Anaphylactic shock to Mediterranean silverside (Atherinaboyeri) caused by non-parvalbumin allergens

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Food allergy represents a growing worldwide health problem. Fish are among the most common triggers of reaction. The global consumption of fish has gradually increased in daily diet [1]. The estimated prevalence of food fish allergy ranges from 0% to 7%, differing according to geographical areas[2]. The subjacent pathophysiologic mechanism is mainly IgE-mediated, where symptoms range from urticaria to anaphylaxis. Sensitization usually starts during childhood and persists lifelong, having avoidance as the only therapeutic option [1, 3]. Even IgE non-mediated reactions (including food protein-induced enterocolitis syndrome) have been associated with the fish intake [2, 4]. Parvalbumin, an acidic calcium-binding protein that is resistant to heat and digestive enzymes is considered a major fish allergen with high cross-reactivity across fish species [1, 5].

Mediterranean silverside (*Atherinaboyeri*) is a fish from the Atherinidae family, belonging to Atherina genus. *A. boyeri*is also named pejerrey and tolerates low-salinity water, living in the Mediterranean and other seas. Here we

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report the case of a patient with a history of immediate hypersensitivity systemic reaction after ingestion of pejerrey, with tolerance to other fish, whose allergologic study suggests an IgE-mediated sensitization to allergens different from parvalbumins. To our knowledge, there is only one previous publication reporting two cases of allergy to pejerreywith tolerance to other fish[6].

A 49-year-old man with history of atopy (drug allergy and food allergy to peach), who, just after intake of a fried pejerrey, experienced dysphonia, dysphagia, itching in the genital area, malar angioedema, and syncope. In the emergency department, blood pressure was 60/40 mmHg and oxygen saturation was 95%. He was treated with intravenous 200 mg of hydrocortisone and 5 mg of dexchlorfeniramine, 0.5 mg of intramuscular epinephrine, and fluid therapy, improving within a few hours. Serum tryptase was not measured during the reaction. Afterwards, he was referred to our allergy department. With a detailed anamnesis, simultaneous use of other drugs, as well as the presence of possible cofactors, were excluded. Since the reaction, the patient avoided ingestion of pejerrey, remaining tolerant of other fish species.

We performed a skin prick-test with a battery of commercial raw fish (tuna, sardine, cod, and hake) and *Anisakis simplex* extracts and prick-by-prick tests with cooked and raw pejerrey. Serum specific IgE to cod, tuna, anchovy and sardine, was determined by the Immulite System and specific IgE to *Anisakis simplex*, hake and recombinant parvalbumins (rGad c 1 and rCyp c 1) were determined by ImmunoCAP.

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Protein extracts from pejerrey body (PB) and pejerrey head and visceras (PHV) were prepared by homogenization in phosphate buffer saline, dialyzation, and lyophilization. SDS-PAGE immunoblotting was carried out in reducing conditions (with 2-mercaptoethanol) with pejerrey extracts, as described by Laemmli[7], using a patient serum dilution of 1/3, a secondary antibody dilution of 1/10000 (SouthernBiothec) and a chemiluminescent detection method (GE Healthcare UK Limited).

The skin prick-tests to all fish extracts tested and *A. simplex* extract was negative. Prick-by-prick tests to cooked and raw pejerrey were positive (10 mm and 7 mm of maximum wheal, respectively). Serum specific IgE to *A. simplex*, cod, hake, tuna, anchovy, sardine, and recombinant parvalbumins (rGad c 1 and rCyp c 1) were all negative.SDS PAGE immunoblotting with PHV extract revealed two IgE-reactive bands, a broad band of 60–55kDa and another of 34-kDa (Figure 1). However, no bands were detected with the pejerrey body extract.

According to the clinical history and *in vivo* and *in vitro* positive results, the patient was diagnosed with an anaphylactic reaction to pejerrey with tolerance to other fish species, and sensitization to allergens different from parvalbumin. Subsequently, we advised the patient to avoid the intake of pejerrey, checking the ingredients listed for all kinds of foods that might contain this ingredient, allowing the ingestion of other fish species.

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Since 1969, parvalbumin has been recognized as the major fish allergen. Parvalbumin is a low molecular weight protein (10–12 kDa) that is water-soluble and calcium-binding in muscle, thermally stable, and able to preserve its allergenic activity even under acidic conditions and after pepsinolysis. The high amino acid sequence homology present among the parvalbuminsof different fish species is responsible for the common cross-reactivity among them. In fact, IgE-binding parvalbumins have been detected in over 90% of fish allergic patients[5, 9]. However minor allergens, such as fish enolase, aldolase, and gelatine, have been demonstrated to be a trigger of monosensitivity or oligosensitivity IgE-mediated reactions to exclusive fish species, without parvalbumin IgE sensitization, and various publications have reported clinical cases of monosensivity or oligosensitivity to specific fish species, highlighting the role of new fish allergens different from parvalbumin [8, 9, 10].

In 2009, Kuehn *et al.* published an anecdotal clinical case of an anaphylactic reaction by the ingestion of marshmallows containing fish gelatine. *In vitro* study detected protein bands of 110 and 210 kDa corresponding to the hetero α-chains and β-chain of tuna collagen, whereas parvalbumin was not identified [8]. Kuehn also reported the first cases of sensitization to fish aldolase (40–50 kDa) and enolase (20 kDa) in patients with a history of an allergic reaction to cod, with a negative parvalbumin *in vitro*study[9]. Subsequently, the importance of fish enolase and aldolase as new fish allergens has been investigated. According to the IgE binding patterns to parvalbumins, fish allergic

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patients have been classified into three IgE-clusters, detecting up 28% of patients that recognize only IgE to enolase, aldolase, and fish gelatine [10]. In 2014, Gonzalez-Mancebo*et al.* published two cases of anaphylaxis to silverside (*Atherinaboyeri*). UsingSDS-PAGE immunoblotting and mass spectrometry in tandem, these authors identify bands of 28.5 kDa and 38 kDa in pejerrey body, corresponding to triosephosphate isomerase β and glyceraldehyde-3-phosphate dehydrogenase (GADPH), respectively [6].

To our knowledge, we report the third described case of anaphylaxis to pejerrey with tolerance to other fish. SDS-PAGE immunoblotting detected IgE reactive bands (55–60 kDa and 34 kDa) in the head and viscera extract of pejerrey, but no bands were detected in the fish muscle. No IgE to recombinant parvalbumins nor binding bands of its molecular weight (12 kDa) were neither detected. Even if an oral challenge test with pejerrey has not been conducted, *in vivo* and *in vitro* studies suggest an IgE-mediated anaphylactic reaction to pejerrey in this patient, with recognition of two allergens different from parvalbumin.

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Conflicts of interest

All authors declare that they have not conflicts of interest.

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Previous Presentations

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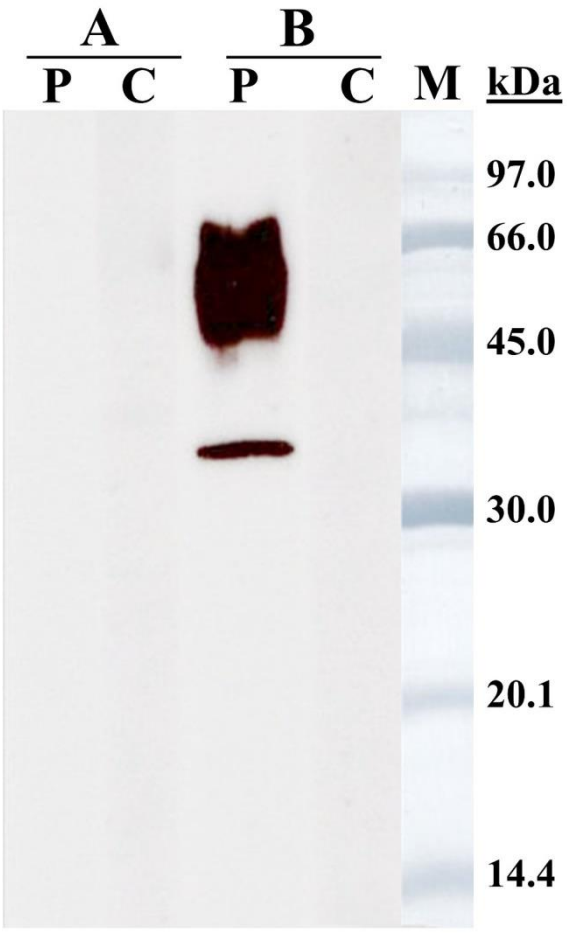
Figure 1. SDS-PAGE Immunoblotting.



A) Pejerrey body extract B) Pejerreyhead+visceras extract. Lane P: patient serum. Lane C: control serum (pool of sera from nonatopic subjects). M: Molecular mass standard.

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