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Study on allergenicity of *Thuja orientalis* pollen grains in rat

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Abstract Cupressaceae pollen allergy is an important cause of pollen allergy throughout the world. Prevalence of allergy to Cupressaceae pollen has increased significantly during the winter over the past 3 decades because of extensive planting of cypress trees for different purposes. *Thuja orientalis* (Cupressaceae) is a naturally grown plant in Iran and is widely cultivated as a common ornamental plant in this country and other ones. Allergenicity of its pollen has been established, but to this day no allergenic component has been detected. The aim of this research is to study allergenicity and evaluate the immunoglobulin E reactivity to *T. orientalis* pollen extracts. Pollen grains were directly collected from mature male cones of trees. Pollen proteins were extracted and were analyzed by SDS-PAGE. Total protein content of pollen extracts was measured by Bradford assay. Immunoblotting using the serum of sensitized rats showed a single immunogenic band at about 44KD in

pollen extracts. Result of this research proved that pollen grains of *T. orientalis* are allergenic.

Keywords Allergy · Cupressaceae · Immunoblotting · Pollen protein content · *Thuja orientalis*

1 Introduction

Respiratory diseases, such as allergic rhinitis, conjunctivitis and asthma, have a worldwide distribution (Longhi et al. 2009). A large population is affected by allergic diseases due to aerobiological disorders such as pollen, spores, house dust mites, insects and different kinds of foods (Celik et al. 2005; Guvensen et al. 2005). Pollen is an important trigger of seasonal rhinitis, conjunctivitis and/or asthma and an exacerbating factor in atopic dermatitis (Kamijo et al. 2009). Anemophilous plants as part of their sexual reproduction release vast amount of small pollen into the air (Mazzitelli and Grilli Caiola 2003; Aboulaïch et al. 2008; Rokade et al. 2010).

Cupressaceae pollen allergy is an important cause of pollen allergy throughout the world. The role of this pollen in winter pollinosis has been clearly demonstrated (Alisi et al. 2001; Sin et al. 2008; Caimmi et al. 2012). This kind of allergy is so-called winter allergic cold (Ianovici 2009).

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The amounts of Cupressaceae pollen released into the atmosphere, especially at the areas where these trees are planted largely for ornamental purposes in gardens and parks are often far greater than those of grass pollen (Pâris-Köhler et al. 2000; Sin et al. 2008). The prevalence of allergy to Cupressaceae pollen has also increased significantly during the winter over the past 3 decades throughout the world (Pâris-Köhler et al. 2000; Diaz de la Guardia et al. 2006; Caimmi et al. 2012). Recent increases in the incidence of this pollinosis are thought to be due to the extensive planting of cypress trees for different purposes and therefore the greater exposure of the population to these plants (Hrabina et al. 2003; Diaz de la Guardia et al. 2006; Caimmi et al. 2012), underestimation of its real prevalence by confusing it with winter colds, and the concomitant effect of environmental pollution on these allergenic particles (Diaz de la Guardia et al. 2006).

Six genera of the Cupressaceae family (*Cupressus*, *Juniperus*, *Chamaecyparis*, *Callitris*, *Thuja* and *Libocedrus*) are a very important cause of allergies in various geographical areas including North America, Japan and Mediterranean countries (Cortegano et al. 2004).

Thuja orientalis L. (Fig. 1), locally named Sarv-e Khomreii or Nosh, is an evergreen species, which grows naturally in China, Korea, Japan and Iran. Also, this species is widely cultivated as a common ornamental plant in Iran and other countries (Hashemi and Safavi 2012). This plant provokes medium to strong allergic reaction, and allergenicity of the pollen has been established (Celik et al. 2005; Shahali et al. 2007). But no scientific research has been done to this day to identify its pollen allergenic components. The aim of this research is to study allergenicity and evaluate the immunoglobulin E (IgE) reactivity to *T. orientalis* pollen extracts.



Fig. 1 *Thuja orientalis*

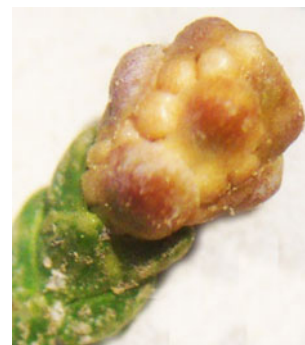


Fig. 2 *Thuja orientalis* mature pollen cone

2 Materials and methods

2.1 Preparation of pollen extracts

During the end of winter 2011 and few days after anther bursting, pollen grains were directly collected from mature male cones (Fig. 2) of trees planted in the Shahid Bahonar University campus. Pollen grains were briefly dried and freeze-dried at -20°C .

The pollen extracts were prepared by incubating pollen grains in 0.1 M phosphate buffered saline, PBS, pH 7.4 in 10 % ratio at 4°C for 18 h. Suspensions were centrifuged at $14,500g$ for 40 min at 4°C , and the supernatants were collected and stored at -20°C until used. The total protein contents of pollen extracts were determined according to Bradford protein assay (1976) using bovine serum albumin (BSA) as a standard.

2.2 Rats, sensitization and sample collection

Fourteen male Wistar rats, 4–6 weeks old, were purchased from the Animal Center of the Shahid Bahonar University (Kerman, Iran) and randomly divided into the control and sensitization groups. The rats were housed in colony cages (7 rats per cage) in room maintained at $24 \pm 2^\circ\text{C}$ with the altering 12:12 h light–dark cycle and fed laboratory diet and water ad libitum.

The first sensitization procedure was initiated 1 week after the arrival of the rats. Animals sensitized by injection of pollen extract on five occasions at weekly intervals through the intraperitoneal route with 75 μg protein with alum and for the negative control group, non-sensitized rat were intraperitoneally treated with PBS. One week after the last injection, animals were decapitated and the blood was collected. Sera were obtained with centrifugation and stored at -20°C until used.

2.3 SDS-PAGE and immunoblotting for the detection of IgE reactivity

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the pollen extract was performed according to Laemmli (1970) using a 12.5 acrylamide separation gel. The samples were run at a constant voltage of 80 V till the dye reached 0.5 cm above the lower end of the gel.

After electrophoresis, proteins were either stained with Coomassie blue or electrotransferred to a 0.45 μm pore size polyvinylene difluoride (PVDF) membrane.

Immunoblot analysis of IgE binding protein fractions was carried out by the method outlined by Towbin et al. (1979).

After blotting, the membrane was stained with Ponceau S red to demonstrate successful transfer of proteins, and membranes were blocked overnight at 4°C in blocking buffer containing 5 % nonfat milk powder in TBS-Tween20. The membrane was washed three times for 20 min in TBS-Tween20 and incubated for 3.5 h with 1:5 diluted pooled sera of allergic subjects in TBS-Tween20. After the incubation, the membrane was washed and incubated for 2 h with 1:10,000 dilution of anti-rat IgE tagged with horse radish peroxidase in TBS-Tween20. The membrane was washed again and then treated by ECL

chemiluminescence method (Amersham Kit). The allergens were revealed after 2 min contact with X-OMAT Kodak film. In all steps, we shook the solution.

3 Results

Bradford protein assay revealed that the total protein content in the pollen extract of *T. orientalis* was 297 $\mu\text{g}/\text{ml}$ in 1:10 (w/v) concentration.

A total of 10 protein bands were detected in the pollen extract by Coomassie blue stain (Fig. 3). The molecular weight of separated proteins ranged from 13.9 to 70 kDa.

The immunoblot analysis was carried out in order to detach the specific IgE binding proteins in pollen extract and showed single immunogenic band at about 44 kDa (Fig. 3). No IgE binding was observed in the Western blot performed with the control serum.

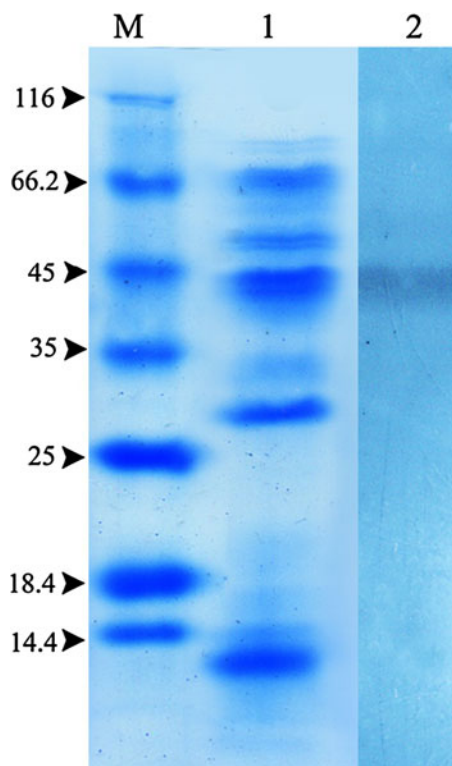


Fig. 3 Pollen protein profile of *T. orientalis* and immunoblots using the pooled sera of an sensitized subjects. *M* marker, lane 1 *T. orientalis* pollen extract, lane 2 Immunoblot showed that the protein with molecular weight at about 44 kDa was allergenic component of *T. orientalis*

Table 1 Allergens from Cupressaceae species

Allergen name	Species	Biological function	References
Cry j 1	<i>Cryptomeria japonica</i>	Pectate lyase	Yasueda et al. (1983); Taniai et al. (1988)
Cry j 2	<i>Cryptomeria japonica</i>	Polygalacturonase	Sakaguchi et al. (1990)
Cha o 1	<i>Chamaecyparis obtusa</i>	Pectate lyase	Kawamoto et al. (2002)
Cha o 2	<i>Chamaecyparis obtusa</i>	Polygalacturonase	Kawamoto et al. (2002)
Jun a 1	<i>Juniperus ashei</i>	Pectate lyase	Midoro-Horiuti et al. (1999)
Jun a 3	<i>Juniperus ashei</i>	Pathogen-related protein (PR-5)	Midoro-Horiuti et al. (2000)
Cup a 1	<i>Cupressus arizonica</i>	Pectate lyase	Di Felice et al. (1994)
Cup a 2	<i>Cupressus arizonica</i>	Polygalacturonase	Di Felice et al. (2001)
Cup a 3	<i>Cupressus arizonica</i>	A thaumatin-like protein	Cortegano et al. (2004)
Cup a 4	<i>Cupressus arizonica</i>	A calcium-binding protein	de Coana et al. (2010)
Cup s 1	<i>Cupressus sempervirens</i>	Pectate lyase	Arilla et al. (2004)
Cup s 3	<i>Cupressus sempervirens</i>	Thaumatin-like protein	Togawa et al. (2006)

4 Discussion

Cupressaceae pollen is an important cause of respiratory allergies in winter. Increased planting of these trees for ornamental purposes in gardens and parks, and also as wind and noise barriers, is probably the main cause of increased prevalence of sensitization to Cupressaceae pollen (Aceituno et al. 2000). The role of Cupressaceae pollen in allergic diseases has often been underestimated because of coincidence the Cupressaceae pollination period with the winter season when upper respiratory tract diseases are common (Hrabina et al. 2003).

Analysis of Cupressaceae pollen extracts in IgE immunoblotting identified the major allergenic components, by IgE from allergic patients (Table 1).

In cypress pollen, four main molecular families of allergens have been described: the group 1 allergens belonging to pectate lyase family, the group 2 to polygalacturonase family, the group 3 to thaumatin-like proteins and the group 4 corresponding to Ca²⁺ binding proteins (shahali et al. 2012).

In this study for the first step, we tried to evaluate the immunoglobulin E (IgE) reactivity to *T. orientalis* pollen extracts in sensitized rats. Western blotting results proved that the pollen of *T. orientalis* is allergenic.

In conclusion, current knowledge of Cupressaceae pollen allergens is concentrated on major allergen sources in Europe, North America and Japan. The increase in allergic diseases, especially in Asia, emphasizes the need for molecular identification,

isolation and characterization of these allergens. To establish effective diagnosis immunotherapy for these kinds of pollinosis, identification and immunological characterization of important allergen molecules is a crucial step. For this purpose, this preliminary study was performed and major allergenic component of *T. orientalis* pollen was determined. More complete identification and structural characterization of this pollen allergen is needed and allow us to improve the diagnostic and therapeutic application of this allergen.

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