and incubated with CNBr[25]-activated discs (40 ml extract solution per g of disc) for 16 hours at 4°C and under agitation. The discs were then washed with the same buffer, 40 ml Tris-HCl 0.1 M buffer (pH 8.0) were added and the discs were again incubated for 1 hour at ambient temperature under agitation. Finally, the discs were washed with a Tween 20 solution (0.05% v/v) in PBS and conserved in phosphate buffer, pH 8.0, at 4°C until used.

Direct RAST. For the assessment of specific IgE to pistachio, cashew nut and mango (skin, pulp and seed), the method described by De Filippi *et al.*[26] was essentially followed. Discs sensitized with *Lolium perenne* and a calibrated serum for this grass were used as controls. The results were expressed as RAST classes.

SDS-PAGE and immunoblotting. These techniques were used for analysing the pistachio, cashew and mango skin and pulp extracts. The mango seed extract was not included in the analyses as no adequate serum was available (according to the RAST results) for the immunodetection of its allergenic proteins. The sera from the patients were pooled according to their specificities as defined by the RAST results. Table II shows the sera contained in each pool.

The proteins were separated by polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate (SDS-PAGE), following the method described by Fling and Gregerson[27] with a 8-to-25% acrylamide gradient. After separation, the proteins were transferred to PVDF membranes (Immobilon-P, Millipore) according to the method of Towbin[28]. After blocking the active sites in the membranes with 1% bovine serum albumin (BSA) in PBS, the membranes were incubated

Table III. Skin test and RAST results with *Anacardiaceae*

Case	Pistachio		Cashew		Mango					
					Skin		Pulp		Seed	
	PT	RAST	PT	RAST	PT	RAST	PT	RAST	PT	RAST
1	9	1	0	0	10	2	8	0	0	0
2	6	2	4	2	10	1	10	0	4	0
3	10	2	0	1	0	0	5	0	0	0
4	5	2	0	1	0	3	0	2	0	0
5	0	0	0	0	5	0	5	0	0	0
6	5	2	0	2	0	3	0	0	5	0
7	6	0	0	0	10	0	0	0	0	0
8	7	0	0	0	6	3	0	0	0	0
9	0	1	0	0	6	3	0	0	0	0
10	6	3	0	2	6	3	6	2	8	1

PT = prick test wheal diameter (mm); RAST class.

overnight at room temperature with the patient sera, diluted 1:3 in PBS containing 1% BSA and 0.05 Tween 20. The membranes were then washed with PBS-Tween 20 and then incubated overnight at room temperature with ^{125}I-labelled HE2 monoclonal mouse anti-human IgE antibody[29]. Finally, the membranes were again washed with PBS-Tween, dried, and autoradiographed.

RAST inhibition. This technique was used for the study of cross-reactivity between pistachio and other *Anacardiaceae.* The procedure described by Yman *et al.*[30] was followed, using discs sensitised with pistachio, cashew and mango skin and pulp extracts as the solid phases and the same extracts and that of mango seed as inhibitors. The tests were carried out with the "B" sera pool (specificity, RAST class ≥2 for mango skin and pulp, pistachio and cashew), so as to have available the most ample possible representation of the various IgE specificities.

A first incubation was carried out with 25 µl of the sera pool and 25 µl of the 2 mg/ml inhibiting extracts (except for the mango seed extract, which was tested at 40 mg/ml concentration) for 30 min at 37°C. The same extract coupled to the disc at 1 mg/ml concentration was used as maximum inhibition control, and 1% BSA as the 0% inhibition control. One extract-sensitised cellulose disc was then added to each well and incubated for three hours at 37°C. After three washings with PBS-Tween, the discs were incubated overnight at room temperature with 50 µl of a ^{125}I-labelled mouse anti-human IgE monoclonal antibody. The discs were finally washed thrice with PBS-Tween and then counted in a Gamma-counter.

RESULTS

Skin tests and RAST. Table III shows the results of the skin tests with the studied members of the *Anacardiaceae* family. Eight patients had positive prick tests to natural pistachio and only one to cashew. As for mango, seven patients had positive tests with mango skin, five had positive tests to mango pulp, and three had positive tests to mango seed. None of the ten control subjects had positive tests with the *Anacardiaceae* extracts. Nine patients had positive prick tests with *Artemisia* and *Parietaria,* and six had positive tests to grass and olive pollens. Only one patient had positive skin prick tests with non-pollen allergens, in this case *Dermatophagoides pteronyssinus.*

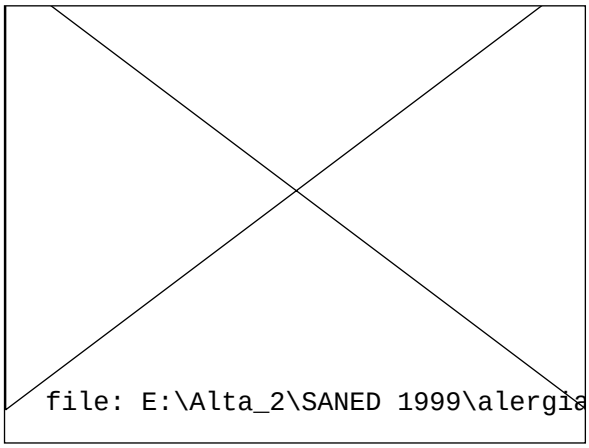

Fig. 1. Electrophoretic patterns of the extracts. The left-hand colum indicates the molecular weights of the proteins included as markers in the same gel: lysozyme (14,4 kDa), soybean trypsin inhibitor (21.5 kDa), carbonic anhydrase (31.0 kDa), ovoalbumin (45.0 kDa), bovine serum albuminm (66.2 kDa) and b-phosphorylase (92.5 kDa). The following amounts of protein were loaded onto each lane: pistachio (P), 125 µg; cashew (A), 125 µg; mango epicarpium (ME), 350 µg; mango mesocarpium (MM), 250 µg. The proteins were stained with Coomassie blue.

The RAST results (expressed as RAST classes) are shown in Table III. Positive specific IgE (RAST class ≥2) to pistachio was present in five patients, to cashew in three, to mango skin in six and to mango pulp in two; there was only one class 1 RAST to mango seed.

SDS-PAGE. The results are shown in Figure 1. Proteins were detected in the 10 - 100 kDa range in all the extracts. In the case of pistachio and cashew, the most abundant proteins are found about the 15, 35 and 50 kDa levels; in mango epicarpium there is a more abundant protein with a molecular weight of *ca.* 24 kDa, and in mango mesocarpium there are three proteins with molecular weights of about 10, 18 and 26 kDa.

Immunoblotting. The results are shown in Figure 2. Generally, it was seen that immunodetection with sera pools with more restricted specificity revealed a lesser number of bands. Thus, the pistachio extract (P) incubated with pool "A" only

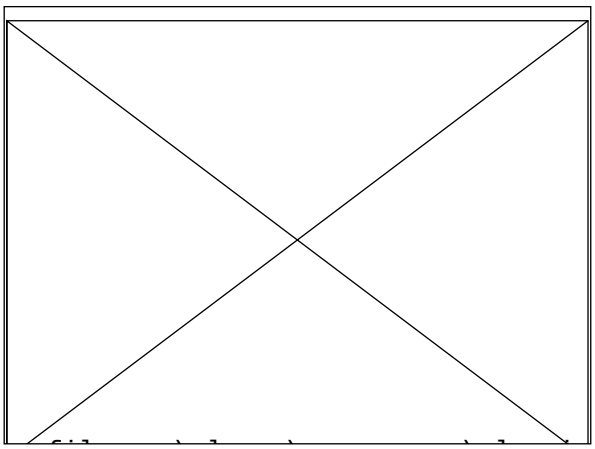

Fig. 2. Immunodetection of the allergenic proteins in the pistachio (P), cashew (A), mango epicarpium (ME) and mango mesocarpium (MM) extracts. On the left are the MW values of the proteins included as markers in the same gel. The letters below the gel indicate the pools of patient sera used.

revealed two bands, with molecular weights of 30 kDa (the strongest one) and 14 kDa (weak), while incubation with the "B" pool revealed, besides these bands, further ones of medium intensity between 40 and 55 kDa. The same was seen with the cashew extract (A): two bands of 29 and 50 kDa MW with pool "A", and further bands with pool "B". In the case of the mango skin extract (ME), incubation with pool "C", which was specific for this extract, yielded only two low MW bands (≈ 10 and 12 kDa), while pool "B" detected a great number of bands, some of them very intense at 43 and 45 kDa. In the mango pulp extract five very intense bands were detected, corresponding to proteins with 9, 41, 43, 70 and 80 kDa MW, besides some weaker ones, when pool "D" was used. No sera specific only for this extract were available.

RAST inhibition. Table IV shows the inhibition values achieved with each of the extracts used as inhibitors, for each of the solid phases used. It should be pointed out that these values might in some instances entail considerable imprecissions, as the differences in c.p.m. between the 0% and 100% values are very small due to the low levels of specific IgE in the sera pools used.

Table IV. RAST inhibition results

Inhibitor	Pistachio	Cashew	Mango		
			Skin	Pulp	Seed
Solid phase					
Pistachio	100	51	97	53	85
Cashew	56	100	100	100	89
Mango skin	35	20	100	34	34
Mango pulp	32	40	80	100	76

Results expressed as % inhibition.

DISCUSSION

Despite the low rate of consumption of members of the *Anacardiaceae* family as compared to other nuts[3], their increasing consumption is leading to a similar increase in the frequency of sensitization. They sometimes cause severe clinical problems (anaphylaxis, glottis oedema), so that many authors recommend that these foodstuff be included in the list of "foods to be considered" in the study of idiopathic anaphylactic reactions. Furthermore, we should not forget the cellular immunity-mediated allergic reactions to members of this botanical family, such as contact dermatitis to poison ivy, poison oak, cashew or mango.

The present study confirms, as already done by other authors, the existence of type I hypersensitivity reactions to members of the *Anacardiaceae* family, both by prick test and by the presence of specific IgE to pistachio, cashew and mango in the patients' sera. The agreement between the skin tests with members of the *Anacardiaceae* family and the RAST results in the present study is moderate, as already pointed out in other studies with this type of fruits[16].

By means of immunoblotting it was shown that the various pools of patient sera recognised various proteins in pistachio, cashew and mango. Generally, it was observed that immunodetection with the pools evidencing more restricted specificity disclosed a smaller number of bands. As in other previous studies[11, 12], the presence of a very intense band of 30 kDa MW was demonstrated, which could represent the major allergen of pistachio.

Our results appear to confirm the existence of

cross-reactivity between the various members of the family. Firstly, there are the prick test results in patients with pistachio allergy, who evidence positive skin tests to other members of the family without the patients having ever eaten cashew or mango. Secondly, the results of RAST inhibition[11] demonstrate the existence of an important allergenic community between the various members of the *Anacardiaceae* family. However, this marked cross-reactivity is not always clinically important, and further studies are required in order to achieve absolute confirmation of the cross-reactivity between pistachio, cashew and mango.

All the patients studied evidenced also seasonal rhinoconjunctivitis, in some cases in association to pollinic bronchial asthma, an association which has been described previously[8, 16-18]. *Artemisia vulgaris* was positive in nine patients, so that the prick test positivity to this pollen might be a valuable orientative datum of a possible food allergy aetiology as responsible for a given allergic picture[16]. The tests with *Parietaria* pollen were also positive in nine patients, an observation that might be explained through a certain degree of cross-reactivity with the *Anacardiaceae,* as demonstrated in several studies[18]. Besides, nine of the patients were also sensitised to fruits or nuts, suggesting that common antigenic determinants are shared with pollens and vegetable foodstuffs.

Although the overall consumption of mango, pistachio and cashew is low in Spain, as compared to other fruits and nuts, we deem it advisable to point out that these fruits may cause IgE-mediated allergic sensitization. The triggered clinical symptoms may be severe. In the case of sensitization to one of these products, it is advisable to avoid all the other members of the *Anacardiaceae* family, because of the possibility of cross-reactivity between them.

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