

Brief communication

Anaphylaxis after ingestion of millet seeds

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We report the case of a patient with anaphylaxis after ingestion of millet seeds, a previously unreported allergen. Skin testing with freshly prepared millet seed extract produced positive intradermal responses at a concentration of 100 pg/ml. The reaction was mediated by IgE antibodies to millet, as demonstrated by elevated specific IgE antibody levels by radioallergosorbent test (RAST) and by significant in vitro histamine release from leukocytes incubated with millet seed extract.

Anaphylactic reactions following ingestion of foods such as nuts and seafoods^{1, 2} have been described by various authors. The severity of such reactions ranges from mild gastrointestinal discomfort to severe respiratory embarrassment with hypotension.^{3, 4} Because of the increasing popularity of "natural foods," we report a patient with anaphylaxis secondary to ingestion of millet seeds, a previously unreported allergen.

PATIENT AND PROCEDURES

Patient history

The patient is a 25-yr-old clerk who was in good health and had no significant allergic history. While dining away from home, approximately 30 min after a "natural food" dinner consisting of pea soup, cole slaw, tangerines, apples, salad dressing, cayenne peppers, celery salt, and a date-millet pudding, she developed symptoms of anaphylaxis. These included periorbital angioedema, ocular injection, tightness of the throat, shortness of breath, wheezing, intense generalized pruritus, and lower abdominal distress.

After treatment at a local emergency room her symptoms resolved at home over the next 2 days. Prior to seeking an allergy evaluation, she deliberately rechallenged herself to all of the items consumed on the evening of her reaction except millet seed and the salad dressing, without any further reactions.

Methods

Suspecting that millet seed sensitivity was the most likely cause of the patient's reaction, we performed skin tests using millet seed extracts prepared in our laboratory from two separate sources of millet seeds. The first extract (millet I) was prepared from millet seeds purchased at a local natural food store, and the second extract (millet II) was prepared from the millet seeds used to make the date-millet pudding. These materials were prepared by extracting 50 gm of finely ground millet seeds with 500 ml of 0.1 M NH_4HCO_3 . Each solution was stirred at 4° C overnight, centrifuged, sterilized by Millipore filtration, and lyophilized. These dry, crude millet seed extracts were reconstituted in either 50% glycerin or Sorenson's phosphate buffer⁵ containing 0.5% human serum albumin for puncture and intradermal skin testing, respectively. Skin tests were graded by the method of Norman.⁶ We also performed puncture skin tests using 1:20 commercial extracts of rye grass, orchard grass, timothy grass, and flaxseed, in addition to mold, tree pollen, and weed pollen extracts. To exclude the possibility of nonspecific irritants in the millet seed extracts, two nonallergic individuals were also skin tested.

Serum IgE antibodies to various cereal grains and grass pollens were measured by radioallergosorbent test (RAST), using commercial extracts for coupling to paper discs.⁵ Mil-

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Supported in part by a grant from the National Institute of Allergy and Infectious Diseases (AI 11483) and the Mayo Foundation.

Received for publication June 11, 1980.

Accepted for publication July 14, 1980.

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*Dr. Yunginger is the recipient of an Allergic Diseases Academic Award (AI 00107).

let seed RAST discs were prepared using reconstituted lyophilized crude millet extracts, while millet pollen RAST discs were produced from a 1:10 extract of this pollen prepared in our laboratory (*Setaria/Panicum* pollen, kindly donated by Greer Laboratories, Inc., Lenoir, N. C.). RAST results were expressed both as percent total counts bound and as percent of binding compared with a control serum from a nonallergic person. Possible cross-allergenicity between the millet seed extracts and either flaxseed or rye grass pollen was measured by RAST inhibition assays⁵ in which both solid-phase millet seed allergens were reacted with the homologous fluid-phase allergens (millet I and II) as well as flaxseed and rye grass pollen.

Leukocyte histamine release studies were performed using only millet II extract, according to the method of May et al.⁷ The histamine content of supernatant fluid was measured spectrofluorometrically.

RESULTS

Table I shows the skin-test results. The patient showed significant intradermal skin-test reactivity with 100 pg/ml of millet II extract; in contrast, there were only $\pm$ irritative reactions to intradermal tests at 1 ng/ml in both nonallergic individuals. With puncture skin testing the patient showed mild reactivity to flaxseed (1+) with the remainder of the pollen and mold allergens tested giving 0 or $\pm$ results (data not shown). Specific IgE antibody levels were 1,200% and 1,030% of negative control for millet I and II, respectively (Table II). There were no significant elevations of IgE antibodies to other cereal grains and grass pollens. Results from RAST inhibition studies are shown in Table III. When either millet I or II was added as a fluid-phase inhibitor, considerable (>75%) inhibition was observed. These results suggest that both millet seed extracts contained similar allergens. To further investigate cross-allergenicity between millet seed and other grains and grasses, we also tested rye, orchard, and June grass pollen, wheat grain, oat grain, and barley grain as fluid-phase inhibitors in the RAST. These extracts showed no significant inhibition (results not shown). Additional RAST inhibition studies were done with flaxseed; the results are shown in Table III. Although slight inhibition (21% to 24%) was produced by flaxseed, this was not substantially higher than the inhibition produced by the 50% glycerin diluent alone (10% to 16%).

Leukocyte histamine release results are shown in Table IV. Significant histamine release (49%) resulted when leukocytes from the patient were challenged with 10 μ g/ml of millet II.

DISCUSSION

With increasing interest in natural food diets more vegetable products not commonly considered as food

TABLE I. Intradermal skin-test results

Test concentration (mg/ml)	Nonallergic controls					
	Patient		No. 1		No. 2	
	Millet		Millet		Millet	
	I	II	I	II	I	II
10 ⁰	ND	ND	$\pm$	$\pm$	0	$\pm$
10 ⁻¹	ND	ND	$\pm$	0	0	0
10 ⁻²	4+	3+	0	0	0	0
10 ⁻³	3+	3+	0	0	0	0
10 ⁻⁴	3+	2+	0	0	0	0
10 ⁻⁵	2+	2+	0	0	0	0
10 ⁻⁶	1+	2+	0	0	0	0
10 ⁻⁷	$\pm$	2+	ND	ND	ND	ND
10 ⁻⁸	0	$\pm$	ND	ND	ND	ND
10 ⁻⁹	0	0	ND	ND	ND	ND

ND = not done.

TABLE II. Direct RAST results

Antigen	% total counts	% negative control
Millet I	9.61	1,200
Millet II	8.46	1,030
Millet pollen	1.87	263
Rye grass pollen	1.64	120
Rye pollen	1.12	130
June grass pollen	2.08	125
Wheat grain	1.37	114
Wheat grass pollen	2.34	119
Oat grain	2.83	135
Barley grain	4.06	180
Flaxseed grain	3.27	146

are being consumed by Americans. Actually, millet seed is one of the oldest cultivated crops; it probably originated from Southern Asia and dates back as far as 2700 B.C.⁸ In the United States approximately 500,000 acres of millet are grown yearly, mostly for use as bird seed, while over 100 million acres are grown annually in mainland China, India, the Soviet Union, and Equatorial Africa, where it is used for food. In India, for example, more than 2½ million tons are used annually for food.⁸ Millet seeds, when compared with other grains, contain high-quality protein and have excellent fat, starch, vitamin, and mineral composition.⁹

We have demonstrated sensitivity to millet seed by skin testing, by measurement of millet-specific IgE antibodies, and by leukocyte histamine release. In our patient, intradermal skin-test reactivity was titrated at 100 pg/ml, while in nonallergic control subjects only irritative responses were noted at the 1 mg/ml concentration.

TABLE III. RAST inhibition results

Inhibitor	Concentration	Volume (μl)	% inhibition	
			Solid-phase millet antigen	
			I	II
Millet I	10 mg/ml	25	86	86
		2.5	74	75
		0.25	18	22
		0.025	7	3
Millet II	10 mg/ml	25	78	82
		2.5	68	69
		0.25	21	16
		0.025	6	7
50% glycerin	1:20	25	16	10
Flaxseed		25	21	24
		2.5	14	13
		0.25	11	16

TABLE IV. Leukocyte histamine release results

Millet II concentration (mg/ml)	% histamine release
10^0	29
10^{-1}	29
10^{-2}	49
10^{-3}	44
10^{-4}	18
10^{-5}	4

The millet seed used to prepare the date-millet pudding was purchased in a nondescript burlap bag that appeared to have been recycled after packaging some other material. We were concerned that the patient may have reacted to a contaminant in the bag; this is why, in part, we also chose to study flaxseed, in that flaxseed sensitivity tends to be sudden and severe. The patient showed significant elevations in IgE antibodies only to millet seed; these IgE antibodies did not recognize any common allergenic component in other cereal grains or grass pollens. The results of the RAST inhibition studies, along with the positive RAST results using millet I extract, suggest

that the patient was reacting to the millet seed itself, and not to a trace contaminant present only in the millet II preparation.

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