

Allergens Responsible for Anaphylaxis After Olive Fruit Ingestion

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Olive is widely consumed as a food and used as raw material to obtain olive oil. Despite its widespread intake, cases of allergy to olive have seldom been reported [1-4]. The olive allergens identified to date include the thaumatin-like protein Ole e 13, which is responsible for occupational allergy in olive oil mill workers [5]. The presence of 7S globulins (vicilins) has also been identified in olive seeds at both the transcriptomic and the biochemical level [6].

We present a case of anaphylaxis after consumption of olive in brine (water, salt, spices, and garlic), where 2 IgE-reactive proteins were detected, namely, the olive thaumatin-like protein Ole e 13 and an olive protein that shares peptides with the vicilin Ara h 1.

The patient was a 19-year-old woman with atopic dermatitis in childhood and persistent mild rhinoconjunctivitis and intermittent bronchial asthma. She was referred to our unit with oral itching, generalized urticaria, lip and eyelid edema, dyspnea, sweating, and dizziness with vomiting. She had eaten olive in brine 30 minutes before the onset of symptoms. She tolerates spices, garlic, and olive oil in different foods (salad, toast), and there were no associated cofactors such as medications, alcohol, or exercise.

The patient underwent skin prick tests (SPTs) with our allergen series (mites, pollens, molds, latex, *Anisakis*, dander [cat, dog, and horse], and Pru p 3). These yielded positive results (a wheal ≥ 3 mm was considered to be positive in the presence of a negative response to the saline control) to *Dermatophagoides pteronyssinus*, cat and dog dander, and *Olea europaea* and grass pollen. Prick-by-prick testing with spices (pepper, cumin, oregano, thyme) and garlic were negative.

We performed prick-by-prick testing with olive, both fresh and in brine. The results were positive, with wheals measuring 9 mm with raw olive and 8 mm with olive in brine.

As the prick test with raw olive was positive, an open oral challenge with olive oil was performed. The challenge yielded negative results.

Allergen microarray immunoassay with 112 allergens (ImmunoCAP ISAC, Phadia, Thermo Fisher Scientific) yielded positive results (ISU-E) only to the following allergens: 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% wt/vol) (50 mM phosphate buffer, 100 mM NaCl, pH 7.5) with magnetic stirring, centrifugation to remove the nonsoluble 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). The separated proteins were subsequently electrotransferred onto a polyvinylidene difluoride membrane

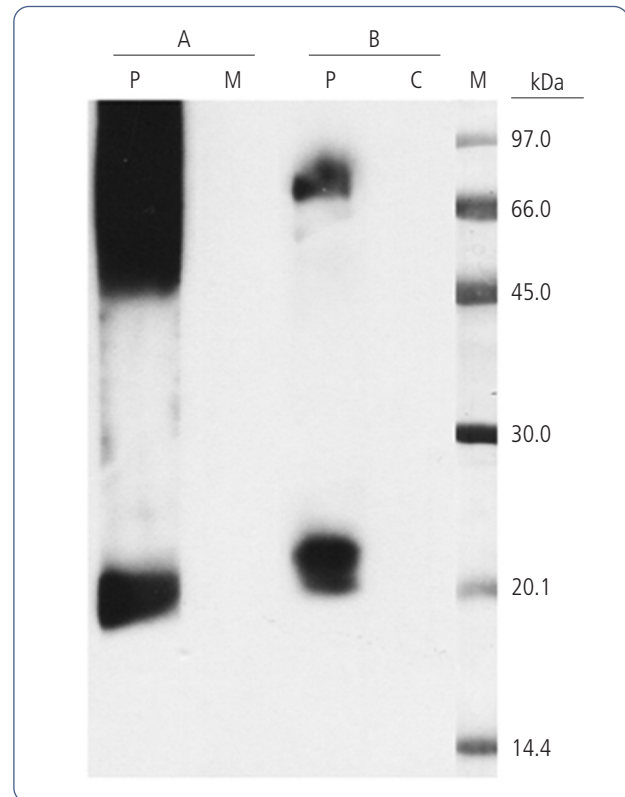


Figure. SDS-PAGE immunoblotting. A, Fresh olive extract. B, Olive in brine extract. Lane P, patient serum; Lane C, control serum (pool of sera from nonatopic individuals); Lane M, molecular mass standard.

(Immun-blott PVDF Membrane, Bio-Rad) and incubated overnight with both patient serum and negative control serum (various sera from nonatopic individuals). The membrane was then incubated with the secondary antibody (antihuman IgE mouse monoclonal antibody; Southern Biotech), and IgE-binding bands were revealed by chemiluminescence following the manufacturer's instructions (Amersham ECL Prime Western Blotting, GE Healthcare).

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

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 revealed the 21-kDa IgE-binding band to be a thaumatin-like protein from *Olea europaea* (Ole e 13) and the 70-kDa IgE-binding band to be a conarachin-like protein (similar to the 7S globulin Ara h 1).

We report the case of a patient who experienced an anaphylactic reaction after ingestion of olive in brine, in which a pair of IgE-reactive proteins were detected, namely, a thaumatin-like protein (already reported, Ole e 13) and a storage protein (conarachin-like protein) as a probable new allergen. We detected a similar IgE-binding profile in extracts of fresh olive and olive in brine.

Ingestion of olive has been followed by anaphylaxis [2,3] and urticaria [4]. Diagnosis of allergy to olive is based on SPT and determination of specific IgE. The characteristics these cases shared with the case we report include sensitization to olive pollen and tolerance of olive oil, probably due to the absence or alteration of proteins during the milling and extraction process.

Ole e 13 is a 21-kDa thaumatin-like protein that was responsible for job-related respiratory allergy in an olive oil mill worker who did not experience allergic symptoms after ingestion of edible olives [9]. Some authors have associated allergy after olive ingestion with lipophilic proteins, although in the case we report, the IgE-reactive proteins were hydrophilic [4].

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

Since the patient did not report symptoms with any other foods, including fruits, legumes, spices, nuts, and seeds, we only recommended avoiding the intake of olives.

In conclusion, we present the first case of allergy after ingestion of olive fruit where the probable allergens were identified.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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