

Paprika Rhinoconjunctivitis Case Reveals New Occupational *Capsicum* Allergens

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No allergens related to paprika or cayenne respiratory allergy have been identified thus far. We describe a previously healthy 28-year woman who developed work-related rhinoconjunctivitis after four years of kebab-restaurant work. The allergy was studied using skin prick tests, serum specific IgE and nasal provocation tests. Specific IgE protein reactions were studied by Western blot analysis. Paprika, cayenne and curry allergens were identified from the strongest immunoblot bands using tandem mass spectrometry. A positive skin prick test, high specific IgE and positive nasal provocation test confirmed occupational rhinoconjunctivitis from Capsicum species. Defensin J1 and Vicilin were identified as major paprika and cayenne allergens in this case. Vicilin was detected also from the curry ingredients. Two new occupational respiratory allergens from the Capsicum species were identified. These differ from previously reported bell pepper allergens. We emphasize that substantial spice handling at work poses an allergy risk. Am. J. Ind. Med. © 2015 Wiley Periodicals, Inc.

KEY WORDS: spice; restaurant; exposure; respiratory; rhinoconjunctivitis

INTRODUCTION

The incidence of symptomatic spice allergy among adults is 4–13 in 10,000 subjects [Chen and Bahna, 2011]. Occupational spice allergies are very rare outside the spice trade. High spice levels during exposure, as well as atopy and sensitization to various plant profilin allergens, have been named as risk factors in work-related airway spice allergy [Lopata et al., 2010; van der Walt, 2010]. Our case of a kebab seller highlights the fact that these allergies can induce symptoms in restaurant workers exposed to significant spice dust concentrations for short periods, and reveals new *Capsicum* species inhalation allergens of paprika and cayenne.

Subject and Methods

After four years of working in an 8 × 8 m² kebab-restaurant, a 28-year-old white kebab-seller, who did not smoke regularly began to increasingly suffer at work from itchy red eyes, together with sneezing, a runny nose, and mild nasal blockage. She did not have any known allergies. Her symptoms at work appeared daily, particularly while handling spices and spice sauces. Each of the approximately one hundred kebab portions that she made daily were seasoned with potato spice mix powder consisting of paprika and curry and some steaming kebab sauces. Every day she prepared liters of several different kebab sauces using *Capsicum* species such as cayenne powder as the ingredient. Three out of the six spice sauces she handled contained tomato. There was no local ventilation at the workplace. She was referred for examinations due to her work-related rhinitis symptoms.

Skin prick tests (SPTs) were performed with commercial common aeroallergens, various vegetables and spices as is moistured with phosphate buffered saline for prick-prick test, histamine hydrochloride, and diluent controls. A positive SPT wheal was defined as ≥3 mm, and ≥half of the diameter of the histamine wheal. Serum IgE was measured using ImmunoCAP[®] (Phadia, Sweden), where

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values of specific IgE below 0.35 kU/l are considered negative.

A nasal provocation test (NPT) was carried out by dropping 0,1 mL exposure agent on both inferior conchae, to verify the suspected causes of allergy. Occupational rhinitis was confirmed if rhinorrhoea and nasal blockage scored in anterior rhinoscopy changed by a total of four or more points in 35 min and the placebo test was negative (score changes two or less points) [Hytonen and Sala, 1996]. Multiple times stronger rhinitis reaction in allergen provocation compared to the placebo provocation confirms the occupational rhinitis in case of mild hyperreactivity. In addition to the scoring criteria, we measured the amount of nasal secretion and nasal inspiratory resistance change in anterior rhinomanometry (Pa/cc/s %, GM instruments NR6-2).

A written informed consent has been received from the patient for the publication. Proteins were extracted from commercial spices and separated by gel electrophoresis (12% Mini-PROTEAN[®] TGX[™] precast gel, BioRad Laboratories, Hercules, CA). The patient's serum IgE reactions towards extracted proteins were studied using Western blot analysis. The main spice allergens were identified using tandem mass spectrometry (QExactive, Thermo Fischer Scientific, Waltham, MA) [Lehto et al., 2010] both from trypsin digested protein gel and immunoblot bands.

RESULTS

In the SPTs, the histamine wheal was 5 and 6 mm at two different test times and revealed no sensitization to common aeroallergens, whereas distinct reactions were observed for some of the tested spices (Fig.1). Cayenne caused 6 and 7 mm positive wheals and hazelnut a wheal of 5 mm. Fennel, cumin, and soya bean gave 3–4 mm reactions, which were considered unsure. Most SPT of vegetables and spices were negative (Swedish turnip, apple, potato, carrot, celery, kiwi fruit, bell pepper, coriander, parsnip, mustard, black and white peppers, turmeric, peanut, and ginger).

Total IgE was normal. Elevated specific IgE (kU/l) was found 6.84 for paprika, 5.48 for chili pepper, 3.74 for tomato, and 9.86 for curry (mixture of coriander, turmeric, fenugreek, cayenne, fennel, cumin, and black pepper).

The nasal provocation test was done to assess the suspected occupational allergy for workers' compensations. Control NPT with phosphate buffer was considered negative, although a 0.24 g secretion appeared. Rhinomanometry was normal (resistance change +27%). The NPT carried out the following day with 1% paprika spice induced positive strong nasal secretion (3.51 g), sneezing, ocular itching, and nasal cyclic reaction (non-specific resistance increase 87%). The NPT with 100% tomato juice on Day 3 was positive, with nasal secretion (0,92 g), and inspection revealed slight

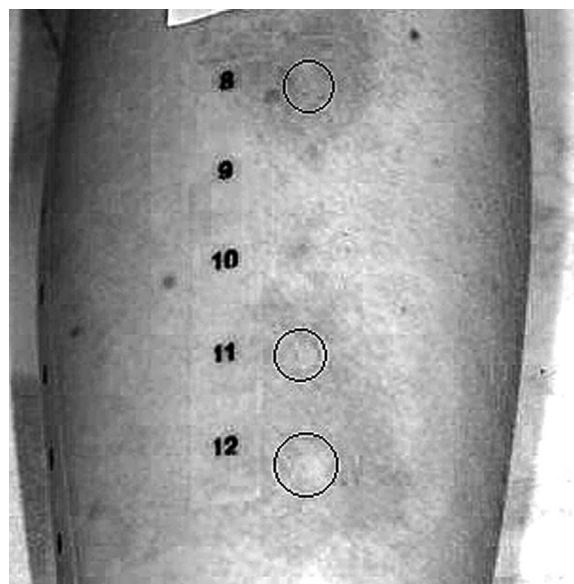


FIGURE 1. Initial skin prick test resulted 5 mm wheals for spice mixtures containing paprika (8,11), no reactions for white and black pepper (9,10), and a 7 mm wheal for cayenne pepper (12).

mucosal congestion increase (resistance increase 130%). To exclude possible irritation effects of the test agent, NPT with paprika was performed for two healthy volunteers, in which cases provocation results were negative.

Western blot of extracts from spice paprika, cayenne, curry, and its ingredients coriander, fenugreek, fennel, black pepper and turmeric revealed the major paprika and cayenne allergen size to be below 10 kDa and minor allergen to be about 50 kDa (Fig. 2). In mass spectrometry the strongest allergen in the *Capsicum* group was identified as 5 kDa Defensin J1. The 50 kDa allergen band was Vicilin, which was found to be the major allergen in fenugreek and curry as well. A minor fenugreek allergen was identified as Profilin-III, which was the only recognized Profilin in this study.

DISCUSSION

The family of *Capsicum* species encompass a wide variety of shapes and sizes of peppers, ranging from mild bell peppers to hot chili peppers, like cayenne. Paprika was suitable for challenge tests to confirm suspected allergy as it lacks the pungent agent capsaicin of chili, which has an inflammatory effect on the nose and can cause rhinitis through sensory neural stimulation [Sanico et al., 1998]. As cayenne was positive in skin prick tests, and CAP to both cayenne and paprika was elevated, suspicion to allergy for both paprika and cayenne was strong. As the plants are botanically closely related, cross-reactivity was likely. Our patient allergens to both paprika and cayenne were similar.

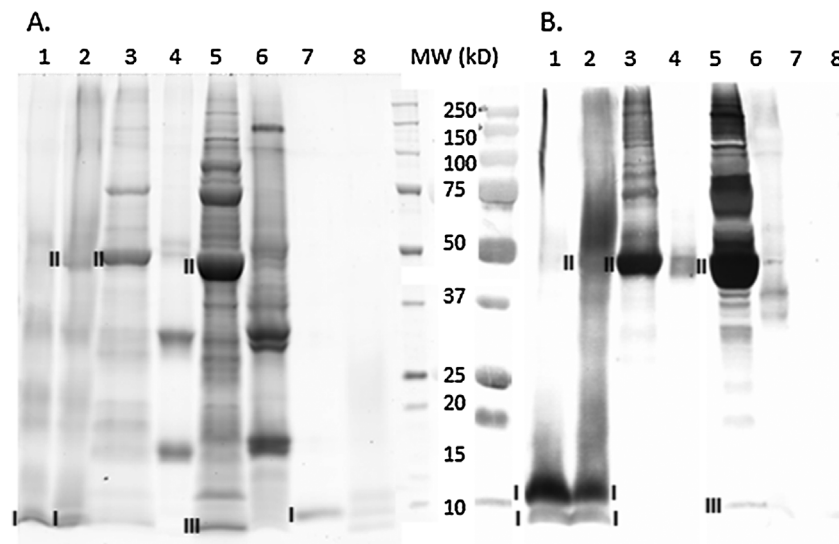


FIGURE 2. Sypro Ruby (BioRad Laboratories) stained protein gel electrophoresis separation (A) and patient serum IgE reactions in Western blot (B) to spice paprika (1), cayenne pepper (2), curry (3), coriander (4), fenugreek (5), fennel (6), black pepper (7), and turmeric (8). In the middle is molecular weight (MW) marker Precision Plus Protein™ Extra Dual Color (BioRad Laboratories). Identified proteins are I: Defensin J1-1, II: Vicilin and III: Profilin-III.

Two major bell pepper and chili fruit allergens from ingested fruits have been previously characterized and sequenced. These are Cap a1, a 23 kDa thaumatin-like protein belonging to a pathogenesis-related family of pollen and fruit proteins that are induced by pathogens and environmental stress; and Cap a2, a 14 kDa labile profilin allergen, which is destroyed during heating and processing. Thaumatin-like proteins are significant allergens in plant food allergy and mainly associated with pollen sensitization. Profilins are plant panallergens, which usually clinically manifest as oral reactions to cross-reacting pollen allergens as well [Asero et al., 2008]. In addition to Cap a1 and Cap a2 allergens, half of the sweet bell pepper strains contain a cross-reactive Bet v1 homologous allergen, which is destroyed during the processing of the vegetable into spice powder [Jensen-Jarolim et al., 1998]. As our patient had no pollen allergy, other allergens than Cap a1 or Cap a2 were likely to explain the daily strong work-related symptoms. In line with this, the only Profilin identification was a minor small size allergen from fenugreek.

Our patient case revealed a new dominant 5 kDa paprika and cayenne allergen: Defensin J1. 5kDa Defensin allergens have been previously found in peanuts (Ara h 12 and 13) and in soybeans (Gly m 2). Plant defensins (also known as gamma-thionins) protect against parasites and form a pathogenesis-related protein family: PR-12. In addition, an unidentified 52 kDa occupational allergen from chili pepper has been reported in spice mill workers [Lopata et al., 2010; van der Walt, 2010]. Our case report confirms this, and identifies the 52 kDa allergen as Vicilin. Vicilin belongs to a

highly thermostable and protease-resistant cupin superfamily of plant storage proteins [Radauer and Breiteneder, 2007]. Vicilin 7s has been previously identified as major fenugreek allergen [Faeste et al., 2010]. In line with this Vicilin was also found as a major allergen in curry containing fenugreek in our patient.

This report identifies the first two occupational inhalation exposure-related allergens from the *Capsicum* species. To our knowledge, this is the first report of a case of occupational allergic rhinitis from paprika that has been confirmed by a challenge test, although asthma in bronchial provocation to paprika has been described previously [Sastre et al., 1996]. In addition, we emphasize the fact that high exposures to spice powders are allergy risks in restaurant work.

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