



IgE response to two new allergen proteins of *Solanum melongena* L. (eggplant)

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ABSTRACT

A number of allergens from eggplant (*Solanum melongena* L.) have been previously identified. In this study, we could detect IgE reactivity of two allergic subjects' sera towards two protein bands of molecular mass of about 35 and 15 kDa. As IgE were reactive to both raw and cooked eggplant extracts, a heat-stable nature of these novel allergens is apparent.

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In recent years, the prevalence of food allergy particularly to different vegetables has dramatically increased. It is estimated that about 4–8% children aged <3 years and 2% adults have food allergy [1,2]. The symptoms of vegetable allergy are often mild and mainly observed in the oral cavity with itching of the oral mucosa, and swelling of the throat, tongue and lips. In some cases, more serious respiratory and skin symptoms are also reported.

Eggplant (*Solanum melongena* L.) is a member of a large family of vegetables and is used in the cuisine worldwide. Several studies have reported allergic reactions to eggplant [3–6]. High incidence of allergic reactions to eggplant was proposed to be mainly due to its high histamine content. Nevertheless, a number of allergens from eggplant have been previously identified [7]. Some of these allergens are heat-sensitive and are therefore destroyed in the cooked eggplant. However, heat-resistant allergens are also identified in the eggplant. We report here two cases of allergic reactions due to consumption of Kamer variety eggplant that it is common in Iran. Laboratory investigations revealed IgE response to two new heat-resistant allergens of eggplant in these individuals.

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First case was that of a 25-year-old man who reported severe itching of lips, eyes, skin, and shedding of tears within 20 min following consumption of a food prepared from the eggplant. These symptoms became severe and lasted for almost an hour. The symptoms were recurrent each time when he consumed any food containing eggplants. He had no family history of allergy.

Second allergic case was reported in a 15-year-old girl who noticed severe itching only in the lips within 5 min following consumption of an eggplant food. This symptom was persisted for nearly 20 min. Similar to first case symptom was recurrent in this patient whenever she was exposed to food containing eggplant. Family history of allergy was negative.

First, we performed skin prick test (SPT) and prick-by-prick test (PPT) in above two individuals by using eggplant extracts. SPT was performed according to Mari et al. [8] with slight modifications by using fresh, raw eggplant extract (30% w/v). This extract was prepared by mixing the eggplant in phosphate-buffered saline (PBS; pH 8)—ascorbic acid (100 mmol/L) for 10 min. Subsequently, a clear 30% w/v extract was obtained by filtration. For PPT, the pulp part of the eggplant was pricked by a sterile lancet. This same lancet was then used to prick the subjects' skin. We have also investigated SPT and PPT for cooked eggplant (15 min in water) in both the subjects. Histamine dihydrochloride (1.5 mg/mL) was used as a positive control and glycerinated PBS was used as a negative con-

Table 1
Results of skin prick test (SPT) and prick-by-prick test (PPT) for raw and cooked eggplant.

	Wheal (bump)/flare (redness) (mm) ^a			
	SPT for raw eggplant	SPT for cooked eggplant	PPT for raw eggplant	PPT for cooked eggplant
Case No. 1(25-year-old man)	4.5/5	5.5/15	2.0/12	3.8/25
Case No. 2 (15-year- old girl)	6.0/10	5.0/10	3.0/30	2.5/20
Histamine dihydrochloride ^b	7–9/5–10		NA ^d	
Glycerinated phosphate-buffered saline ^c	0–1/0		NA	

^a Diameter.^b Positive control.^c Negative control.^d NA: not applicable.**Table 2**
Results of IgE ELISA to eggplant extracts.

Absorbance values (A ₄₀₅)		
	Raw eggplant	Cooked eggplant
Case No.1(25-year-old man)	0.864 ^a	0.132
Case No.2 (15-year- old girl)	0.667	0.384
Histamine dihydrochloride	–	–
Glycerinated phosphate-buffered saline	–	–

^a Mean of triplicates.

trol. We found that SPT and PPT were positive in both the subjects for raw as well as cooked eggplant (Table 1). Based on the data of Pramod and Venkatesh [6] and SPT results, we surmised that the molecular weight of the suspected allergen(s) in the Kamer variety eggplant was >10 kDa.

We then performed ELISA to detect IgE antibodies to eggplant extracts in the subjects [9]. Microtiter wells were coated with the 15 µg of raw and 30 µg of cooked eggplant extracts. The extracts were dialyzed against 100 mmol/L carbonate-bicarbonate buffer (pH 9.6) at 4 °C prior to coating. The coated wells were then incubated with 1:3 diluted 100 µL sera from allergic subjects or normal individuals. Alkaline phosphatase-conjugated mouse anti-human IgE (Sigma–Aldrich) at 1:1000 dilution was used a secondary antibody. We observed that both the allergic subjects had high titers of IgE antibodies to both raw and cooked eggplant extracts (Table 2). Such IgE response was negative in normal subjects.

We then carried out immunoblot to detect the reactivity of IgE from the allergic subjects towards proteins of eggplant (Fig. 1A and B). Ultrafiltration method was used to concentrate the eggplant extract by using DIAFLO YM10 membrane (molecular weight cut-off: 10,000; Millipore, Bedford, USA) and was dialyzed extensively against water at 4 °C for 24 h. This concentrated eggplant extract was then subjected to 12% SDS-PAGE for the separation of the proteins [10]. The proteins were then transferred onto nitrocellulose membrane at 4 °C overnight. After blocking, the membrane was incubated at 4 °C overnight with allergic or normal serum diluted 1:3 in Tris-buffered saline/1% BSA/0.05% Tween-20. The secondary antibody used was alkaline phosphatase-conjugated murine anti-human IgE at 1:1000 dilution.

We could detect IgE reactivity of two allergic subjects' sera towards two protein bands of eggplant extract with molecular mass of about 35 and 15 kDa (Fig. 1B). As expected, normal serum did not react to any protein bands (Fig. 1B). As IgE were reactive to both raw and cooked eggplant extracts, a heat-stable nature of these novel allergens is apparent. Based on the molecular mass, these proteins appear to be distinct from the other identified allergens of eggplant reported elsewhere including lipoxygenase and protein proteinase inhibitor [6]. The nature of these proteins is not yet known and needs further investigation. To our best knowledge, this study is the first descriptive report of food allergy to the Kamer variety eggplant that is widely cultivated perennial herb and common vegetable in Iran.

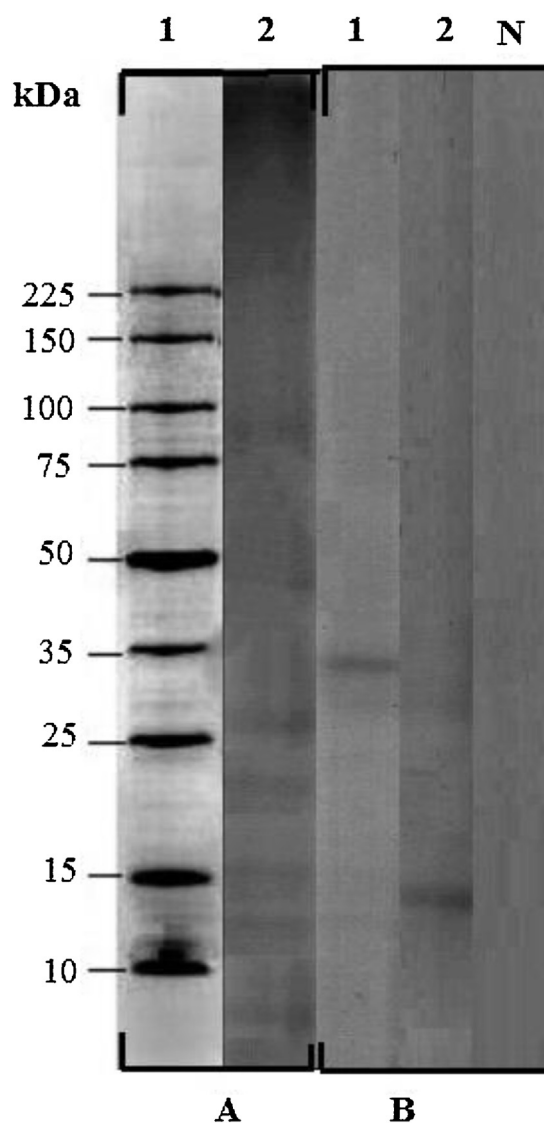


Fig. 1. IgE immunoblot towards raw eggplant extract. (A):SDS-Polyacrylamide gel electrophoresis (SDS-PAGE) silver staining gel: Lane 1, molecular weight markers; lane 2, raw eggplant extract (~10 µg protein). (B): IgE-immunoblotting: N, normal serum; lanes 1 and 2 serum from case numbers 1 and 2, respectively.

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