

Rice (*Oryza sativa*) allergy in rhinitis and asthma patients: A clinico-immunological study

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Abstract

Sensitization to foods varies in different countries reflecting a possible interaction of genetic factors, cultural and dietary habits. Rice is a major food consumed world wide and needs evaluation for IgE mediated reactions. The present study was carried out to identify rice allergy in patients of rhinitis and asthma and identify the allergenic proteins in raw and cooked rice. Of 1200 patients screened using standard questionnaire, 165 presented with history of rice allergy. Of these, 20 (12.1%) patients demonstrated marked positive skin prick test (SPT) and 13 showed significantly raised specific IgE to rice compared to normal controls. Double blind placebo controlled food challenge (DBPCFC) confirmed rice allergy in 6/10 patients. Immunoblot with hypersensitive individual patients' sera showed 14–16, 33, 56 and 60 kDa proteins as major IgE-binding components in rice. Boiled rice retained four IgE reactive proteins of 16, 23, 33 and 53 kDa. In summary, IgE-mediated rice allergy affects 0.8% [(0.42–1.58) at 95% CI] of asthma and rhinitis cases. The subjects with severe SPT reactions (4 mm or above) and specific IgE, 6.9 ng/ml to rice demonstrated positive blinded food challenge with clinical symptoms.

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1. Introduction

Food allergy is defined as an IgE-mediated immune response to food proteins. It has become a major health problem affecting 6% of young children and 3–4% of adults. In young children, the common causal foods are cow's milk, egg, peanut, wheat, soy, tree nuts, fish, and shellfish while adults mostly suffers with allergies to shellfish, peanut, tree nuts and fish (Sampson, 2004). The incidence of perceived food hypersensitivity varies

(1.4–19.1%) largely across different countries probably due to differences in the diagnostic criteria, study design and population variation, etc. (Crespo and Rodriguez, 2003). Food allergy is greatly influenced by genetic factors, cultural and dietary habits, and exposure to new allergenic products early in life. For example, peanut allergy is common in UK, France, and Switzerland but rare in Italy, Singapore and Israel (Dalal et al., 2002).

Rice is a cereal grain from the family Poaceae providing 25–80% of the daily calories consumed by more than half of the world population. Despite this, only a few cases of true rice allergy are established by double blind placebo controlled food challenges

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(DBPCFCs). The association of rice seed proteins with allergic reaction was first reported from Japan for patients with history of asthma induced by exposure to rice flour and eczema exacerbated by rice ingestion (Shibasaki et al., 1979). IgE-mediated rice allergy is estimated at about 10% in atopic subjects in Japan (Besler et al., 2001). In Malaysia, next to shrimp, rice is the most common allergenic food. Rice is the fourth most important allergen after shellfish, peanut and soy in Thailand, whereas in Japan and Indonesia it is ranked fifth and sixth, respectively, as an allergen (Hill et al., 1997). Prevalence of rice allergy is much lower in Europe and the USA, where rice is primarily recognized as a cereal-associated allergen source (Lehrer et al., 1999; Andre et al., 1994; Varjonen et al., 1995). Rice hypersensitivity has also been reported from countries like France, Sweden, Denmark, Estonia, Lithuania, and Russia (Andre et al., 1994; Eriksson et al., 2004).

Rice-induced allergy is IgE mediated in cases of bronchial asthma (Arai et al., 1998; Lezaun et al., 1994) and atopic dermatitis (Ikezawa et al., 1992; Di Lernia et al., 1992; Lezaun et al., 1994) in contrast to non-IgE-mediated reactions. Two different routes of exposure leading to immediate hypersensitivity reactions have been documented: (1) ingestion of the cooked rice and (2) inhalation of vapors during its boiling. Most reports describe contact urticaria with raw rice (Di Lernia et al., 1992; Lezaun et al., 1994) whereas reports of immediate hypersensitivity reactions after ingestion of rice are scarce (Arai et al., 1998; Wuthrich et al., 2002). Rice is washed before cooking, hence the water soluble protein allergens cause contact urticaria in patients. A case of anaphylaxis has also been reported after inhalation of rice flours (Fiocchi et al., 2003). Earlier, rice seed proteins of 14–16, 26, 32–33 and 56 kDa were demonstrated IgE-reactive using suspected rice allergy patients' sera (Urisu et al., 1991; Usui et al., 2001).

In India, a large population complains of allergy or allergy-like reactions after ingestion of rice recipes. But knowledge about rice allergy and IgE reactivity to individual proteins is lacking. The present study was aimed at identifying the rice allergy among patients with allergic rhinitis and bronchial asthma. Immunoreactivity to rice proteins was also investigated using hypersensitive patients' sera.

Methods

Subjects

A total of 1200 referred cases of respiratory allergy (asthma, rhinitis or both) aged 5–60 years visiting out patient department, Vallabhbhai Patel Chest Institute, Delhi, during April 2002–September 2004, were

screened for food allergies, using a standard questionnaire (Vally et al., 1999). The detailed history of patients including suspected food and dose of food eliciting allergic reaction, symptoms, family history of allergy, onset and duration of symptoms was recorded by questionnaire. Routine physical examination, chest X-ray, blood analysis and pulmonary function test (PFT) were carried out for each patient. PFT included forced vital capacity (FVC), FVC/ FEV₁, peak expiratory flow rate (PEFR), 1 s forced expiratory volume (FEV₁), and forced expiratory flow at 25–75% of vital capacity (FEF 25–75%).

The diagnosis of asthma and allergic rhinitis was ascertained as per American Thoracic Society guidelines and criteria of Adomopoulous et al., respectively (American Thoracic Society, 1991; Adamopoulous et al., 1995).

Informed consent of each subject was obtained for participation in this study as per the guidelines of the Indian Council of Medical Research, New Delhi. The study protocol was approved by the human ethics committee of the institute.

Preparation of extracts

The extracts were prepared from food items including rice (*Oryza sativa* variety Parmal). Food and other aeroallergens were extracted in 1:20 w/v ammonium bicarbonate buffer (50 mM, pH 8.0), by continuous stirring at 4 °C for 4 h. The extracts were centrifuged at 10,000g, supernatant filtered through 0.22 µm filter (Millipore corp), and lyophilized. The extracts were standardized following protocols of the Antigen Laboratory, Institute of Genomics and Integrative Biology, Delhi. The protein content of rice was 28 mg/g of dry powder as estimated by Lowry's method (Lowry et al., 1951).

Skin testing and sera collection

Skin prick tests (SPTs) were performed with extracts of 38 commonly consumed foods (Pulses, Cereals, Vegetables, Fruits, Milk, Egg, Chicken and Fish) and 23 common inhalants (Pollens, Fungi and Insects) on patients of suspected food allergy (history). Briefly, a drop of each extract (1:10 w/v in 50% glycerinated PBS) was placed on the forearm and the skin was pricked with a 26.5G needle. Histamine diphosphate, 5 mg/ml (in 50% glycerinated PBS) served as positive control. Skin tests were graded after 20 min in comparison to positive control. Skin reactions equal to or more than weal size (>3 mm) of positive control were graded as positive. Ten ml venous blood was collected from each patient, sera separated and stored at –20 °C.

Food challenge

Open food challenge. Open food challenge (OFC) was done with rice in SPT positive subjects. About 15–60 g of cooked rice was given to the patients. The open provocation took place under medical observation for at least 2 h. During the observation, blood pressure, pulse rate, forced expiratory volume in 1 s and peak expiratory flow were measured. Subjects showing positive reaction on open provocation were confirmed by DBPCFC.

DBPCFC. For food challenge, exact matching of the placebo test food and the active test food was chosen (Vlieg-Boerstra et al., 2004). Wheat flour was chosen as a vehicle for delivering the active test food. Rice (active food) was mixed with wheat (placebo) in the ratio of 3:7 to make it indistinguishable. The mixture was steamed for 10 min. As placebo test food, wheat flour paste alone was used. Approximately 2–40 g of active (test) food or placebo was given orally to patients for DBPCFC. Pulmonary function evaluation was done after 30 min and FEV₁ was recorded before and after placebo and active test food intake. Neither the patient nor the technician making recordings was aware about ingredients of placebo or active test food. A positive challenge was defined as one where symptoms such as wheeze, chest tightness, shortness of breath, coughing, a drop in FEV₁ of at least 15% for asthma (Roberts et al., 2002), and sneezing, runny noses for rhinitis occurred within 1 h of the exposure. Medication and resuscitation equipment were available in the food challenge area in the clinic. The patients were kept under observation for at least 2 h after the food challenge.

Enzyme linked immunosorbent assay

Specific IgE was determined in patients' sera following Voller et al. (1980). Rice extract (1 µg/100 µl) was coated with carbonate buffer (pH 9.6) overnight at 4 °C in microtiter plate. Non-specific sites were blocked with 3% defatted milk, washed with PBS-Tween 20 and incubated at 4 °C with hypersensitive patients' sera (1:10 v/v). After washing with PBS-Tween 20 and PBS, antihuman IgE horse radish peroxidase was added (1:1000) and incubated for 4 h at 4 °C and color developed with *O*-phenylene diamine (Sigma, St. Louis, MO). The reaction was stopped with 50 µl of 5 N H₂SO₄ and read at 492 nm.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis and immunoblot

To study the protein profile of rice, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was done following the method of Laemmli

(1970). Rice protein (100 µg) was loaded on a 12% reducing gel and run at 120 mV. The gel was silver stained to visualize the separated proteins.

SDS-PAGE separated proteins were transferred onto nitrocellulose membrane (NCM) as described by Towbin et al. (1979). The NCM strips were blocked with 3% defatted milk, washed with PBS-Tween 20 and incubated with individual and or pooled patients' sera (1:5 v/v) overnight at 4 °C. The strips were washed and incubated with 1:1000 diluted antihuman IgE-peroxidase conjugate (Sigma, St. Louis, MO). IgE binding components were detected by adding diaminobenzidine with hydrogen peroxide in sodium acetate buffer (pH 5.0).

Results

Screening of rice allergy patients

Out of 1200 cases screened using a standard questionnaire, 165 gave convincing history of rice allergy. Of these, 22 were suffering from asthma, seven allergic rhinitis and 136 with both. SPTs were performed in 165 patients with raw rice, food and inhalant allergen extracts. Rice extract elicited marked positive reactions in 20 (12.1%) patients. The patients also demonstrated positive reactions with other foods such as *Phaseolus mungo* (blackgram) 6, *Lens culinaris* 4 (lentil), *Cicer arietinum* (chickpea) 2, *Phaseolus lunatus* (lima bean) 2, *Pisum sativum* (pea) 2, *Zea mays* (maize) 2, *Musa paradisiaca* (banana) 2 and pollens viz. *Ehretia aspera* 2, *Artemisia scoparia* 2 and *Cynodon dactylon* 1.

Specific IgE against raw rice was raised (6–28.8 ng/ml) in 13/20 patients whereas 12/20 patients' samples showed elevated IgE against boiled rice (6–26.4 ng/ml). Specific IgE, three times or more of normal control was taken as cut off for positive enzyme linked immunosorbent assay (ELISA). Specific IgE determined against wheat (placebo for DBPCFC) were similar to normal controls (≤ 1.0 ng/ml).

Oral challenge tests for rice allergy

Out of 20 SPT positive patients, 15 agreed to participate in the open (oral) food challenge. Of these ($n = 15$), ten patients showed immediate positive reaction on open provocation with rice. One of the positive patients showed systemic reaction after ingestion of rice. DBPCFC with rice was carried out in these ten patients. 6/10 cases demonstrated positive challenge with 15% or more fall in FEV₁. The patients presented with symptoms such as rhinorrhoea, sneezing, breathlessness, chest tightness, oral thrush, abdominal pain, and skin rashes (Table 1). Patients no. 7–9, 13 showed mild symptoms and ($<15\%$) fall in FEV₁, hence were

Table 1. Analysis of rice sensitive cases by skin prick test, ELISA and oral food challenge tests

No.	Age/sex	Symptoms (history)	SPT dia. (mm)	Specific IgE (ng/ml)		OFC results	DBPCFC		Symptoms after DBPCFC
				Raw	Boiled		Results	Reaction dose	
1	35/F	A, GI, U	7	19.2	16.6	+	+	2	A, GI, U, Ana
2	40/F	A, Rhi	4	24	26.4	+	+	35	A, U
3	32/M	A, Rhi, GI	5	9.6	6.9	+	+	29	A, Rhi, GI
4	13/M	A, Rhi	4	28.8	24	+	+	33	A, U
5	21/M	A, Rhi, U	6	14.4	7.2	+	+	26	A, U
6	43/F	A, Rhi, U	5	12	7.2	+	+	37	A, U
7	19/M	A, Rhi, GI	3	21.6	19.2	+	–	40	A
8	40/F	A, Rhi, U	4	18	10.8	+	–	40	A, Rhi
9	37/M	A, Rhi, GI	5	12	4.8	+	–	40	Rhi, U, GI
10	16/M	A	3	1.9	4.8	–	ND	ND	ND
11	39/F	A	3	6	6.4	–	ND	ND	ND
12	14/F	A	4	9.1	6.9	–	ND	ND	ND
13	39/M	A, U	3	7.2	6	+	–	40	U
14	60/M	A, GI	3	7.2	6.4	–	ND	ND	ND
15	27/M	Rhi, GI	5	3.6	2.4	–	ND	ND	ND

M = male, F = female; A = asthma, GI = gastrointestinal symptoms, U = urticaria, Ana = anaphylactic reaction, Rhi = rhinitis; SPT = skin prick test; OFC = open (oral) food challenge; DBPCFC = double blind placebo controlled food challenge; ND = test not done. SPT positive wheal diameter 3 mm or more; Specific IgE for normal control with raw rice extract = 2 ng/ml; Specific IgE for normal control with boiled rice extract = 1.7 ng/ml; three times of normal control was taken as ELISA positive for specific IgE, i.e. 6 and 5.1 ng/ml for raw and boiled rice, respectively.

designated as DBPCFC negative. The symptoms appeared within 15–40 min of active food (rice) intake. Patient 1 showed anaphylactic reaction within minutes of oral challenge with active food. Patient was given 8 mg decadron intravenously and nebulized twice with salbutamol and ipratropium bromide. The symptoms were controlled after treatment in the emergency unit.

Immuno-reactivity of SDS-PAGE separated proteins

SDS-PAGE of raw rice seed extract showed 21 silver stained bands from 12 to 97 kDa whereas boiled rice extract showed 11 protein bands (Fig. 1). The proteins of 14–16, 19 and 29 kDa were prominent in boiled rice extract but the proteins higher than 29 kDa appeared as faint bands. The 29 kDa protein was absent in raw rice extract and appeared on boiling. The rice wash water (extract) showed six bands with 19 and 33 kDa as prominent bands.

Immunoblotting with hypersensitive-pooled patients' sera showed 12 IgE-reactive bands in raw rice extract (Fig. 2). Proteins of 14–16, 33, 56 and 60 kDa reacted with 80% or more individual patients' sera, and were designated as major IgE-binding components. A 23 kDa protein was recognized by 70% of the patients' sera. Immunoblot of boiled rice with pooled patients' sera showed only four IgE-binding proteins of 16, 23, 33 and

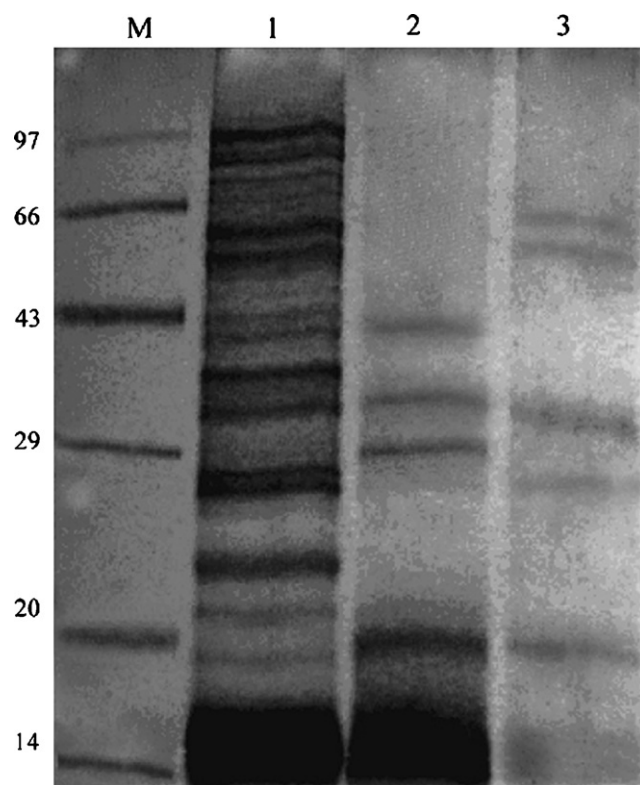


Fig. 1. SDS-PAGE protein profile of rice extract. One hundred microgram protein was loaded and bands were visualized with silver staining. M: Molecular weight marker. Lane 1 = Raw rice extract, Lane 2 = Boiled rice extract, Lane 3 = Rice wash water.

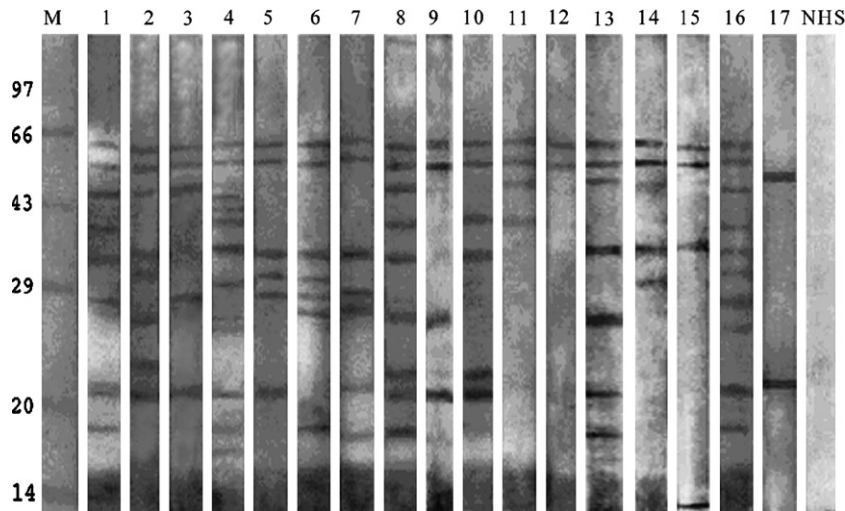


Fig. 2. IgE immunoblot of raw rice extract using hypersensitive patients' sera. M: Molecular weight marker. Lane 1 = Raw rice extract probed with pooled patients' sera, Lane 2–16 = Probed with individual patients' sera, Lane 17 = Boiled rice extract probed with pooled sera, NHS = Normal human sera.

53 kDa. At 53 kDa a new IgE-reactive band appeared in boiled rice extract (Fig. 2). However, no IgE-binding band was observed in rice wash water with pooled patient's sera.

Discussion

Rice allergens induce IgE-mediated reactions varying from urticaria, asthma to anaphylactic shock (Di Lernia, et al., 1992; Arai et al., 1998; Fiocchi et al., 2003). Inhalation of vapors from cooking rice was sufficient to trigger an asthmatic attack in some patients (Gonzalez-Mendiola et al., 2003). Most of the earlier studies reported isolated cases of rice allergy in which only one patient was tested (Arai et al., 1998; Wuthrich et al., 2002; Fiocchi et al., 2003; Orhan and Sekerel, 2003). In the present study, out of 1200 referred cases of allergy/asthma, 165 (13.8%) gave history of rice allergy. Of 165 patients tested, 20 (12%) showed positive skin reactions and 13 (7.8%) had significantly elevated specific IgE, i.e. three times or more of normal control. In the present study, none of the rice positive patients elicited SPT positive reactions with wheat. Some of the rice positive patients demonstrated marked positive SPT with food and pollen extracts. This may be due to co-sensitization or cross-reactivity between them. In earlier studies by Lewis and coworkers, 39% showed positive skin reactions to rice among 1196 cereal allergic adults (Lewis and Imber, 1975). Another study on 580 patients demonstrated adverse reactions to food where 17% had positive RAST with rice (Andre et al., 1994). In a study of patients with specific food-dependent exercise-induced anaphylaxis (FDEIAN), 32/54 cases showed either positive SPT or CAP to rice extract (Romano et al.,

2001). The lower rate of skin and specific IgE-positivity in our study than those of the above mentioned groups might be due to selection bias of the respiratory allergy patients only.

In various studies, specific IgE against rice varied from 4.6–18.5 KU/l by CAP-FEIA in patients with positive food challenge (Wuthrich et al., 2002; Fiocchi et al., 2003; Orhan and Sekerel, 2003). In the present study, patients with positive blinded challenges showed elevated specific IgE (6.9–26.64 ng/ml) by ELISA which is slightly lower than the earlier studies, which might be due to differences in level of sensitization and or calibration methods.

In the present study, rice proteins of 14–16, 33, 56 and 60 kDa demonstrated IgE reactivity with most of the patients' sera (>80%). Findings of the present study are consistent with the previous reports where similar molecular weight proteins were identified as major allergens (Urisu et al., 1991; Usui et al., 2001). Immunoblotting with boiled rice using pooled patients' sera showed four IgE-reactive bands of 16, 23, 33 and 53 kDa. These proteins seem thermo-stable and therefore retain their allergenicity. The 16 and 33 kDa proteins showing IgE-reactivity in boiled rice are the major allergens as identified in raw rice extract. The 14–16 kDa major allergen in rice were characterized as α -amylase inhibitors (Urisu et al., 1991). An allergen of 26 kDa recognized less frequently was identified as α -globulin, the major seed storage protein (Limas et al., 1990). The 33 kDa allergen from *Oryza sativa* L. japonica has been cloned and expressed as a novel glyoxalase (Usui et al., 2001) and demonstrated in various plant tissues.

Despite the risk involved, DBPCFC test is considered the gold standard to confirm hypersensitivity reaction to

food (Vlieg-Boerstra et al., 2004). Positive DBPCFCs were recorded in 55% of milk, 52.5% of chickpea and only 28% of soy sensitive cases (Patil et al., 2001; Perry et al., 2004). In the present study, out of 10 rice sensitive (SPT, ELISA and OFC+ve) cases, 6 (60%) demonstrated positive blinded challenge (DBPCFC) with clinical symptoms such as breathlessness, cough, sneezing, gastrointestinal problem (vomiting and stomach upset) and urticaria (Table 1). One patient developed a severe shock during the oral challenge test. For DBPCFC, boiled rice was used as active test food masked with placebo. Immunoblotting with boiled rice showed only four IgE-reactive bands. This may be due to the loss of allergenic activity of heat labile proteins in rice. All six positive cases in DBPCFC showed high specific IgE to raw/boiled rice extract.

In the present study, rice allergy was established by history SPT, ELISA and open and blinded food challenge. Perception of rice allergy (history) has been confirmed in 3.6% of cases. In the blinded challenge, four patients with minor symptoms did not show > 15% decrease in FEV₁. Taking this into account, history of allergy was confirmed in 6.06% [(3.1–11.16) at 95% CI] of cases. The discrepancy between the self-reported allergic reactions to rice and challenge proven symptoms may be due to their strong tendency to casually relate situations of daily life with ingested foods. Besides, the processing of the recipe of the active food may have led to reduced allergenicity.

In conclusion, rice is an important allergen source afflicting overall 0.8% [0.42–1.58 at 95% CI] of IgE-mediated reactions in asthma and rhinitis patients. The patients with positive skin reactions (4 mm or above) and raised specific IgE to rice ≥ 6.9 ng/ml showed positive blinded food challenges with clinical symptoms. The major allergens of 14–16, 33, 56 and 60 kDa may be explored for diagnostic and or therapeutic potential in rice allergic cases.

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