Allergens responsible of Olive fruit ingestion anaphylaxis

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Olive fruit is widely consumed as food and it is also used as crude material to obtain olive oil. In spite of its common intake, cases of allergy to olive fruit have been seldomly reported [1-4]. Some olive fruit allergens have been identified as the thaumatin-like protein Ole e 13, which is responsible of occupational allergy in olive oil mill workers [5]. Most recently, a study has identified the presence of 7S globulins (vicilins) in olive seeds at both the transcriptomic and biochemical level [6].

We present a case of anaphylaxis after eating olive in brine (water, salt, spices and garlic) where two IgE reactive proteins were detected, the olive thaumatin-like protein Ole e 13 and an olive protein which share peptides with the vicilin Ara h 1.

A 19-year -old female, with a previous atopic dermatitis in childhood and persistent mild rhinoconjunctivitis and intermittent bronchial asthma. She was referred to our Unit due to oral itching, generalized urticaria, lips and eyelids edema, dyspnea, sweating and dizziness with an emetic episode. She had eaten olive in brine 30 minutes before the symptoms appeared. She tolerates spices, garlic, olive oil in different foods (salads, toasts…), and there were no associated co-factors such as medications, alcohol or exercise.

The patient underwent skin prick tests (SPTs) with our allergens battery (mites, pollens, molds, latex, *Anisakis*, dander from cat, dog and horse and Pru p 3) with positive results (a wheal ≥3 mm was considered to be positive in the presence of a negative response to

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the saline control) to *Dermatophagoides pteronyssinus*, cat and dog dander, and *Olea europea* and grass pollen. Prick-by-prick test with spices (pepper, cumin, oregano, thyme) and garlic were negative.

We performed prick-by-prick test with olive fruit, both fresh and in brine. The results were positive, with wheals measuring 9mm with raw olive and 8mm with olive in brine. As prick test with raw olive was positive an open oral challenge with olive oil was performed with negative results.

Allergen microarray immunoassay with 112 allergens (ImmunoCAP ISAC^{TM}; Phadia, Thermo Fisher Scientific Uppsala, Sweden) was carried out and the results were positive exclusively to (ISU-E): Ole e 1: 11, Phl p 1: 1.1, Can f 1: 1.4, Fel d 1: 19, Der f 1: 1.6, Der f 2: 5.2, Der p 1: 17, Der p 2: 11 and Der p 23: 2.7.

Extracts from fresh olive and olive in brine were prepared by delipidation with acetone, homogenization in phosphate buffered saline (20% W/V) (50 mM phosphate buffer, 100 mM NaCl, pH 7.5) with magnetic stirring, centrifugation to remove the non soluble materials, dialysis of the supernatants against distilled water and lyophilization.

SDS-PAGE was carried out according to the method described by Laemmli [7], under reducing conditions (with 2-mercaptoethanol). Subsequently the separated proteins were electrotransfered onto polyvinylidene difluoride membrane (Immun-blott® PVDF Membrane, Bio-Rad, USA) and incubated overnight with both the patient serum and a negative control serum (various sera from non atopic subjects). Then the membrane was incubated with the secondary antibody (anti-human IgE mouse monoclonal antibody; Southern Biotech), and IgE binding bands revealed by chemiluminescence method following manufacturer´s instructions (Amersham^{TM} ECL Prime Western Blotting. GE Healthcare).

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The IgE-binding analysis revealed an area between > 100 kDa- 45 kDa and a band of approximately 20 kDa in fresh olive extract, and IgE-reactive bands with molecular masses of about 70 kDa and 21 kDa in olive in brine extract (Figure 1).

Protein was identified by mass spectrometry, as previously reported [8], as well as by searching a nonredundant protein sequence database (NCBI) using the Mascot program (http://www.matrixscience.com) in the Proteomic Service of Complutense University of Madrid, which is a member of the ProteoRed Network. Research conducted with protein databases identified 21kDa IgE-binging band as thaumatin-like protein from *Olea europaea* (Ole e 13) and 70 kDa IgE binding band as a Conarachin-like protein (similar to the 7S globulin Ara h 1).

We present a patient with an anaphylactic reaction after the ingestion of olive in brine where a pair of IgE-reactive proteins have been detected, a thaumatin-like protein (already reported, Ole e 13) and a storage protein (Conarachin-like protein) as a probable new allergen. We detected similar IgE-binding profile in fresh and olive in brine extracts.

After olive ingestion anaphylaxis [2,3] and urticaria [4] have been described in the literature. Skin prick test and specific IgE have been used for diagnosis. The described cases have in common with our patient the sensitization to olive pollen and olive oil tolerance probably due to the absence or alteration of proteins during the milling and extraction process.

Ole e 13 is a thaumatin-like protein of 21 kDa, which has been described to be responsible for job-related respiratory allergy in an olive oil mill worker who does not experience allergic symptoms after ingestion of edible olives [9]. Some authors have related allergy to olive ingestion with lipophilic proteins, however in our case the IgE reactive proteins are hydrophilic [4].

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Vicilins (7S globulins, storage proteins from cupin superfamily) are well known legume allergens that could cause ingestion anaphylaxis [10] and there is evidence of the presence of 7S vicilins in olive seeds[6]. The tolerance to olive ingestion showed by the Ole e 13-sensitized patient described by M. Torres *et al.* [9]and the anaphylactic character of the vicilin proteins led us to point to the 70kDa-IgE-reactive protein detected as the most probable cause of the allergic reaction in our patient.

Since our patient does not report symptoms with any other foods, including fruits, legumes, spices, nuts, or seeds, we only recommend avoiding the intake of olives.

In conclusion, we present the first case of allergy to olive fruit ingestion where the probable allergens have been identified.

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Figure 1. SDS-PAGE Immunoblotting. A) Fresh olive extract. B) Olive in brine extract. Lane P: patient serum Lane C: control serum (pool of sera from non atopic subjects) Lane M: Molecular mass standard.