Case report

Fenugreek allergy caused by cross-reactivity with peanut: An *in vitro* analysis

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Fenugreek is often hidden in processed foods, where it is included in the generic term *spice* on food labels. Cross-reactivity of peanut and fenugreek should be considered in the management of peanut allergy. (J Allergy Clin Immunol Global 2024;3:100292.)

Key words: *Fenugreek, food allergy,* in vitro *cross-reactivity, peanut, spice*

Fenugreek (*Trigonella foenum-graecum*), a spice that is often used in curry and other dishes, belongs to the family Fabaceae (ie, the same family as peanuts and other legumes). Dried fenugreek seeds are roasted, ground into a powder, and used as a spice, dye, and medicine. Spice allergies affect about 0.04% to 0.13% of the general population, mostly owing to cross-reactions with pollen allergens (mainly PR-10 and profilin), resulting in a secondary spice allergy called celery-birch-mugwort-spice syndrome.[1,2] Many reported cases of fenugreek allergy included suspected clinical cross-reactivity with peanuts.[3-5] *In vitro* cross-reactivity between peanut and legumes other than fenugreek has been reported[6]; however, there have been no reports of *in vitro* cross-reactivity between peanut and fenugreek based on serum from patients with fenugreek allergy. Here, we report a pediatric case of fenugreek allergy and examine the origin and causative allergen.

We conducted skin prick tests, measurements of allergen-specific IgE levels, and oral food challenge (OFC) to diagnose food allergies. For the skin prick test, a commercial peanut allergen (Torii Pharmaceutical Co, Ltd, Tokyo, Japan), various spices purchased from a supermarket (10 mg/mL), and causative foods were used. Allergen-specific IgE levels were measured by using the ImmunoCAP system (Thermo Fisher Scientific/Phadia AB, Uppsala, Sweden). Sensitization to allergen was defined as skin prick tests resulting in a wheal diameter 3 mm or larger or an IgE level of 0.35 kU_A/L or higher. In the OFCs, the peanuts and spices were used in hamburger steaks or sauces. A result was deemed positive determined if obvious objective allergic reactions were observed. To identify the causative allergen, we performed Western blotting and ELISA inhibition. For the *in vitro* assay, peanut and fenugreek allergens were extracted by using ammonium bicarbonate.

Abbreviations used
Ara h: *Arapis hypogaea*
OFC: Oral food challenge

In compliance with the Declaration of Helsinki, we obtained approval from the NHO Sagamihara National Hospital ethics committee (approval no. 2019-022) and written consent from the patient's guardian.

A 6-year-old Japanese boy had a history of anaphylaxis to peanuts at age 2 years. He visited our department because of recurrent episodes of emesis and urticaria after consuming curry or fried noodles with a spice source after age 4 years. At the time of the allergic symptoms, he did not take any medication and had no other health problems. There was no contamination of peanuts in either food; because the common ingredients were spices, a spice allergy was suspected. The boy had cow's milk allergy in infancy that resolved by 2 age years and hay fever after age 5 years but no bronchial asthma or atopic dermatitis.

Skin prick tests were positive for peanut (wheal diameter 18 mm), fenugreek (18 mm), cumin (2 mm), curry (5 mm), and fried noodle source (5 mm) (see Table E1, *A* in the Online Repository at www.jaci-global.org). The patient's allergen-specific IgE levels were as follows: peanut, 292 kU_A/L; fenugreek, 8.89 kU_A/L; *Arapis hypogaea* (Ara h) 1 (7S globulin), 54.6 kU_A/L; Ara h 2 (2S albumin), 131 kU_A/L; Ara h 3 (11S globulin), 17.4 kU_A/L; Ara h 6 (2S albumin), 93.9 kU_A/L; Ara h 8 (PR-10), less than 0.10 kU_A/L; and Ara h 9 (lipid transfer protein), less than 0.10 kU_A/L. Other inhaled allergen IgE levels are shown in Table E1, *B* (in the Online Repository). In peanut OFC, the patient developed emesis twice and moderate abdominal pain after consuming 31 mg of peanut protein. In fenugreek OFC, he developed emesis twice, urticaria, and throat pain after consuming 16 mg of fenugreek protein. In cumin OFC, no symptoms were observed after the patient had consumed 45 mg of cumin protein.

The fenugreek protein band pattern observed in SDS-PAGE differed from that of peanut (Fig 1, *A*). Fluorescence ELISA showed a notable increase in fluorescence intensity for peanut

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Received for publication January 10, 2024; revised March 19, 2024; accepted for publication April 23, 2024.

Available online June 12, 2024.

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2772-8293

https://doi.org/10.1016/j.jacig.2024.100292

and fenugreek but only a slight increase in intensity for cumin, compared with the negative controls (see Fig E1 in the Online Repository at www.jaci-global.org).

Western blotting using patient serum showed binding of various proteins from 10 kDa to 100 kDa in peanut, and prominent binding at approximately 50 kDa in fenugreek (Fig 1, *A*). IgE binding to the 50-kDa protein in fenugreek was inhibited by the patient's serum preincubated with peanut (Fig 1, *B* [*lane 4*]). In ELISA inhibition, fenugreek did not inhibit IgE binding in the peanut solid phase (Fig 2, *A*), but peanut inhibited IgE binding in the fenugreek solid phase at a concentration lower than that of fenugreek (Fig 2, *B*).

We diagnosed the patient with fenugreek allergy and considered that the allergy to be due to cross-reactivity with peanuts.

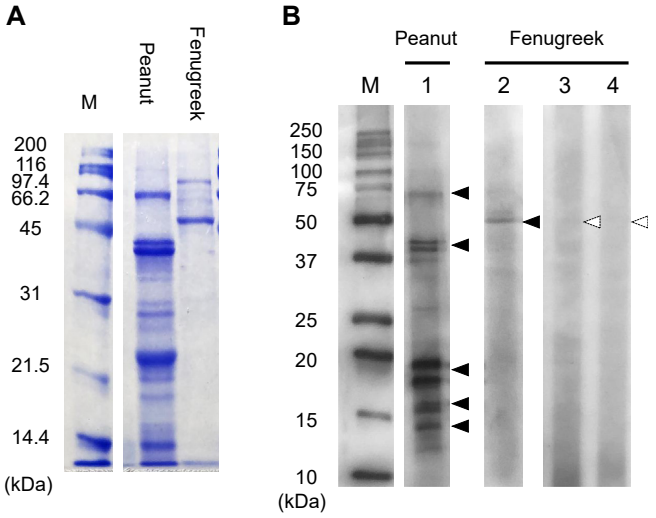

FIG 1. SDS-PAGE (**A**) and immunoblotting (**B**) results. **A,** Peanut and fenugreek samples (approximately 2.5 μg of protein). **B,** Immunoblotting against peanut (*lane 1*) and fenugreek (*lanes 2, 3, and 4*). Lanes 1 and 2 depict the results for sera plus PBS; lane 3 depicts the results for sera plus fenugreek as an inhibitor; and lane 4 depicts the results for sera plus peanut as an inhibitor. Closed triangles indicate specific IgE binding; open triangles indicate inhibited binding. *M*, Molecular weight standards.

Previously, cases of fenugreek allergy involving possible cross-reactivity to peanuts have been reported,[3-5] and several potential allergens in fenugreek have also been characterized.[7] The approximately 50-kDa protein to which this patient reacted most strongly was thought to be the 7S vicilin-like protein Tri fg 1, as previously reported. This allergen has been confirmed to have a partial homology with peanut Ara h 1 (7S vicilin-type globulin).[5,7] In our case, blotting inhibition assay using the patient's serum revealed *in vitro* cross-reactivity between peanut and fenugreek, associated mainly with the approximately 50-kDa protein. We also found that in ELISA inhibition, peanut allergenicity was higher than that of fenugreek, suggesting that primary peanut allergy caused the fenugreek allergy. In a study that investigated sensitization to other legumes in 195 children with peanut allergy, 61 of 92 children (66%) were sensitized to fenugreek.[8] Of those 61 children, 6 (10%) had fenugreek allergy. Although it was not possible to confirm the intake of fenugreek in all study subjects, some children with peanut allergy exhibited clinical cross-reactivity to fenugreek.

In summary, we have described a child with peanut allergy who was diagnosed with fenugreek allergy. *In vitro* cross-reactivity between peanut and fenugreek was demonstrated by inhibition assays. We concluded that peanut was the primary sensitizer and the patient's fenugreek allergy was secondarily caused by cross-reactivity to peanuts.

It should be noted that fenugreek is hidden in processed food, often included on food labels in the generic term *spice*. Spices are widely used in various cuisines such as curry worldwide. Therefore, cross-reactivity between peanut and fenugreek should be considered in the management of peanut allergy. Further studies should investigate the pathogenic mechanism and prevalence of fenugreek allergy in patients with peanut allergy.

DISCLOSURE STATEMENT

Supported by funding from the Practical Research Project for Allergic Disease and Immunology from the Japan Agency for Medical Research and Development (grant 17ek0410019h003) and Research on Food Labeling Related to Food Allergy in 2018, Consumer Affairs Agency, Government of Japan. The

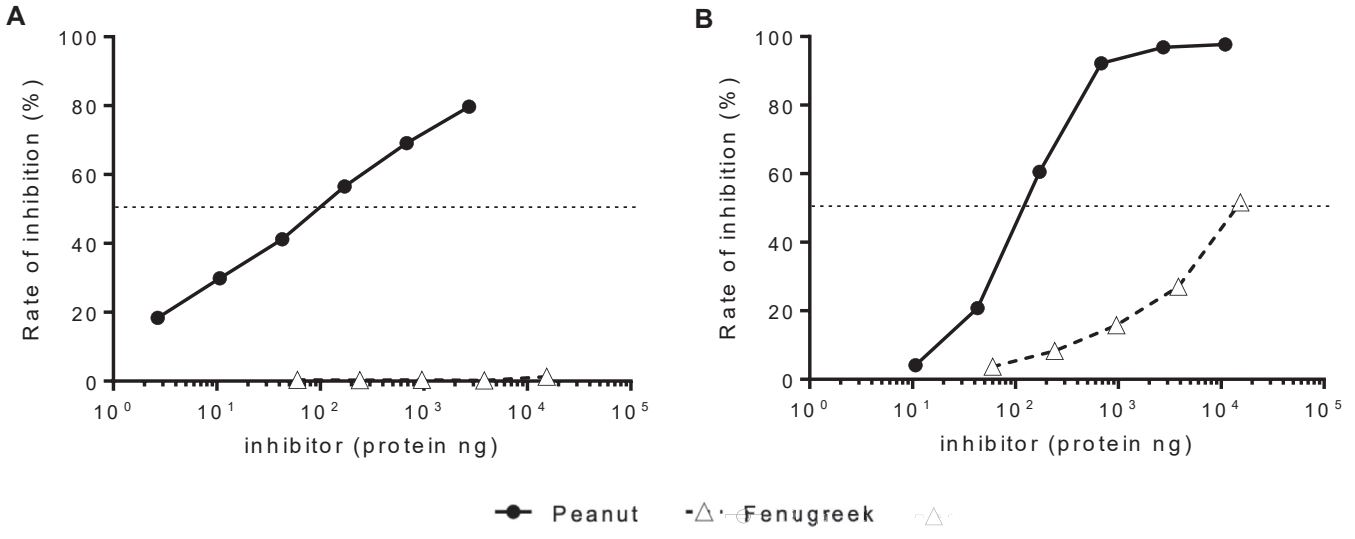

FIG 2. ELISA inhibition against peanut (**A**) and fenugreek (**B**) solid phases. Dotted line indicates 50% inhibition rate.

funding sources were not involved in the study design; collection, analysis, and interpretation of data; writing of the report; or decision to submit the article for publication.

Disclosure of potential conflict of interest: M. Ebisawa is on the scientific advisory board of Novartis, Sanofi, and ARS-Pharmaceuticals and has received lecturing fees from Viatris. S. Sato has received lecturing fees from Viatris. The rest of the authors declare that they have no relevant conflicts of interest.

We would like to thank Dr Fukutomi and the entire staff of the Department of Pediatrics and Clinical Research Center for Allergy and Rheumatology, NHO Sagamihara National Hospital. We would also like to thank Editage (www.editage.com) for English language editing.

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