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Fish collagen allergy: a pitfall of the prick-to-prick test with raw fish

S. Chikazawa ¹, T. Hashimoto¹, Y. Kobayashi², and T. Satoh¹

¹Department of Dermatology, National Defense Medical College, Tokorozawa, Japan, and

²Department of Marine Biosciences, Tokyo University of Marine Science and Technology, Tokyo, Japan

Address correspondence: Dr. Sakiko Chikazawa,

Department of Dermatology, National Defense Medical College, 3-2 Namiki, Tokorozawa
359-8513, Saitama, Japan.

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TEL: +81-4-2995-1665

FAX: +81-4-2996-5209

E-mail: schika@ndmc.ac.jp

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To the Editor:

A 20-year-old Japanese woman presented with a history of nausea and wheals after eating baked Japanese amberjack. She also had a history of urticaria after eating fish fingers, although the actual species of fish involved was unknown. Four months before admission to our department, she experienced generalized erythema and wheals with throat discomfort after eating baked eel.

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Other medical past histories were unremarkable. Laboratory tests revealed positive immunoglobulin (Ig)E specific for tuna, salmon (6.37 IU/ml, class 3 and 117 IU/ml, class 6, respectively; normal <0.27 IU/ml, FEIA), cod, plaice (4.47 UA/ml, class 3 and 1.15 UA/ml, class 2, respectively; normal <0.34 UA/ml, CAP-RAST), but negative for sardine, Pacific mackerel, horse mackerel, shrimp, squid, octopus, and crab. *Anisakis*-specific IgE also showed negative results. We were unable to measure IgE against Japanese amberjack and eel. We then performed the prick-to-prick test with raw fish, including Japanese amberjack, tuna, salmon, Japanese sea perch, and red sea bream. However, the patient responded to none of these fish.

The most common fish allergen is known to be parvalbumin¹. It is a thermostable, calcium-binding sarcoplasmic protein of various species of fish. In addition, fish collagen has also been identified as an important allergen for fish allergy². Inconsistent results of specific IgE and skin-prick tests despite apparent allergic episodes to fishes have prompted us to explore causative allergens and fish in detail. To this end, we attempted to identify specific IgE to parvalbumin and fish collagen by enzyme-linked immunosorbent assay (ELISA), as reported previously³. Briefly, parvalbumin and fish collagen (type I collagen) were purified from the meat

and skin of Pacific mackerel, respectively. Purified allergens (1 µg/ml) were coated on a microtiter plate followed by incubation with serum from the patient and enzyme reaction using an ELISA POD Substrate TMB Kit Popular® (Nakalai Tesque, Kyoto, Japan), and the color developed was measured by absorbance at 450 nm. Concomitantly, we performed ELISA with crude extracts from Pacific mackerel and horse mackerel following homogenization of fish meat with phosphate buffer (10 mM, pH 7.0) and heat treatment (100°C, 10 min). As a result, the patient was found to have specific IgE to fish collagen, but not parvalbumin (Fig. 1A). In addition, she had Pacific mackerel- and horse mackerel-specific IgE (Fig. 1A) that had not been detected by the FEIA method.

Collagen is a protein that becomes water-soluble with heat treatment. Therefore, collagen does not exist as the water-soluble protein in raw materials³. In addition, IgE-binding epitopes of collagens are known to be heat-stable⁴. We thus examined whether the prick-to-prick test with heat-treated fish would result in positive reactions. Fish samples were treated in a microwave oven (500 W ×15 s /10g sample) and subjected to prick-to-prick test together with raw fish. As expected, we observed wheal reactions in the skin-prick test with heated fish, including Japanese

amberjack, tuna, salmon, Japanese sea perch, red sea bream, and horse mackerel, while no responses were detected with untreated raw fish (Table 1 and Fig. 1B). Purified fish collagen, but not parvalbumin, also induced a positive reaction (Table 1).

Fish collagen is an important allergen for fish allergy. While fish collagens from various species of fish cross-react with each other, they are not cross-reactive with collagens from mammals^{2,3}.

Nevertheless, even if eating fish is avoided, patients with fish collagen allergy carry a risk of exposure to causative allergens in products such as gelatin, a denatured collagen that is used as a supplement for foods and probably for cosmetics, although this is not as commonly used as bovine and porcine collagens.

The prick-to-prick test is a reliable test for immediate-type food allergy, although oral challenge test is needed to exactly confirm causative foods. In our patient, the initial skin prick test with raw fish did not result in positive reactions. We additionally performed the prick-to-prick test with heated fish to make the collagen soluble, based on the assumption that negative results with raw fish could have been due to its insolubility. Indeed, negative skin reactions with raw fish became

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positive after heat treatment. It should also be noted that, despite negative results for IgE against Pacific mackerel and horse mackerel as assessed using FEIA methods, we detected positive results for specific IgE against these fish by ELISA. This could be explained by the fact that we used heated fish extracts for ELISA assays.

Our results suggest that the prick-to-prick test with raw fish risks overlooking fish collagen allergy. Performing the prick-to-prick test with heat-treated fish in addition to raw fish is recommended in patients with suspected fish allergy, although it may have a higher risk for adverse events than ordinary skin prick test with standardized samples.

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FIGURE LEGENDS

Figure 1. Results of specific IgE from ELISA and skin prick tests. **A)** IgE against fish collagen, Pacific mackerel, and horse mackerel was detected. **B)** Prick-to-prick test was performed with raw and heated fish. The diameter of positive control (1% histamine hydrochloride) was 7x7 mm.

Table 1. Results of prick-to-prick test

Fish	Skin reactions		Specific IgE for fish meat
	Untreated	Heated	
Japanese amberjack	-	+	Not tested
Tuna	-	+	6.37 IU/ml
Salmon	-	++	117 IU/ml
Japanese sea perch	-	++	Not tested
Red sea bream	-	++	Not tested
Horse mackerel	-	++	<0.34 UA/ml
Purified fish collagen (1 μ M)	++		
Purified parvalbumin (1 μ M)	-		

-: Equivalent to negative control
 +: < 50% of positive control
 ++: > 50% of positive control
 Positive control: 1% histamine dihydrochloride
 Negative control: saline

