

Anaphylaxis induced by pine nuts in two young girls

Ibáñez MD, Lombardero M, San Ireneo MM, Muñoz MC. Anaphylaxis induced by pine nuts in two young girls.

Pediatr Allergy Immunol 2003; 14: 317–319. ©2003 Blackwell Munksgaard

Pine nuts are the seeds of *Pinus pinea*. There are few reported cases of allergy to pine nut. We describe two young girls with anaphylaxis caused by small amounts of pine nuts. Specific IgE to pine nut was demonstrated by skin prick tests and RAST but no IgE to other nuts and pine pollen was detected. The patients had IgE against a pine nut protein band with apparent molecular weights of ~17 kDa that could be considered as the main allergen. Our patients were monosensitized to pine nut and the 17-kDa protein could be correlated with the severe clinical symptoms.

M. Dolores Ibáñez¹, Manuel Lombardero², Mercedes Martínez San Ireneo¹ and M. Carmen Muñoz¹

¹Servicio de Alergia, Hospital Universitario Niño Jesús and ²Departamento de I+D, ALK-Abelló, S.A., Madrid, Spain

Key words: allergens; anaphylaxis; pine nut

M. Dolores Ibáñez, Servicio Alergia, Hospital Niño Jesús, Avenue. Menéndez Pelayo 65, 28009 Madrid, Spain

Tel.: +34 91 5035935

E-mail: dibanezs@medynet.com

Accepted 18 December 2002

Pine nuts (piñon nuts, pignoli, pignolia) are the seeds of *Pinus pinea*. They are consumed as roasted nuts or added to vegetable salads and to different kinds of sauces. There are few cases described of allergy to pine nut and its allergens have been rarely investigated. We report two cases of allergy to pine nut and the *in vitro* study of the allergens involved.

Case reports

The first case is a 23-month-old girl who had suffered two episodes of angioedema of the eyelids, lips, face and feet, exhibiting moderate respiratory difficulty, in the last 3 months. A third episode happened immediately after sucking pine nuts and the patient experienced angioedema, wheezing and acute dyspnea. This time, she had to be treated in the emergency room with subcutaneous epinephrine and intravenous corticosteroids and antihistamines. After the last attack, the parents related the first two reactions with the intake of small amounts of pine nuts. The patient tolerated normal food in her diet, including almond, walnut, hazelnut and other nuts.

The second patient is a 15-year-old girl who had had, when she was 4 years old, severe urticaria and angioedema of the lips and eyelids and laryngeal edema with hoarseness and dizzi-

ness after ingestion of a single pine nut. When she was 14 years old, she suffered an episode of anaphylaxis with vomiting, itching of the mouth, edema of the tongue and larynx, general urticaria, severe respiratory difficulty, cyanosis and thoracic pain after eating a sausage outside the home environment. She needed treatment in the emergency room with epinephrine and corticosteroids. The sausage may have contained pine nuts. She tolerated all foods including peanut, almond, sunflower seed and the rest of tree nuts.

Methods and results

Pine (*Pinus radiata*) pollen and nut extracts were prepared by extraction in 1.8% NaCl at 10% w/v ratio with magnetic stirring for 90 min at 4°C. After centrifuging, the supernatants were dialyzed, filtered through a 0.22 µm pore filter and used for skin testing (after 50% glycerination) and *in vitro* techniques.

Skin prick tests (ALK-Abelló S.A., Madrid, Spain) were performed with common allergens (grass, tree and weed pollens, mites, molds, epithelia and latex) and food (peanut, almond, hazelnut, chestnut, walnut, sunflower seed, egg, milk, and fish) and negative results were obtained in both cases. A skin prick test with pine pollen extract was also negative but a prick with a pine nut was positive (7 and 10 mm, respectively).

A prick-by-prick test with a pine nut was positive with a wheal of 12 × 16 mm in the first case, and 9 × 17 mm in the second case.

Total serum IgE was 70 and 200 kU/l in each case. Specific IgE (CAP System, Pharmacia, Uppsala, Sweden) against peanut, almond, hazelnut, chestnut, walnut, sunflower seed, pistachio, latex, echinococcus and inhalants was negative in both cases. Specific IgE (direct RAST using paper disks sensitized to the corresponding extracts) against pine nut was positive in both cases (7 and 1.7 kU/l, respectively) and negative for pine pollen. IgE immunodetection was carried out using enhanced chemiluminiscence (Amersham Pharmacia Biotech, Amersham, UK) after SDS-PAGE of pine nut extract under nonreducing conditions and electrotransfer of proteins from the polyacrylamide gel to nitrocellulose membranes using standard techniques (1). Figure 1 shows the results of the IgE-immunodetection experiment. The patients (notably case 1) had IgE against a pine nut protein double band with apparent molecular weights of ~17 (main)/14 kDa. Other bands of higher molecular weights were also detected more weakly although they have some nonspecific binding (lane 3, Fig. 1).

Discussion

There are few reported cases of allergy to pine nut, although many of them are anaphylactic reactions (2–9). Oral challenge tests were positive in four of the reported cases, two of them in children (7). In our cases, the severity of the symptoms and their clear relationship with pine nut advised against the challenge test. They suffered a very severe life-threatening anaphylactic reaction after eating a small quantity of this nut. We suspect that the second patient had an anaphylactic reaction due to a hidden pine nut content in the sausage. Near fatal reactions due to eating hidden allergens are not rare. There are many reports on peanut, an aggressive hidden allergen, but it is necessary to keep in mind that other nuts are able to induce this type of reaction. It is very important to mark clearly on food labels the amount of each potential allergen.

There are some publications that describe cross-reactivity between pine nut and other nuts (4,6) or pine pollen (8). In our cases, the two young girls were monosensitized to pine nut. We have demonstrated specific IgE to pine nut by a standard skin prick test, prick by prick and RAST, but no IgE to other nuts and pine pollen was detected. These data suggest that the allergen or allergens involved in the patients' reactions do

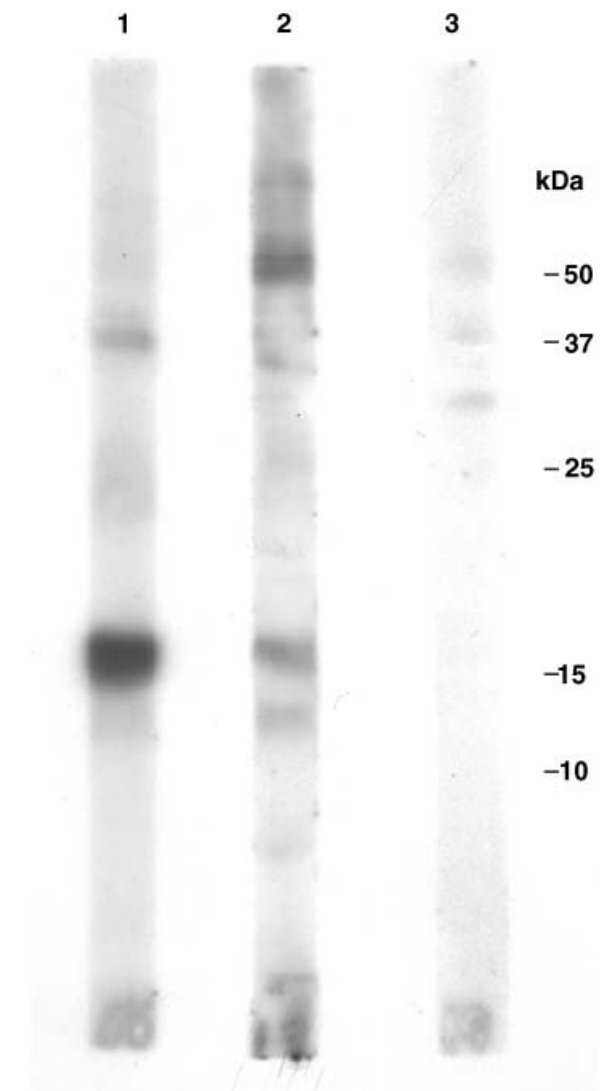


Fig. 1. IgE immunoblotting of pine nut extract with the patient's sera. Next, 70 µg of pine nut protein was loaded per lane in the SDS-PAGE and a 1/10 dilution of the sera was used. 1: case 1; 2: case 2; 3: negative control (nonatopic serum). On the right, the molecular weight in kDa, obtained from prestained markers run in parallel, is indicated.

not cross-react with allergens from pine pollen or other nuts.

Allergens of pine nut have been studied and characterized by immunoblotting assays in a few reports. Some of them described pine nut allergens with molecular weights of 50 and 66–68 kDa. De las Marinas et al. (4) reported an important allergenic band with a molecular weight of 50 kDa, which disappeared after blotting inhibition with an almond extract. Other authors (6) found bands of 30 and 44 kDa that disappear in the inhibition of immunoblotting with peanut.

The prevalence of sensitization to the pollen of *Pinus pinea* is low even in areas where this tree is

widely distributed. Pine nut allergy has been described in only one patient with pine pollinosis but the allergens were not studied in this case (7). Senna et al. (8) described a pine nut-allergic patient with pine pollen-specific IgE but without clinical symptoms of allergy to this pollen. In this study, soluble pine nut extract caused the disappearance of any reactivity against pine pollen extract components in the immunoblotting, but soluble pine pollen extract only inhibited IgE binding of the serum against allergens of pine nut with a molecular weight greater than approximately 35 kDa.

In agreement with a previous study by Garcia-Menaya et al. (9), a 17-kDa allergen band was detected in our study. Their patient, like our patients, was not sensitized to pollen or other nuts, and it is possible that this protein could be considered as the main pine nut allergen. In contrast to that work, we were able to electrotransfer the pine nut proteins from the SDS-PAGE gel to nitrocellulose membranes using standard methods (1). A possible explanation for this is that we used methanol in the transfer buffer.

In summary, we describe two cases of anaphylaxis induced by pine nut due to an IgE-mediated reaction, which was demonstrated by both *in vivo* skin tests and *in vitro* tests. Our patients were monosensitized to pine nut and the 17-kDa

protein could be correlated with their severe clinical symptoms.

References

1. TOWBIN H, STAEGELIN T, GORDON J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 1979; xx: 4350–4.
2. SANTOS JM, UNGER L. Severe allergic reaction to pignolia nut. *Ann Allergy* 1958; 16: 459–61.
3. FINE AJ. Hypersensitivity reaction to pine nuts. *Ann Allergy* 1987; 59: 183–4.
4. DE LAS MARINAS D, VILA L, SANZ L. Allergy to pine nuts. *Allergy* 1998; 53: 220–2.
5. KOEPKE JW, WILLIAMS PB, OSA SR, DOLEN WK, SELNER JC. Anaphylaxis to piñon nuts. *Ann Allergy* 1990; 95: 473–6.
6. AÑÓ MA, MASELLI JP, SANZ ML, FERNÁNDEZ-BENÍTEZ M. Allergy to pine. *Allergol Immunopathol* 2002; 30: 104–8.
7. ARMENTIA A, QUINTERO A, FERNÁNDEZ-GARCÍA A, SALVADOR J, MARTÍN-SANTOS JM. Allergy to pine pollen and piñon nuts: review of three cases. *Ann Allergy* 1990; 64: 49–53.
8. SENNA G, RONCAROLO D, DAMA A, MISTRELLO G. Anaphylaxis to pine nut and immunological cross-reactivity with pine pollen proteins. *Invest Allergol Clin Immunol* 2000; 10: 44–6.
9. GARCIA-MENAYA JM, GONZALO-GARIJO MA, MONEO I, FERNÁNDEZ B, GARCÍA-GONZÁLEZ F, MORENO F. A 17-kDa allergen detected in pine nuts. *Allergy* 2000; 55: 291–3.