

Case Report



A case of pediatric anaphylaxis caused by gummy tablets containing fish collagen

Rumi Ueno ¹, Yuri Takaoka ^{1*}, Naoshi Shimojo ^{2,3}, Fumiaki Ohno ^{2,3}, Tomohiro Yamaguchi ¹, Kayoko Matsunaga ³, and Makoto Kameda ¹

¹Department of Pediatrics, Osaka Habikino Medical Center, Osaka, Japan

²General Research and Development Institute, Hoyo Co., Ltd., Aichi, Japan

³Department of Integrative Medical Science for Allergic Disease, Fujita Health University Bantane Hospital, Aichi, Japan



Received: Feb 26, 2020

Accepted: Sep 14, 2020

*Correspondence to

Yuri Takaoka

Department of Pediatrics, Osaka Habikino Medical Center, 3-7-1 Habikino Habikino-city, Osaka, Japan.

Tel: +88-72-957-2121

Fax: +88-72-957-8002

E-mail: zvb11075@nifty.com

Copyright © 2020. Asia Pacific Association of Allergy, Asthma and Clinical Immunology. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<https://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ORCID iDs

Rumi Ueno

<https://orcid.org/0000-0002-1734-1501>

Yuri Takaoka

<https://orcid.org/0000-0002-0403-3366>

Naoshi Shimojo

<https://orcid.org/0000-0002-5366-6899>

Fumiaki Ohno

<https://orcid.org/0000-0001-6261-4479>

Tomohiro Yamaguchi

<https://orcid.org/0000-0002-3842-4939>

Kayoko Matsunaga

<https://orcid.org/0000-0001-5096-3006>

Makoto Kameda

<https://orcid.org/0000-0001-9746-4650>

<https://apallergy.org>

ABSTRACT

Fish collagen is one of the major allergens involved in fish allergies; however, it has not been well-established whether fish collagen can cause anaphylaxis among individuals with fish allergies, especially children. A 9-year-old girl experienced anaphylaxis after consuming 2 fruit-flavored gummy tablets. Contacting the manufacturing company revealed that fish-derived collagen was used in the gummy tablets, and an oral fish collagen challenge test confirmed that the anaphylaxis was induced by fish collagen. Immunoblot analysis showed a 120-kDa band in a sample of the fish collagen in the gummy tablets, and no reactive band of the pork gelatin was observed. Additionally, salmon collagen and other fish samples that provoked symptoms were detected as a 120-kDa band in the immunoblot analysis. The 120-kDa band was identified as fish collagen by mass spectrometry confirming that it was the causative antigen of our patient's allergy. This case study confirms that fish collagen can cause anaphylaxis in children with fish allergies. Physicians and allergic individuals need to be aware that processed foods may contain fish collagen to avoid allergic reactions.

Keywords: Anaphylaxis; Food allergy; Fish products; Food hypersensitivity; Immunoblotting

INTRODUCTION

Fish allergies are common worldwide. Parvalbumin and collagen have been reported as fish allergens. Fish collagen is an approximately 120-kDa protein consisting of 3 spirally wound polypeptide chains, which form gelatin when the helical structure loosens and fragments upon heating or enzymatic degradation [1, 2]. Fish gelatin retains as much allergenicity as fish collagen [1, 2]. Fish collagen is used widely in various processed products.

Fish collagen-specific IgE antibodies have been found in 50% of Japanese patients with fish allergies [3]. The risk of fish collagen-induced symptoms was considered low in people with fish allergies with no positive reactions (0/30, 0/3, respectively), in studies by Hansen et al. [4] and André et al. [5]. On the other hand, 4 cases of fish collagen allergy, in 3 adults and 1

Conflict of interest

The authors have no financial conflicts of interest.

Author Contributions

Conceptualization: Yuri Takaoka. Data curation: Rumi Ueno, Yuri Takaoka, Tomohiro Yamaguchi. Formal analysis: Rumi Ueno, Yuri Takaoka, Naoshi Shimojo, Fumiaki Ohno. Methodology: Rumi Ueno, Yuri Takaoka, Naoshi Shimojo, Fumiaki Ohno. Project administration: Yuri Takaoka. Visualization: Rumi Ueno, Yuri Takaoka, Naoshi Shimojo, Fumiaki Ohno. Writing - original draft: Rumi Ueno. Writing - review & editing: Rumi Ueno, Yuri Takaoka, Naoshi Shimojo, Fumiaki Ohno, Tomohiro Yamaguchi, Kayoko Matsunaga, and Makoto Kameda.

child, have been reported recently: 1 involving eel consumption [6] and 3 involving ingestion of processed products (sweets and dietary supplements) containing fish collagen [7-9].

In this study, we describe the case of a girl who presented with anaphylaxis after inadvertently consuming 2 gummy tablets containing fish collagen. This case may be useful in elucidating the pathogenesis of fish allergies in children.

CASE REPORT

A 9-year-old Japanese girl had medical history included wheezing and facial swelling after consuming grilled mackerel at 7 years of age and emergency treatment of itching in her mouth, generalized urticaria, and coughing after consuming raw salmon at 8 years and 6 months of age. As she was suspected to have a fish allergy, she avoided fish-related products. At the age of 8 years and 9 months, she experienced throat discomfort, generalized urticaria, coughing, and respiratory distress after consuming 2 fruit-flavored gummy tablets. She was admitted to the Emergency Department of a nearby hospital, where she received a nebulized bronchodilator.

After discharge, we performed a prick-by-prick test using a bifurcated needle (Tokyo MI Shokai, Tokyo, Japan), positive (histamine dihydrochloride, 10 mg/mL) and negative (saline) controls (Torii Pharmaceutical, Tokyo, Japan), and test substances. The test results for the same type of gummy tablets that had been taken by the patient, heated mackerel, and heated salmon were positive (wheal diameter ≥ 3 mm more than that of the negative control), measuring 13, 9, and 7 mm, respectively, compared to 0 and 7 mm for the negative and positive controls, respectively. The specific immunoglobulin E (IgE) antibodies for flatfish, salmon, mackerel, fish gelatin, carp parvalbumin, and cod parvalbumin, measured by ImmunoCAP FEIA (Thermo Fisher Scientific, Waltham, MA, USA), were 10.2, 11.9, 1.05, 12.3, 1.95, and 1.50 UA/mL, respectively.

Further, we performed an oral food challenge (OFC) test for fish allergy. The patient developed pharyngeal and laryngeal discomfort, abdominal pain, and urticaria of her mouth and face after ingesting 2 g of heated flatfish. She was given another OFC test of fish collagen 5 months after ingesting the gummy tablets. This caused a more severe anaphylactic reaction than the previous OFC of fish itself (**Table 1**).

Table 1. Patient response to the oral fish collagen challenge test

| Time after OFC (min) | Dose of fish collagen* (g) | Vital sign | | Symptoms and signs | Treatment |
|----------------------|----------------------------|----------------------|-----------|--|--|
| | | SpO ₂ (%) | BP (mmHg) | | |
| Before OFC | | 98 | 97/61 | No symptoms | |
| 0 | 0.3 | | | | |
| 1 | | 100 | 103/57 | Discomfort in the pharynx and larynx | Oral antihistamines |
| 15 | | 100 | 97/64 | Pharyngeal obstruction and moderate abdominal pain | Nebulized adrenaline |
| 50 | | 100 | 106/84 | Cold distal limbs | Infusion of normal saline and prednisolone |
| 115 | | 100 | 103/57 | Redness and a wheal around the face | |
| 180 | | 98 | 94/51 | All symptoms and signs resolved | |

OFC, oral food challenge test; SpO₂, percutaneous oxygen saturation; BP, blood pressure.

*A sample of the fish collagen used in the gummy tablets was provided by the manufacturer; 1 g was added to 3 mL of water and melted in a 500-W microwave for 30 seconds. The patient consumed 1 g of the product, equivalent to 0.3 g of fish collagen.

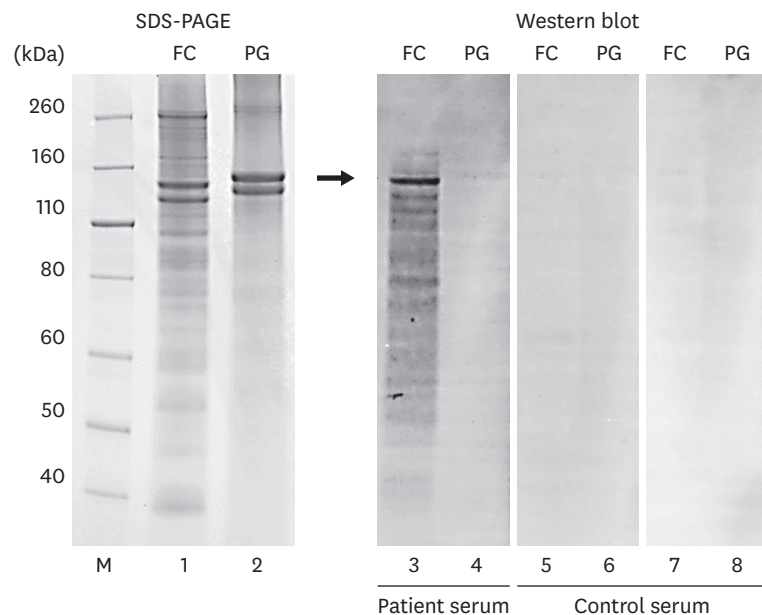


Fig. 1. Interaction between the fish collagen in the gummy tablets and serum from the patient and control. Lanes M, 1, 2, SDS gel stained with Simply Blue; Lanes 3–8, Immunoblot; M, marker; FC, fish collagen in the gummy tablets; PG, pork gelatin; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Immunoblotting was performed according to the method of Hayashi et al. [10] Proteins were resolved on 4%–12% Bis-Tris SDS gels (Thermo Fisher Scientific, Waltham, MA, USA) and transferred to an Immobilon-P polyvinylidene fluoride membrane (pore size, 0.45 μm ; Millipore, Bedford, MA, USA). The membranes were incubated with the patient or volunteer serum and subsequently with a secondary antibody (peroxidase-labeled anti-human IgE [epsilon] antibody; Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA). Bound secondary antibody was detected using the Pierce ECL Plus Western Blotting Substrate (Thermo Fisher Scientific, Waltham, MA, USA). The IgE antibodies in the patient's serum interacted with an approximately 120-kDa protein in the fish collagen sample, but not with pork gelatin.

In order to confirm that fish collagen was the antigen responsible for the patient's fish allergy, immunoblotting was performed to determine whether her serum contained IgE antibodies to the fish collagen in the gummy tablets and to commercially available pork gelatin by the method of Hayashi et al. [10] (**Fig. 1**). The 2 negative controls were stored blood specimens of volunteers without allergies to fish or collagen. This study was approved by the Ethics Committee of the Osaka Habikino Medical Center (approval number: 914-2) and Fujita Health University (approval number: HM16-371). The assays showed that the patient's serum reacted strongly to a protein of approximately 120 kDa and multiple bands in the fish collagen sample, but not with pork gelatin.

A similar experiment was performed using heated flatfish, mackerel, salmon, and salmon collagen as the antigen or antigen source (**Fig. 2**). Salmon collagen was extracted according to the method of Hamada et al. [2]. The IgE antibodies in the patient's serum reacted with a 120-kDa protein in the flatfish, mackerel, salmon, and salmon collagen samples.

The 120-kDa band from the flatfish was identified by mass spectrometry as collagen alpha-1(I) chain-like of *Paralichthys olivaceus* (accession number XP_019961379.1, the National Center for Biotechnology Information [Bethesda, MD, USA] gene database). The salmon and mackerel collagen were not registered in the database but were similar to the collagen alpha-1(I) chains of *Oncorhynchus nerka* and *Sinocyclocheilus anshuiensis* (accession numbers XP_029491415.1 and gi|1025094027, respectively). The protein coverage for each band of

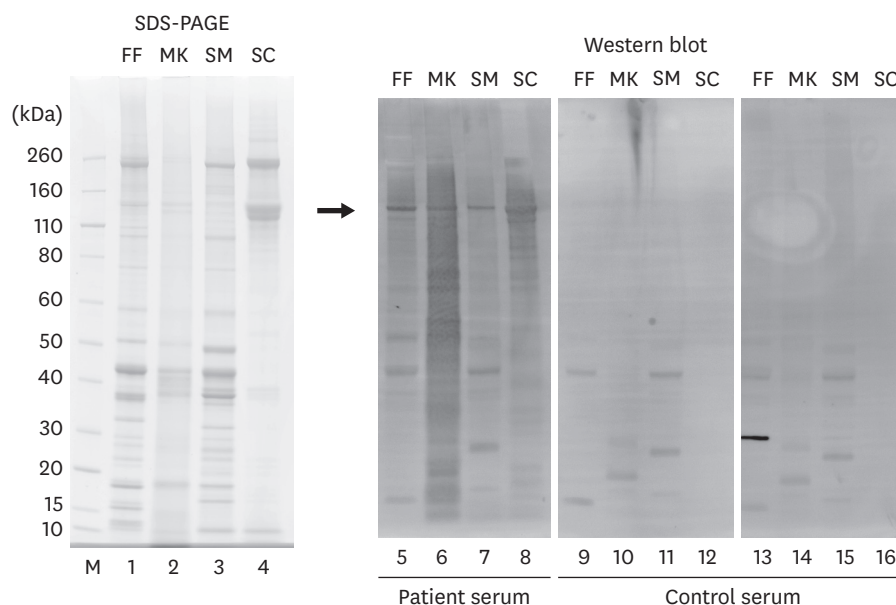


Fig. 2. Interaction between proteins in heated flatfish, mackerel, salmon, and salmon collagen with serum from patient and controls. Lanes M, 1–4, SDS gel stained with Simply Blue; Lanes 5–16, Immunoblot; M, marker; FF, flatfish; MK, mackerel; SM, salmon; SC, salmon collagen; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The IgE antibodies in the patient's serum reacted with an approximately 120-kDa protein in all samples.

flatfish, salmon, and mackerel were 31.7% (458aa/1447aa), 3.2% (46aa/1449aa), and 1.4%, (20aa/1451aa), respectively.

DISCUSSION

This is the first child fish collagen anaphylaxis case diagnosed by oral fish collagen challenge test (**Table 1**). Care should be taken when performing OFC test in patients with a suspected fish collagen allergy, and a small amount of fish collagen should be used. According to the manufacturer, there is about 0.38 g of fish collagen per 4-g tablet, which is relatively high, as compared with 80–280 mg of fish collagen in an 80 g piece of fish fillet [11]. Hence, ingestion of even a small amount of a tablet could provoke a severe reaction.

Multiple bands were present in the main 120-kDa band in the immunoblot of fish collagen for the gummy tablets, but not in the immunoblot of pork gelatin indicating fish collagen/gelatin and pork gelatin do not show cross-reactivity (**Fig. 1**) [1].

The collagen in the heated flatfish, mackerel, and salmon samples was identified as the antigen by immunoblotting and mass spectrometry indicating fish collagen is the antigen responsible for fish allergy and a high-level cross-reactivity among the collagens of different fish species (**Fig. 2**) [2]. IgE antibodies in the patient's serum also reacted with additional proteins; these proteins may also be done in the development of fish allergies.

The cause of this case of pediatric anaphylaxis following the ingestion of gummy tablets was confirmed to be fish collagen by oral fish collagen challenge test. Immunoblot assays

indicate that fish collagen can cause symptoms in children allergic to fish. Physicians need to be aware that some processed products contain fish collagen so that they can warn patients with fish allergies.

ACKNOWLEDGEMENTS

We are grateful to the pediatricians of Osaka Habikino Medical Center, namely Junko Kumon, Tamana Nakano, Yohei Fukasawa, Yuki Tsurinaga, Amane Shigekawa and Yukinori Yoshida, for their contribution to the treatment of the patient.

REFERENCES

1. Sakaguchi M, Toda M, Ebihara T, Irie S, Hori H, Imai A, Yanagida M, Miyazawa H, Ohsuna H, Ikezawa Z, Inouye S. IgE antibody to fish gelatin (type I collagen) in patients with fish allergy. *J Allergy Clin Immunol* 2000;106:579-84.
[PUBMED](#) | [CROSSREF](#)
2. Hamada Y, Nagashima Y, Shiomi K. Identification of collagen as a new fish allergen. *Biosci Biotechnol Biochem* 2001;65:285-91.
[PUBMED](#) | [CROSSREF](#)
3. Kobayashi Y, Akiyama H, Hige J, Kubota H, Chikazawa S, Satoh T, Miyake T, Uhara H, Okuyama R, Nakagawara R, Aihara M, Hamada-Sato N. Fish collagen is an important panallergen in the Japanese population. *Allergy* 2016;71:720-3.
[PUBMED](#) | [CROSSREF](#)
4. Hansen TK, Poulsen LK, Stahl Skov P, Hefle SL, Hlywka JJ, Taylor SL, Bindslev-Jensen U, Bindslev-Jensen C. A randomized, double-blinded, placebo-controlled oral challenge study to evaluate the allergenicity of commercial, food-grade fish gelatin. *Food Chem Toxicol* 2004;42:2037-44.
[PUBMED](#) | [CROSSREF](#)
5. André F, Cavagna S, André C. Gelatin prepared from tuna skin: a risk factor for fish allergy or sensitization? *Int Arch Allergy Immunol* 2003;130:17-24.
[PUBMED](#) | [CROSSREF](#)
6. Tamura M, Matsui K, Kobayashi Y, Ogita C, Tsuboi K, Kusakabe M, Azuma K, Abe T, Yoshikawa T, Sekiguchi M, Azuma N, Kitano M, Sano H. A case of eel collagen allergy. *Allergol Int* 2018;67:138-40.
[PUBMED](#) | [CROSSREF](#)
7. Fujimoto W, Fukuda M, Yokooji T, Yamamoto T, Tanaka A, Matsuo H. Anaphylaxis provoked by ingestion of hydrolyzed fish collagen probably induced by epicutaneous sensitization. *Allergol Int* 2016;65:474-6.
[PUBMED](#) | [CROSSREF](#)
8. Abe N, Ito T, Kobayashi T, Egusa C, Maeda T, Okubo Y, Tsuboi R. A case of anaphylaxis due to fish collagen in a gummy candy. *Allergol Int* 2020;69:146-7.
[PUBMED](#) | [CROSSREF](#)
9. Kuehn A, Hilger C, Hentges F. Anaphylaxis provoked by ingestion of marshmallows containing fish gelatin. *J Allergy Clin Immunol* 2009;123:708-9.
[PUBMED](#) | [CROSSREF](#)
10. Hayashi E, Sowa-Osako J, Fukai K, Natsumi A, Yagami A, Sato N, Shimojo N, Nakamura M, Matsunaga K, Tsuruta D. Case of anaphylaxis caused by black ginger in a dietary supplement. *J Dermatol* 2019;46:e56-7.
[PUBMED](#) | [CROSSREF](#)
11. Hamada Y, Shiomi K. Collagen: a new fish allergen. *Jpn J Pediatr Allergy Clin Immunol* 2001;15:47-53. Japanese.