

The Allergenic Potency of Japanese Hop Pollen Is Increasing With Environmental Changes in Korea

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Purpose: The sensitization rate to Japanese Hop (Hop J) in respiratory allergy patients has increased in recent years in Korea. We evaluated changes in the allergenic potency of Hop J pollen collected in 1998 and 2009. **Methods:** Thirty-five patients with allergic rhinitis and/or asthma were enrolled. Group I included 21 subjects sensitized to Hop J at an initial visit and group II included 14 subjects who developed a new sensitization. Hop J pollens were collected in 1998 and 2009 (98 and 09 extracts) and both urban and suburban environments (urban and suburban extracts). Serum specific IgE levels to Hop J pollen extracts were compared using an enzyme-linked immunosorbent assay (ELISA). IgE binding components were compared by IgE immunoblot analysis. **Results:** Serum specific IgE levels to the 09 and urban extracts in both groups increased significantly compared to those of the 98 and suburban extracts. IgE immunoblot demonstrated that the major 10 kDa allergen was intensified in group I, while it was newly generated in group II with additional components ranging from 12-95 kDa. When the 98 and 09 extracts were compared, intensification of the major allergen of 09 extract had occurred in both groups. The IgE binding components of the urban extract was stronger than those of suburban one. **Conclusions:** The allergenic potency of Hop J pollen may be increased with environmental changes.

Key Words: Allergens; environment; Japanese Hop pollen

INTRODUCTION

The Fourth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC) demonstrated that the global average temperature has been increasing with the increased concentrations of anthropogenic greenhouse gases since the mid-20th century.¹ Several studies have demonstrated that environmental changes, particularly climate change can result in increased pollen production, extension of the pollination period, and increased levels of the allergenic components of major pollens.²⁻⁴ The urbanization of Asian countries, including Korea and China, is rapidly increasing; this will accelerate the environmental greenhouse effect. Moreover, recent studies have shown that atmospheric temperature and CO₂ content are increasing⁵ and the prevalence of allergic diseases sensitive to pollens is increasing in this area.^{6,7}

Japanese hop (Hop J), a weed of the family Cannabis and or-

der Urticales, is one of the principal allergenic plants in Far Eastern region that induces pollinosis between the middle of August and the end of September.^{6,8-10} According to aerobiological studies, the pollen counts of Hop J are greater than those of mugwort or ragweed pollens during the pollination period in these areas.^{11,12} It is not cross-reactive to other weed pollens when evaluated by ELISA inhibition.¹³ Weed pollen counts are likely correlated with asthma and rhinitis symptoms, which were closely related with that of Hop J.⁷ Furthermore, a recent study demon-

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strated that skin reactivity to Hop J increased significantly in respiratory allergy patients; furthermore, the sensitization period in the past 10 years in this country has been increasing.¹⁴ Therefore, we hypothesized that Hop J pollen may be the weed pollen most sensitive to environmental changes in this area.

The objective of this study was to confirm the changing patterns of allergenic potency within Hop J pollen collected two times, 1998 and 2009.

MATERIALS AND METHODS

Patients

Thirty-five patients with allergic rhinitis and/or asthma sensitive to Hop J pollen by skin prick test were enrolled from Ajou University Hospital, Suwon, Gyeonggi province, South Korea. The study subjects were divided into two groups, 21 who were sensitized to Hop J at the initial visit (group I) and 14 who had developed a new sensitization to Hop J pollen over the past 10 years (group II). They had suffered from seasonal aggravation of asthmatic and rhinitis symptoms. Symptoms were newly developed or aggravated during the follow-up period in Group II subjects. Patients' sera were collected. This study was approved by the ethical committee of Ajou University Medical Center, Suwon, Korea and written informed consent was obtained from each subject.

Preparation of Hop J pollen extracts

Hop J pollens were prepared individually and collected from Gyeonggi province during the middle of August and the end of September in 1998 and 2009 (98, 09 extracts). During the 2009 season, pollen was collected in two different environments: urban (Seoul as a representing metropolitan city), and suburban (Gyeonggi province surrounding Seoul), where we measured the temperature and CO₂ concentration. During the growing season, the mean temperatures and CO₂ concentrations are on average 1.09 ± 0.55°C and 20 ± 9.67 ppm lower in suburban (11.04 ± 9.79°C, 420.40 ± 3.51 ppm) compared with that in urban (12.12 ± 10.19°C, 440.40 ± 8.96 ppm). The mean humidity is lower in urban (66.75%, 71.9%). Air pollutants including particulate matter, nitrogen dioxide and ozone are more abundant in urban area. The pollens were defatted and extracted by magnetic stirring (24 h at 4°C) in phosphate-buffered saline (PBS), 1:5 wt/vol at 4°C for 1 h followed by centrifugation at 5,000 rpm. The supernatant was dialyzed (The cut-off molecular weight: 6,000 Da) against 4 L distilled water at 4°C for 48 h. Pollens were then lyophilized, and subjected to enzyme-linked immunosorbent assay (ELISA) and immunoblot analysis.

ELISA for serum specific IgE antibodies to Hop J pollen extracts

Changes in serum specific IgE levels to Hop J were determined by ELISA according to previously described methods.¹⁵ Briefly, microtiter plates (Costar, New York, NY, USA) were coated with

four different kinds of Hop J pollen extracts with carbonate buffer (10 µg/mL) and left at 4°C overnight. Each well was washed three times with 0.05% Tween-PBS (PBS-T), and blocked by incubation with 200 µL/well of 10% fetal bovine serum-PBS for 1 h at room temperature (RT). The wells were then incubated for 2 h at RT with 50 µL of either the subjects' or control sera (diluted 1:5). After washing three times with PBS-T, biotin-labeled goat anti-human IgE antibody (Vector Co., St. Louis, MO, USA) was added to the each well and incubated for 1 h. After washing, the wells were incubated with 100 µL of 1:1,000 (vol/vol) streptavidine peroxidase (Sigma Co. St. Louis, MO, USA) for 30 min and then washed again as before. The colorimetric reaction was developed with TMB (3,3', 5,5'-tetramethylbenzidine) substrate solution for 10 min at RT. The reaction was stopped by the addition of 100 µL of 2 N sulfuric acid, and the absorbance at 450 nm read by an automated reader (Benchmark, Bio-Rad, Hercules, CA, USA). The amounts of specific antibodies were calculated from control curves that were created using the OD of serial dilutions of positive control samples that showed higher antibody titers using a spline fit program (Microplate Manager, Bio-Rad), and were expressed as arbitrary units (AU).

IgE immunoblot analysis using Hop J pollen extracts

IgE immunoblot analyses were performed under reducing conditions according to methods described previously.¹⁵ Hop J pollen extracts were mixed with sample buffer (Tris-HCl 31 mM/L, 10% glycerol, 1% SDS, 0.0025% bromophenol blue, 2.5% mercaptoethanol; pH 6.8) and heated in boiling water for 5 min. A standard marker (10 to 170 kDa; Lonza, Switzerland) and the 30 µg Hop J pollen extracts were loaded onto 12% Tris-glycine gels for separation of the antigens. Electrophoresis was performed with a Bio-Rad PowerPac 1,000 for 180 min at 120 V. The gel was fixed and stained with Coomassie brilliant blue. For immunoblotting, the proteins were transferred onto a polyvinylidenedifluoride (PVDF) membrane (Millipore, Bedford, MA, USA) in transfer buffer (Trisbase 25 mM/L, glycine 193 mM/L, and methanol 20%) with a Bio-Rad transfer apparatus set at 200 mA for 90 min. The blotted PVDF membrane was sliced into 4 mm widths. These pieces were blocked with 20% skim milk in 0.1% Tris-buffered saline (TBS)-Tween (TBST) for 2 h to block nonspecific binding. Each membrane was then incubated overnight at 4°C with subjects' or control sera that had been diluted 1:2 v/v with 5% skim milk-TBST. After washing, the membranes were incubated with anti-human IgE conjugated to biotin and streptavidine-alkaline phosphatase (1:1,000 diluted, Sigma Co.) for 1 h at RT. After washing with TBST, membranes were developed using BCIP/NBT alkaline phosphatase substrate (Sigma Co.). The band intensities were measured using the image J software package (National Institutes of Health, USA).

Statistical analysis

Statistical differences between the two groups were assessed using the Mann-Whitney test and Fisher's exact test with the SPSS software package (Chicago, IL, USA), version 12.0. Wilcoxon signed rank tests were applied to evaluate statistical differences between two values. A *P* value of 0.05 or less was regarded as statistically significant.

RESULTS

Characteristics of the study subjects

There were no significant differences in age, sex, or smoking

Table 1. Comparison of clinical features between the two groups

	Group I (n=21)	Group II (n=14)	<i>P</i> value
Age (year)*	38.48 ± 11.54	40.07 ± 10.64	0.80 [†]
Sex (M/F)	9/12	4/10	0.49 [‡]
Smoking history, n (%)	1 (5)	2 (17)	0.55 [‡]
FEV1 (% pred)*	104.86 ± 12.68	101.70 ± 15.38	0.51 [†]
Total IgE (KU/L)*	1,008.90 ± 1,539.34	855.14 ± 1,146.64	0.92 [†]
TEC (μg/L)*	320.24 ± 256.49	308.43 ± 366.30	0.47 [†]
Response to Hop J, (n) [†]			0.99 [‡]
3+	3	2	
4+	5	4	
5+	5	3	
6+	8	5	

*All values were presented as mean ± SD; [†]The response was assessed by skin prick test with hop J extract; [‡]Difference between means was assessed by Mann-Whitney test; [§]Calculated by Fisher's Exact Test. TEC, total eosinophil count; Hop J, Japanese hop.

history. The baseline FEV1, serum total IgE levels, total eosinophil counts and positive results to Hop J pollen extract on skin prick test between the two groups were not significantly different (Table 1).

Comparison of serum specific IgE levels to four kinds of Hop J pollen extracts in group I and II

The serum specific IgE levels to the 98 extracts were not significantly different between the two groups (*P*=0.893); however, serum specific IgE levels to the 09 extracts were significantly higher than those of the 98 extracts in both groups (*P*<0.001, *P*=0.006, respectively; Fig. 1A). Moreover, when serum specific IgE levels to the 09 Hop J extracts from urban and suburban areas were compared, those to the urban extracts were significantly higher than those to the suburban in both groups (*P*<0.001, *P*=0.001; Fig. 1B).

Comparison of IgE binding components using four different Hop J pollen extracts

Fig. 2 shows the distribution of the IgE binding component in group I, in which a major IgE binding component at 10 kDa was detected. In addition, this component was newly generated (data not shown), and other IgE binding components ranging from 12-95 kDa were generated in group II. When these IgE binding components were compared between the 98 and 09 extracts in groups I and II, the intensity of the major IgE binding component at 10 kDa was further intensified (1.39 and 1.79× the mean optical density increased in group I and II) in the 09 extract in both groups. The new IgE binding components were more prominent within the 09 extract. In addition, when we compared the 09 Hop J extracts derived from urban and subur-

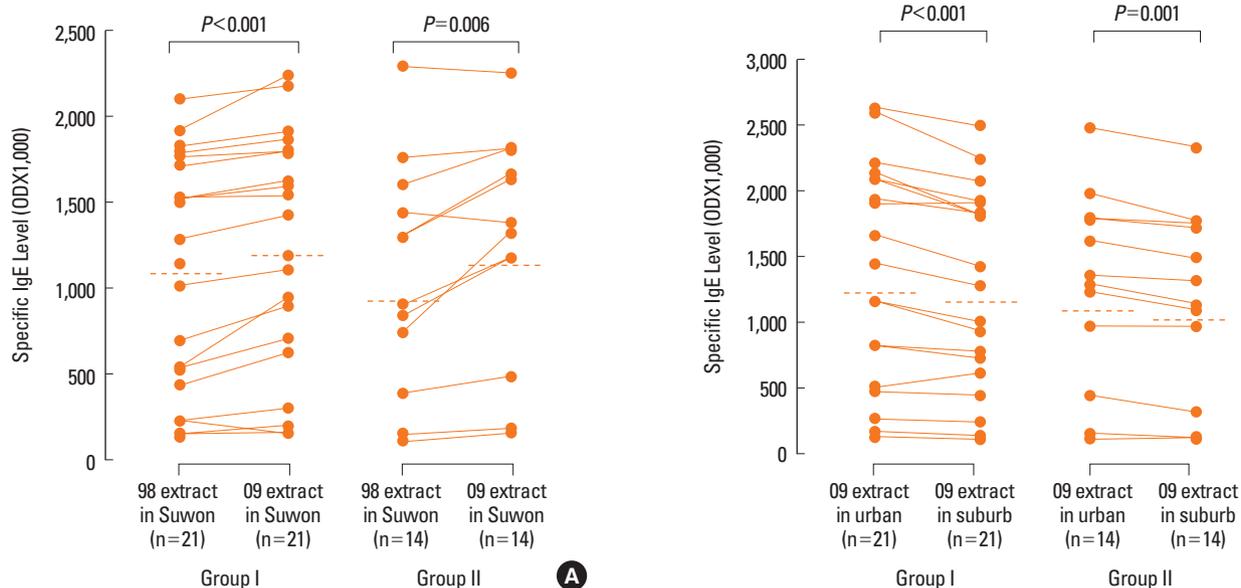


Fig. 1. Comparison of serum specific IgE levels against four different Hop J pollen extracts, i.e., the 98 and 09 extracts (A), and the urban and suburban extracts (B), in groups I and II subjects using enzyme-linked immunosorbent assay. *P* values were determined by a Wilcoxon signed rank test. Horizontal bar indicates the mean value.

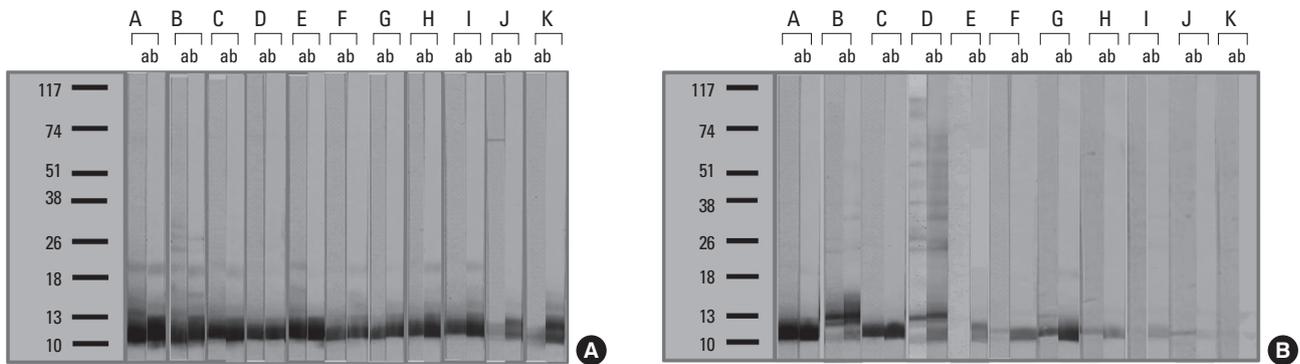


Fig. 2. Changes in specific IgE binding components, as determined by IgE immunoblot, using the 98 and 09 extracts in group I (A) and II (B) subjects. a, 1998 year Hop J pollen extracts; b, 2009 year Hop J pollen extracts.

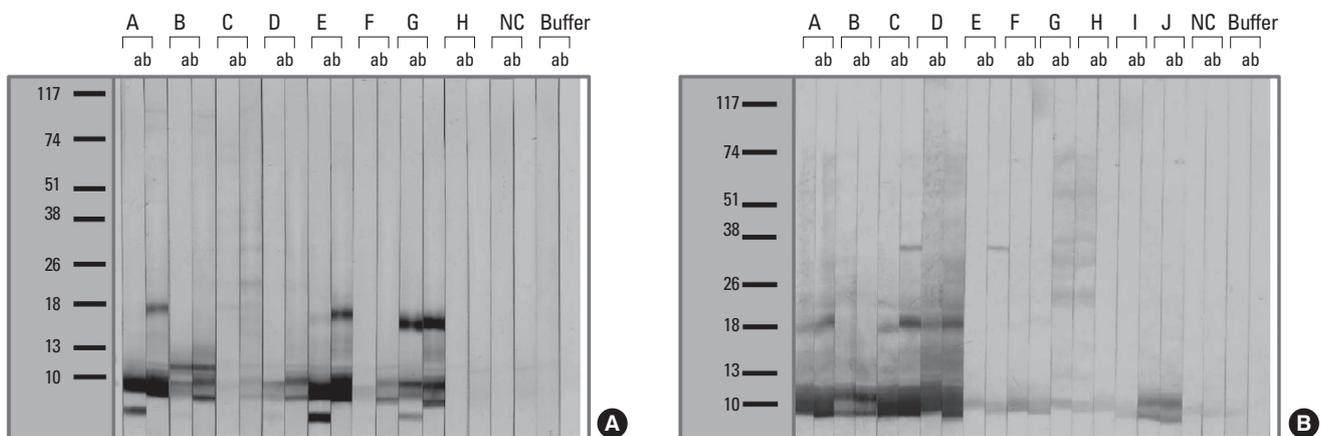


Fig. 3. Changes in specific IgE binding components as determined by immunoblot using the Urban and Suburban extracts in Group I (A) and Group II (B). a, Suburban Hop J pollen extracts; b, Urban Hop J pollen extracts.

ban areas, both the major 10 kDa IgE binding component and the new IgE binding components (12-95 kDa) were enhanced in the urban extracts in both groups (Fig. 3).

DISCUSSION

Hop J pollen is one of the most prevalent weed pollens during the autumn in Far Eastern region.¹⁰ Its count in the air in this country has been steadily increasing and the pollination period of them has been extended.^{7,16} According to the data from Korean Meteorological Administration, average temperature has increased by more than 1.5°C over the past 100 years, which is on average 0.07°C higher in the 2000s compared with that in the 1990s in Suwon. The average CO₂ concentration for the year, 2009 recorded 392.5 ppm, an increase of 21.8 ppm relative to the annual average of 370.7 ppm for 1999. We found that the air temperature and CO₂ content were significantly higher in urban than suburban areas,⁵ which may be due to global warming and environmental changes resulting from rapid urbanization in this region.¹⁷ Based on these findings, Hop J pollen is the most sensitive to environmental changes, climate changes in

particular, thus, it is likely that its sensitization rate will increase further with environmental changes and urbanization in the Far East.

Considerable research has been conducted on the impact of climate changes on the increasing allergenic potency of ragweed and birch pollens,¹⁸⁻²¹ but no such report regarding Hop J pollen has to our knowledge been published. First, we demonstrated that 14 group II subjects who showed an initial negative response to the Hop J pollen on a skin prick test became sensitized with an aggravation of seasonal symptoms during the follow-up period. This suggests that Hop J pollen can induce sensitization in exposed adult patients, which will become more prevalent as the environmental changes further.

To observe the effects of urbanization the IgE-binding capacity of Hop J pollen extracts, we compared serum specific IgE levels to Hop J pollen extracts from urban and suburban areas collected in 2009. The serum specific IgE levels to the urban extracts were significantly higher than those to the suburban extracts in both groups. Similarly, serum specific IgE levels to the 09 extracts were significantly higher than those to the 98 extracts. Furthermore, IgE immunoblot analysis demonstrated

that the intensity of the major 10 kDa IgE binding component to the 09 extracts was greater than that to the 98 extracts in both groups I and II. Although we collected Hop J pollen two times in the same place and weeks in 1998 and 2009, allergen contents within pollens may vary depending on location or time during which they were collected and an influence of annual difference should be considered. Based on these findings, we suggest that the allergenic potency of the major 10 kDa allergenic component of Hop J pollen may be increasing with environmental changes. Further studies will be needed to investigate the exact mechanism by which this allergen is augmented as the environmental changes, and to develop a therapeutic strategy to prevent further increases in Hop J pollinosis.

It is well known that the increased allergenic potency of major pollens with climate change is caused by higher temperature and CO₂ levels.^{2,3,22} Moreover, the increased presence of outdoor air pollutants resulting from higher energy consumption and vehicle exhaust emissions may account for the increasing prevalence of allergic respiratory diseases in polluted urban areas.²³⁻²⁵ Ahlholm et al.²⁶ found that IgE-immunoblot responses to the major allergen of birch pollen were stronger in the sera of patients exposed to birch pollen grown at higher temperatures. Singer et al.²⁷ showed that Amb a 1 allergen concentrations increased in response to ragweed plants cultivated under higher CO₂ concentrations. In the present study, we did not perform the *in vitro* experiments that would explain how the CO₂ content and/or temperature increased the allergenic potency of Hop J pollens. However, we found a higher CO₂ content and temperature in urban areas as compared with suburban areas in this country,⁵ and the allergenic potency of the urban extracts was higher than that of suburban extracts. The Hop J pollen count in the air of Far East nations is increasing, and Hop J pollen is the most sensitive to environmental changes.^{7,11} We speculate that global warming, air pollution, and urbanization may have a significant adverse impact on allergic respiratory diseases such as allergic rhinitis and asthma in this area. Further comprehensive research is essential to clarify how changes in climate, in parallel with air pollution and urbanization, will increase Hop J pollinosis. In addition, studies should focus on the development of therapeutic interventions.

In conclusion, the allergenic potency of Hop J pollen may be increased with environmental changes in the past decade; These changes will be highlighted in far East Asian countries in the coming years.

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