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Identification of the Most Common Allergens of *Acer velutinum* Pollen

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ABSTRACT

Pollens have been identified as potent inducers of allergic diseases worldwide. *Acer velutinum* (Persian maple) tree is an important source of allergic pollens in Iran. This study aimed to identify the immunoglobulin E (IgE)-reactive components of *A. velutinum* pollen extract in patients with maple allergy. We aimed to evaluate its allergenic components; using IgE in the serum of patients with maple allergy.

Twenty-two patients with a clinical history of reaction and a positive skin-prick test to maple pollen extract were included in this study. Identification of IgE-binding proteins in *A. velutinum* pollen extract was performed by immunoblotting using sera from sensitive patients.

A protein band with a molecular weight of around 70 kDa was the most IgE-reactive allergen in *A. velutinum* pollen extract detected by this method.

Identification of a protein with a molecular weight of about 70 kDa, as the most reactive allergen of *A. velutinum* pollen extract, can be considered as a potential allergen for designing diagnostic kits or as a target for immunotherapy of allergic patients with maple allergy.

Keywords: Hypersensitivity; Immunoglobulin E; Pollen

INTRODUCTION

Allergic rhinitis, inflammation of the mucous membrane of upper airways, is one of the most prevalent atopic disorders. It is characterized by sneezing, itching, nasal congestion, and rhinorrhea.[1] Currently, about 10% to 40% of the population is affected by such conditions.[2] Aeroallergens including house dust mites, pollens, animal dander, and molds are the most common causes of allergic rhinitis.[3]

Pollen allergens are considered a major risk factor for both seasonal allergic rhinitis and asthma.[4,5] Sapindaceae family (maple), with approximately 135 genera of trees and shrubs, is identified as one of the allergenic trees in temperate climate zones which have not been extensively characterized so far.[6] Maple tree pollens are considered as tropical and warm regions around the world, mainly in Eastern North America and Eastern Asia. It spread in Iran as ornamental trees.[6,7] The velvet maple, *Acer velutinum* is one of the largest maples in the world and the most frequent maple species in Iran. *A. velutinum* is also the most prevalent one in Shiraz, southeast Iran.[8]

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Some studies have examined hypersensitivity to maple pollen extract to determine the prevalence of maple pollen aeroallergy among patients with allergic rhinitis by skin prick test (SPT).[9-11] Few studies have investigated the allergenic components of some maple species.[12] To the best of our knowledge, there is no study on the characterization of allergenic components of *A. velutinum*. As this species is prevalent in Shiraz, southwest Iran, we aimed to evaluate its allergenic components; using IgE in the serum of patients with maple allergy.

MATERIALS AND METHODS

Patient Selection

Twenty-two patients with allergic rhinitis who were referred to Imam Raza clinic, Shiraz, Iran, were enrolled in this study. The patients were all positive by SPT with maple extract. Five healthy subjects were selected as negative controls. The protocol of this study was approved by the Ethics Committee of Shiraz University of Medical Sciences (IR.SUMS.REC.1395.S288). Written informed consent was obtained from all participants.

SPT was performed using a commercial extract (Greer, US). After 15 min, the mean diameter of the wheal reaction was measured and compared with saline as negative control and histamine as the positive control. Patients with a wheal diameter>3 mm larger than negative control were considered reactive. Serum samples of patients with positive SPT were then collected and stored at −20ºC.[13,14]

Preparation of *A. velutinum* Pollen Extract

The pollen grains of *A. velutinum* were collected from February to April. Pollen extract was prepared based on a previously established method.[13] Pollens were defatted using repeated changes of diethyl ether by continuous stirring (overnight). One gram of defatted pollens was mixed with 9 mL of phosphate-buffered saline (PBS) and 1mL protease inhibitor 10× (Sigma, Germany) by continuous stirring overnight at 4ºC.

The supernatant was separated by centrifugation at 15000 RPM for 30 min, dialyzed against deionized water, and filtered through a 0.22 μm membrane under sterile conditions. The extract was then freeze-dried. The final powder was dissolved in distilled water. The protein concentration of the extract was measured using the Bradford assay.[15]

SDS-PAGE and Western Blotting

Protein separation of *A. velutinum* was performed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) 12.5%, and the separated protein bands were transferred into nitrocellulose membrane (74 mA, 55 min). After washing and blocking the membrane with skim milk (5% in PBS) for 2 h, different strips of the membrane were separately incubated with the sera of patients with maple allergy or negative control pooled serum (1:5 dilution) overnight at 4ºC.

Horseradish peroxidase (HRP)-conjugated anti-human IgE antibody (Invitrogen, USA, Catalog No. A18799) (1:150 v/v in PBS containing 3% skim milk) was added to the blotted membrane strips and incubated for 2 h at room temperature. Washing was performed by PBS-Tween 20 for removing the unbound antibodies and detection was performed using ECL (Bio-Rad, USA) and documented with G: box imaging system (Syngene, UK).

RESULTS

A. velutinum was detected as the main Acer species in Shiraz. The Voucher number of this species was 781 that detected by the Pharmaceutical Science Research Center of our university. Twenty-two patients with allergic rhinitis, 9 males and 13 females with mean age of 35.36±12.6 years and an age range of 15-68 years were included in the study. The results of SPT with maple extract were positive for all patients (Table 1).

The protein component of *A. velutinum* pollen extract was determined by Coomassie blue staining. Several protein bands in the *A. velutinum* were separated by SDS-PAGE with the molecular weight range of approximately 10 to 250 kDa (Figure1).

The allergenic profile of maple pollen extract was studied by Immunoblotting. A band with a molecular weight of around 70 kDa was detected in *A. velutinum* pollen extract by serum IgE of 4 patients with maple allergy. The negative control pool showed no reaction to the pollen extract (Figure 1).

Table 1. Clinical characteristics and skin reactivity of allergic patients to *A. velutinum* commercial extract

Patients Number	Age (Years), Sex	Symptoms	Wheal diameter (mm)
1	33, F	NS, ES	5
2	36, M	NS, LS	7
3	28, M	NS, LS	7
4	22, F	NS, ES	5
5	68, F	NS, SS	7
6	59, F	NS, LS	5
7	53, F	NS, SS	5
8	47, M	NS, ES, LS	5
9	33, M	NS, ES, LS, SS	13
10	19, F	NS, ES, LS	5
11	35, M	NS, ES	5
12	32, F	NS, ES, LS, EaS	5
13	32, M	NS	4
14	46, M	NS	4
15	25, M	NS	5
16	31, F	NS, ES, LS	5
17	15, M	NS, LS	10
18	39, F	NS, ES	9
19	33, F	NS, ES	7
20	32, F	NS, ES	4
21	27, F	NS, ES	14
22	33, F	NS	6

M: male; F: female; NS: nose symptoms; ES: eye symptoms;
LS: lung symptoms; EAS: ear symptoms; SS: skin symptom

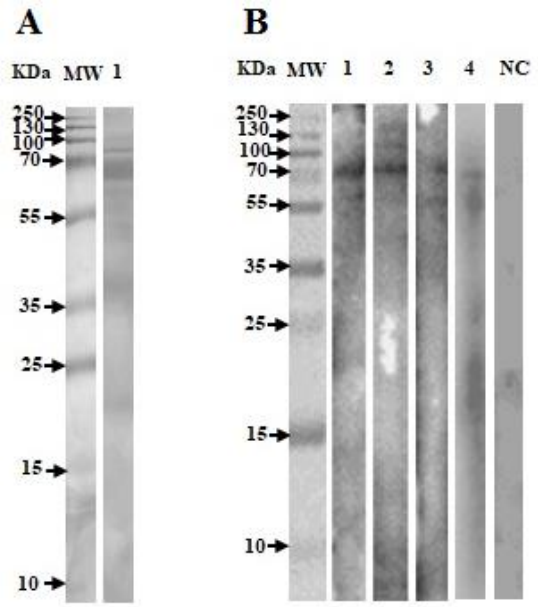

Figure 1. A: SDS-PAGE of A. velutinum pollen extract. MW: molecular weight size marker; B: Immunoblotting of the A. velutinum pollen extract using the serum of allergic patients (lanes 1-4), MW: molecular weight size marker, and NC: negative control.

DISCUSSION

Allergic reaction to maple is common among patients with allergic rhinitis in Iran.[16] As far as we know, the protein allergenic profile of *A. velutinum* is not yet characterized and most studies only have investigated the frequencies of patients with an allergic reaction to maple extract with SPT. In Mazandaran, north of Iran, about 3.6% of patients with allergic rhinitis had positive SPT with maple extract.[10] Maple allergy was also reported 3.03% and 25.5% in two separated studies carried out in Turkey by SPT.[8,9] In New York, the prevalence of hypersensitivity to maple pollen extract was reported about 32.8%.[17]

In the current study for the first time, we detected an IgE-reactive protein band with a molecular weight of about 70 kDa in four patients with maple allergy. SDS-PAGE revealed several bands from the *A. velutinum* pollen extract, among which ~70 kDa protein was identified as the immune-dominant band. Four patients showed IgE reactions to *A. velutinum* pollen extract. When we used mixed maple extract in the skin prick test, patients who were sensitive to maple could detect common allergen between maple species and show positive skin prick test. Among all patients with positive SPT, only 4 patients had specific IgE against *A. velutinum* species. No detectable reaction to common antigen among maple species in other patients might be due to low IgE titer or low antigen concentration. The characterization of this protein is remained to be identified and should be addressed in future studies. In a study performed by Ribeiro et al, a similar fraction with ~71 kDa was detected in *A. negundo* (boxelder maple), a species of maple native to North America, pollen extract by IgE serum of seven out of eight patients with maple allergy.[12] We hypothesized that this 71 kDa fraction might be similar to ~70 kDa protein detected in our study.

In conclusion, this 70 kDa protein as the most reactive allergen of *A. velutinum* pollen extract can be considered as a potential allergen for designing diagnostic kits or as a target for immunotherapy of allergic patients with maple allergy.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest concerning research, authorship, and/or publication of this article.

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