

CORRESPONDENCE

Clinical monosensitivity to salmon and rainbow trout: a case report

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To the Editor,

Seafood plays an important role in human nutrition; the increasing consumption of certain fishes due to their healthy effects has led to a raising in the number of cases related to allergic reactions (1).

The major fish allergen is parvalbumin (2), an acidic, calcium-binding 12 kDa protein resistant to heat and gastrointestinal digestion. Fish allergic patients are often sensitized to more than one species due to the cross-reactivity of their beta-parvalbumins. However, until now, only few cases of monosensitivity to fish have been described (3–7).

We report the case of a 14-year-old boy with a history of food allergy (milk) from the first year of life. At age 4, he presented his first allergic reaction to fish, after ingesting a few appetizers containing smoked salmon. In a few minutes, he suffered from rush cutaneous and lip edema, which regressed after oral administration of corticosteroids. Skin prick tests, performed with commercial extracts (Lofarma, Milano, Italy), and prick by prick tests showed negative results to mollusks, crustaceans, and fishes, including salmon.

Two years later, few minutes after the ingestion of broiled salmon, he showed a new allergic reaction including lip and nose edema, which regressed after cortisone treatment.

At the age 11, a small quantity of broiled rainbow trout (Salmonidae) determined a sudden and severe intense throat itching; this reaction regressed spontaneously in a few minutes. After this new event, the boy stopped consuming salmon, rainbow trout, and other freshwater fishes. He had safely consumed codfish, halibut, anchovies, tuna fish, and mackerel, apart from a case of reaction to fish sticks (containing only cod fish) where a cross-contamination with salmon in the production chain was hypothesized. As monosensitization to fish is quite unusual, the patient was tested at molecular level to confirm the specific pattern of reactivity by immunoblotting. IgE multiplex test for the most usual allergens showed a positive response only for *Gadus callarias* (cod fish) muscle IU = 0.03. Further investigation by ISAC test showed the presence of serum-specific IgEs versus the allergens Onc m from rainbow trout (*Oncorhynchus mykiss*) ISU = 2.10 and Sal s from salmon (*Salmon salar*) ISU = 2.22. ISAC tests that were reported negative were obtained with fishes from the families of *Gadidae* (cod fish, Gad m), *Soleidae* (sole, Sol so), and *Scombridae* (tuna and similar fishes, Thu a). Finally, a negative result was found for *Anisakis simplex* (Ani s).

Proteins from codfish (*Gadus morhua*), salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), sole (*Solea vulgaris*), and tuna fish (*Thunnus albacares*) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions. Fish species were from a local supermarket and were analyzed as such (raw) and cooked

(boiled in water at 100°C for 20 min) to simulate the usual domestic preparation. All the samples were freeze-dried, ground, and the resulting flour was suspended in sample buffer (containing 0.063 M TRIS-HCl pH 6.8, 1.88% glycerol, 0.5% SDS, 2.5% β -mercaptoethanol) at a final concentration of 7.5 mg/ml (w/v), corresponding to 4 mg/ml of proteins approximately. A pre-stained molecular weight standard solution (broad range; Bio-Rad, Richmond, CA, USA) was run in parallel to the samples.

SDS-PAGE and immunoblotting were performed according to Ballabio et al. (8). Briefly, after SDS-PAGE, proteins were transferred to a PVDF membrane by Western blotting, blocked, and washed with gelatin solutions to prevent non-specific adsorption of the immunological reagents.

The membrane was then immersed in 10 ml of 0.25% gelatin solution containing 0.3 ml of the patient's serum. Antigen-IgE complexes were detected using 10 μ l of goat antihuman IgE antibodies labeled with alkaline phosphatase in the presence of the specific substrates. To verify the specificity of immunoreactivity, immunoblotting was performed under identical conditions using the serum of two children highly allergic to foods different from fish (results not shown) with faint unpecific immunoreactivity.

Figure 1 shows the electrophoretic profiles of fishes included in the study, tested as such (left panel) and after boiling (right panel). All the samples yielded a complex protein pattern, with some differences from the qualitative and quantitative point of view. Raw codfish, rainbow trout, and salmon showed some protein bands having low molecular weight (MW); in particular, two bands (L and M) were less abundant or absent in sole and tuna fish. Parvalbumin was only visible in codfish and rainbow trout (band L, MW 12–14 kDa).

Cooked samples presented several differences in their electrophoretic pattern when compared to raw fish; in particular, cooked samples showed a larger number of protein bands in the MW region from 6 kDa to 30 kDa. A band having a MW of about 12–14 kDa, corresponding to parvalbumins, was clearly evident in all the samples analyzed, although the intensity of this band in the sole was very low (band L).

Immunoblotting analysis (Fig. 2) showed that serum allergen-specific IgEs were mainly reactive for two protein bands having a molecular weight of approximately 35 kDa (G) and 75 kDa (C) in raw salmon. These two protein bands were also immunoreactive in the other fish species tested, with the exception of codfish, in which the IgE recognized the 35 kDa (G) protein and a second band having a MW > 100 kDa (B). Several bands were recognized by serum IgEs in rainbow trout after cooking: A (170 kDa), B (120 kDa), C (75 kDa), E (50 kDa), F (40 kDa), G (35 kDa), and H (30 kDa). These IgE-reacting proteins were

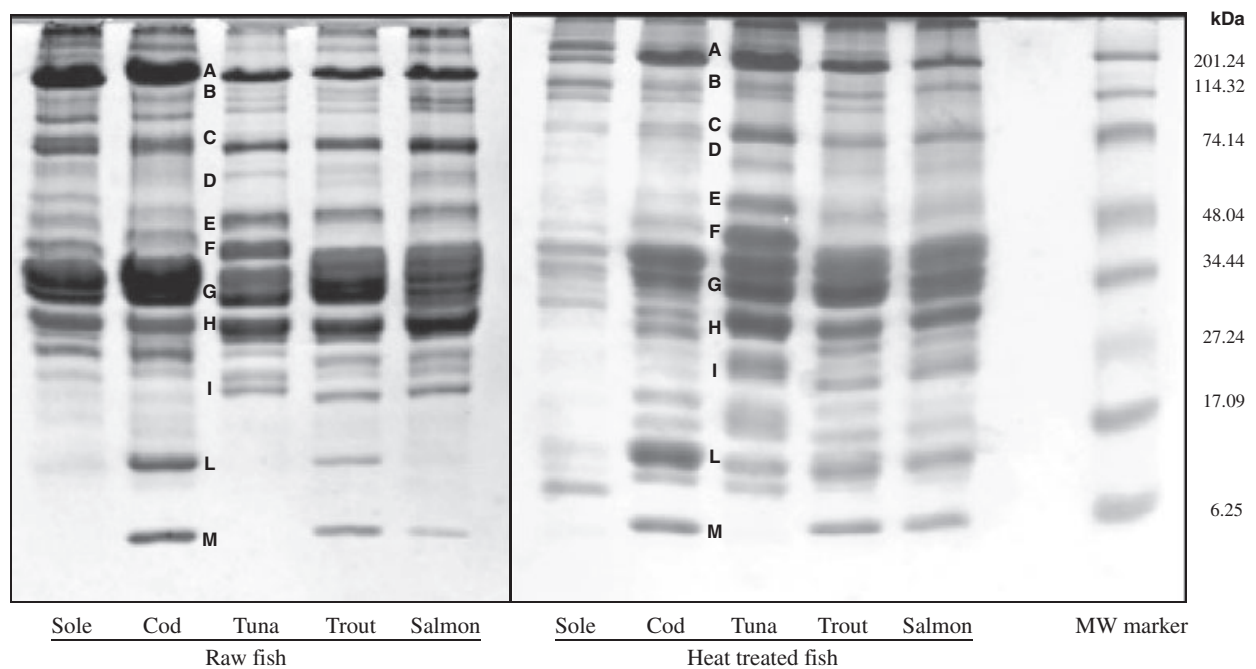


Figure 1 SDS-PAGE under reducing conditions on a 9–19% polyacrylamide gradient gel stained with Coomassie brilliant blue G-250 of raw (left panel) and cooked (right panel) fishes.

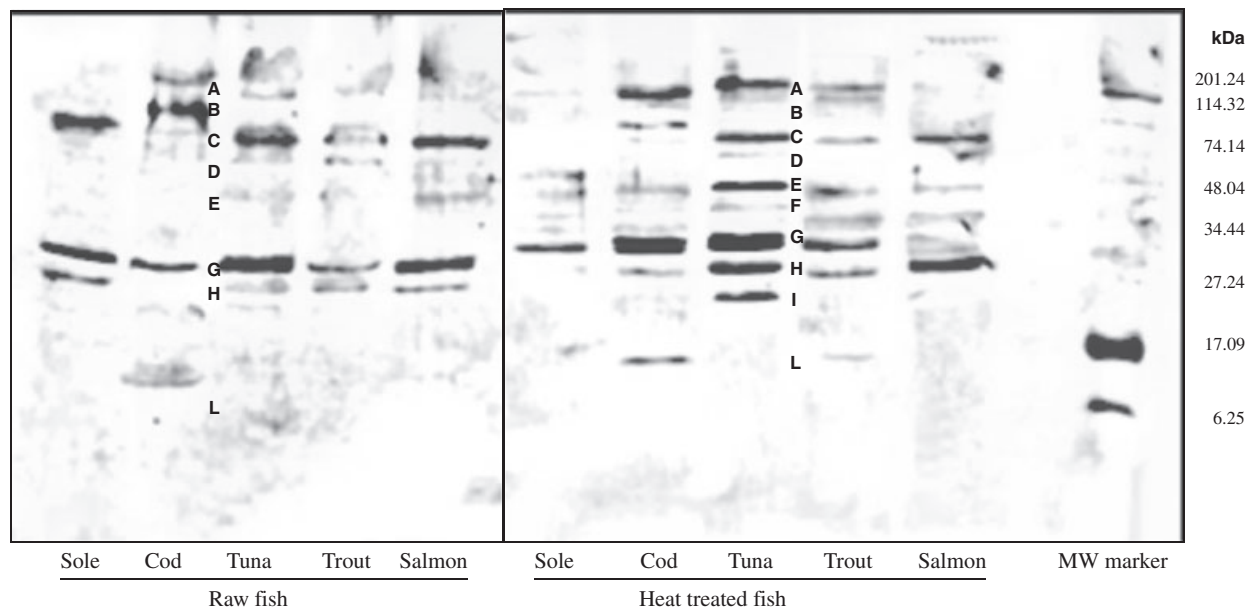


Figure 2 Immunoblotting of fish samples illustrated in Fig. 1 obtained by incubation with the serum of the allergic subject diluted in 0.25% gelatin solution (1: 100 v/v).

also observed in codfish and tuna fish, while salmon pattern showed only two immunoreactive bands (C and H). Two antigenic bands having MW 34 (our band G) and 74 kDa (our band C) were also identified by Bardina et al. (9) in cooked salmon; the band of 50 kDa (band E) could

correspond to beta-enolase, which has been previously described as an important fish allergen (10).

In agreement with data previously reported for cooked salmon (9), the higher number of immunoreactive protein bands observed in cooked fishes in comparison with those

found in raw fishes suggests the possible formation of new antigenic epitopes after cooking. It is well known that significant alterations in protein structure occurs during heat treatment, with possible formation of new intra/intermolecular interactions, that can create new allergenic epitopes. Further reaction could be associated with the presence of different ingredients during the technological treatment, as in the case of fish stick where carbohydrate (from flower) and proteins (from fish) can interact producing new molecules, such as Maillard derivatives.

The case reported here describes a patient clinically monosensitized to salmonid fishes (salmon and trout). Although parvalbumin is the main salmon allergen described in the literature, the circulating IgEs from our patient did not recognize it in the two salmonid fishes analyzed. These results confirm data reported by other authors, who indicated a different immunoreactivity pattern in some monosensitized subjects (3–7). The immunoblottings showed in Fig. 2 indicate that the serum allergen-specific IgEs reacted not only with salmon and trout proteins but also with other fish species, in particular tuna and codfish after cooking. Curiously enough,

the children included in this study were able to normally tolerate codfish apart from the event associated with fish sticks.

Even though the possible cross-contamination of cod stick by salmon cannot be ruled out (the assessment was impossible due to the elimination of the product), it is also possible that monosensitized subjects, not reactive to parvalbumin, could develop an allergic reaction in particular conditions. Technological treatments of fish or a specific combination of ingredients could determine a different degree of molecular modification of the allergens and then a change of clinical reaction. The strongest reactivity of serum IgEs versus cooked fish samples observed in immunoblotting could support this hypothesis.

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