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Food allergy to pumpkin seed

To the Editor:

Pumpkin (*Cucurbita maxima*) belongs to the Cucurbitaceae family. Its seeds contain large quantities of proteins (35%) and unsaturated fatty acids (50%, mainly linoleic acid and oleic acid), tocopherols, and phytosterols. The seeds are usually consumed toasted as an appetiser, in salads, or as an additive to bread. The flour obtained by grinding the seeds is mixed with water and used as bait by fishermen.[1] Unlike the other members of the Cucurbitaceae family,[2] pumpkin seed is not a common cause of food allergy, and there have been few reports of allergic reactions after ingestion.[1]

We report the case of a 33-year-old man referred to our clinic who, on several occasions, had presented facial oedema and erythema accompanied by a sensation of dyspnoea 15 min after eating toasted pumpkin seeds (*Cucurbita maxima* species). Each episode required emergency medical care with antihistamines and corticosteroids. He tolerated pumpkin, melon, watermelon, and nuts (peanut, hazelnut, almond, sunflower seed, and walnut) with no problems. He also reported facial oedema and erythema after eating peach, apple, unpeeled pear, and orange. He tolerated squeezed juices of these same fruits peeled. He tolerated other fruits and vegetables without problems. He also had a 3-year history of pollen-induced rhinoconjunctivitis, and had taken oral antihistamines on demand. He had never received pollen-specific immunotherapy.

He underwent skin prick testing with the most common commercial pollen extracts in our practice area, namely, profilin, lipid transfer protein (LTP) of peach (30 μg/mL) (ALK-Abelló, Madrid, Spain), fruit, and nuts (Leti, Barcelona, Spain). The patient presented positive results for grasses, olive, cypress, plantain, *Chenopodium*, pumpkin seed, almond, hazelnut, and LTP of peach, melon, and apple. The result of prick-prick testing with pumpkin seed was positive (wheal size, 22 × 20 mm). Prick-prick testing with the two main pumpkin species of the Mediterranean area (*Cucurbita pepo l.* and *Cucurbita maxima duchesne*) were positive.

Total IgE was 1272 kU_A/L. Specific IgE was determined (CAP System, Phadia, Uppsala, Sweden) against grasses (>100 kU_A/L), olive (9.09 kU_A/L), and the recombinant allergens of timothy (*Phleum pratense*): rPhl p 1(72.30 kU_A/L), rPhl p 5 (72.30 kU_A/L), rPhl p 7 (polcalcin), and rPhl p 12 (profilin of *Phleum pratense*) (<0.35 kU_A/L).

The results of determination of specific Ig (ADVIA-Centaur) against apple profilin (Mal d 4), 2S albumin (Sin a 1, the major mustard allergen), vicilin (Len c 1, the major lentil allergen), and chitinases (Prs a 1, a class 1 chitinase of avocado and Cas s 5, a class 1 chitinase of chestnut) were negative. Specific IgE against peach LTP, Pru p 3, was 0.65 kU_A/L.

To confirm the diagnosis, we suggested the patient to carry out a controlled specific challenge test with pumpkin seeds, but he declined the proposal.

In order to study the pumpkin seed allergens recognised by our patient, we first obtained pumpkin seed extract at 10% (w/v) in sodium chloride 1.8%, for 90 min at 4 °C with magnetic shaking. After centrifugation, the supernatant was filtered through 0.2 μm pore size microfilter and kept at −20 °C until used.

Both the extract and the molecular weight markers were analysed using SDS-PAGE (acrylamide content, 16%) under non-reducing conditions, according to the method described by Laemmli.[3] The polyacrylamide gel proteins were electrophoretically transferred to nitrocellulose paper strips,[4] which were then saturated with 5% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 1 h at room temperature and incubated for 18 hs with the patient's serum diluted 1:5. A nitrocellulose strip containing milk thistle extract incubated with 5% BSA in PBS was used as a negative control. The strips were washed in 0.1% Tween-20 in PBS and incubated for 2 h at room temperature with human anti-IgE monoclonal antibody (HE-2) obtained from ascites and diluted 1:3000.[5] The strips were washed again and incubated a third time at room temperature with peroxidase-marked rabbit anti-mouse IgG (RAM-HRP, Calbiochem) diluted 1:5000. Finally, the strips were washed and proteins capable of fixing IgE were detected using chemoluminescence (ECL, Amersham Bioscience) according to the manufacturer's instructions.

Figure 1 shows the results of immunodetection. The patient's serum IgE recognised proteins of different molecular weights in the extract. The most intense band corresponded to a protein weighing approximately 12 kDa.

In order to determine whether the 12-kDa band corresponded to an LTP present in the extract, immunodetection was performed with a specific polyclonal antibody of Pru p 3. However, the polyclonal antibody was unable to recognise any bands in the extract; therefore, it was not possible to demonstrate the existence of an LTP that was homologous to Pru p 3.

Pumpkin seed allergy is rare. Previous studies on allergy to this[2] and other members of the Cucurbitaceae family[6] have pointed to profilin as one of the major allergens. However, although the patient we report had rhinoconjunctivitis caused by grass pollen, he presented neither profilin-specific antibodies, nor antibodies against other food allergens such as 2S albumins, vicilins, or chitinases. He

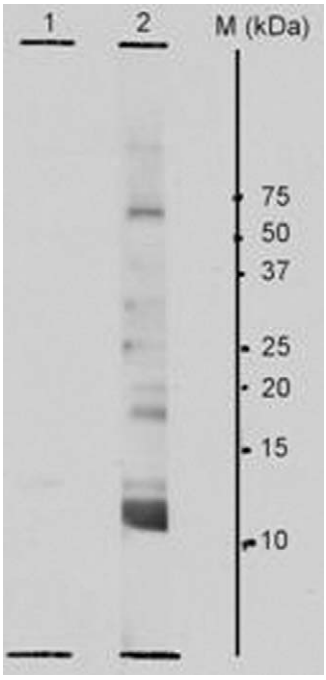

Figure 1 Immunoblot analysis of the binding of the IgE antibodies with pumpkin seed. 1. Negative control, 2. Patient's serum. Numbers on the right indicate the position of molecular markers in kDa.

did present significant, although low, levels of IgE to LTP. Furthermore, in the immunologic work-up, the patient's serum recognised a band of approximately 12 kDa, which could correspond to an LTP of pumpkin seed. Given that pumpkin seeds are usually consumed toasted, as in this case, the allergen causing the symptoms appears to be a heat-stable protein that does not undergo structural modifications at high temperatures. The fact that the polyclonal antibody Pru p 3 recognised no bands tells us that either the 12-kDa protein detected does not belong to the LTP family or this LTP is very different from that of peach. Different degrees of sequence identity, ranging from 30 to 95%, have been found among the family members of different species,[7] and this could explain the low degree of similarity in this case.

In conclusion, we present a case of allergy to pumpkin seed in which an IgE-mediated hypersensitivity mechanism was demonstrated in vivo and in vitro. A protein of approximately 12 kDa seems likely to be responsible for the clinical picture. More complex protein identification methods will be necessary to accurately determine the group of food allergens this protein belongs to.

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