

Tree Pollen Sensitization and Cross-Reaction of Children with Allergic Rhinitis or Asthma

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Objectives: Tree pollen causes allergic rhinitis and asthma. We investigated children who diagnosed as rhinitis or asthma, living in Busan, for tree allergen sensitization, component allergen, oral allergy syndrome, and the relationship between pollen counts and allergen sensitization.

Methods: Pollen were collected in Busan, from January 1 to December 31, 2017, using a Rotorod sampler and enumerated using a microscope. We conducted a study of children with rhinitis or asthma at Busan St. Mary's Hospital in 2017, administered an ISAAC questionnaire, and an oral allergy syndrome survey. Serum specific Ig E tests were performed.

Results: Among the 57 patients, the mean age was 9.3 years. The pollen counts in decreasing order were as follows: pine, alder, oak, juniper, beech, ginkgo, and birch. For sensitization, birch and alder 35.1%, Japanese cedar 19.3%, juniper 17.5%, pine 10.5%, and Japanese cypress 8.2%. The component Ig E was tested in 27 patients. Bet v 1 had a high correlation with birch, alder, and peach. Bet v 2 showed a statistically significant correlation with all tree pollen except cypress. Bet v 4 did not have any apparent correlation. Bet v 6 had the same pattern as Bet v 2, but correlation coefficient was higher than that of Bet v 2. Oral allergy syndrome was noted in 7 patients, including peach, peanut, apple, tomato, kiwi, and sesame.

Conclusions: Alder and juniper are clinically important tree pollens in Busan. These pollens cause sensitization to birch and Japanese cedar by cross-reaction.

Key Words: Allergy, Busan, Pollen

Pollen-related allergic diseases are called pollinosis. The typical diseases include allergic rhinitis, allergic conjunctivitis, and bronchial asthma, while atopic dermatitis and oral allergy syndrome are also thought to be associated according to recent studies.¹⁻⁴ In the US and Europe, studies regarding the distribution of pollen and related diseases have been continuously conducted since 1930's.⁵⁻⁷ In South Korea, similar

studies regarding the regional distribution of pollen have also been conducted since 1960's.⁸⁻¹⁰ In particular, a nationwide pollen-related study involving children has been conducted by the Korean Academy of Pediatric Allergy and Respiratory Disease since 1997.¹¹ Tree pollen is a typical allergen causing pollinosis. In South Korea, there have also been several studies regarding pollen sensitization and

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allergic diseases in children.¹²⁻¹⁵ Sensitization was mostly detected by skin prick tests,¹²⁻¹⁹ while studies regarding sensitization using serum specific immunoglobulin E (serum specific IgE) tests are scarce. In a study by Yang et al., serum specific IgE in children with allergic rhinitis under the age of 18 years was detected by the MAST (multiple allergen simultaneous test),¹⁸ but not by the ImmunoCap tests. ImmunoCap test has been newly developed, with a quantitative technique, and it can be tested even the patients had medications such as antihistamines. It can be tested even the patients have active skin disease or dermatographism.

In the case of allergic sensitization-related studies conducted only in Busan, South Korea, there was a study of allergic sensitization using pollen collection and skin prick tests for 15 years from 1998,¹³ but there has been no recent studies conducted in Busan.

This study aimed to investigate the distribution of tree pollen in the Busan area, to examine the status of pollen sensitization in children with allergic rhinitis or bronchial asthma through ImmunoCap tests, and to investigate the recent trend of clinically important tree pollen in children with allergic diseases in the Busan area. This study will be helpful when the physicians in Busan evaluate allergen sensitization.

MATERIALS AND METHODS

Subjects

This study was performed after Busan St.

Mary's Hospital Institutional Review Board approval (BSM2016-14). The subjects of this study were children under the age of 18 years who visited the Department of Pediatrics at Busan St. Mary's Hospital in Busan, South Korea, from January 1 to December 31, 2017. The 57 subjects were children who were diagnosed with allergic rhinitis or bronchial asthma by pediatricians and were suspected of having tree pollen allergies with symptoms worsening in the spring.

Methods

1) History taking and physical examination

After obtaining consent from the children and their guardians, data on age, sex, residential area and family history of allergic diseases were acquired. In addition, allergic rhinitis, asthma, and atopic dermatitis were examined, the International Study of Asthma and Allergies in Childhood (ISAAC) written questionnaire and an oral allergy syndrome survey were also conducted.

2) Pollen collection

Pollen was collected from Busan St. Mary's Hospital, located in Yongho-dong, Nam-gu, Busan, South Korea for 1 year from January 1, 2017 to December 31, 2017. A rotorod sampler (Sampler Technologies Inc., Minneapolis, MN, USA) was placed 1.5 m above the roof of the hospital, and glycerin-applied glass rods for collection were placed in the sampler. Then, pollen was collected daily from 8 am to 8 am the next day (the sampler operates for 144 minutes per day). The collected glass rods were classified by the days of the week (except for Sundays) and

were collected in collecting boxes.

The glass rods in the sampler were replaced for collection. Each collected glass rod was placed at the grooves of a microscope support, covered with a 22 mm x 22 mm cover glass, stained with Calberla's fuchsin staining solution (10 mL glycerin, 20 mL 95% alcohol, 30 mL distilled water, 0.2 mL basic fuchsin), and then identified within 30 minutes. Pollen grains/m³ was calculated with a 400× optical microscope and recorded.

Pollen was classified according to size, shape, and surface pattern of the grains. Pollen was largely categorized on the basis of their origin, as follows: trees, grasses, and weeds. The tree pollen collected included pine, alder, oak, juniper, beech, ginkgo and birch.

3) Measurement of serum specific antibody levels using ImmunoCap

Serum specific IgE was measured using the ImmunoCap system (ThermoFisher, Uppsala, Sweden), and was investigated for pine (*Pinus strobus*), alder (*Alnus incana*), juniper (*Juniperus sabinooides*), Japanese hop (*Humulus scandens*), ragweed (*Altemisia vulgaris*), birch (*Betula verrucosa*), Japanese cedar (*Cryptomenia japonica*), cypress (*Chamaecyparis obtusa*), orchard grass (*Dactylis glomerata*), house dust mite (*Dermatophagoides farina*), peach (*Prunus persica*), and tomato (*Lycopersicon lycopersicum*). Sensitization was defined as class 1 (≥ 0.35 kUA/L). Oak, Beech and Ginkgo were not investigated.

4) Birch pollen component allergen test

Birch pollen component IgE was tested along

with serum specific IgE measurements. Bet v 1 (PR-10), Bet v 2 (Profilin), Bet v 4 (Polcalcin), Bet v 6 (Isoflavonreductase-like and phynylcoumaranbenzylic ether reductase) were tested.

Statistical analysis

Statistical analysis was performed using the SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). The correlations between pollens were estimated by Pearson correlation. Statistical significance was set at $P < 0.05$.

RESULTS

Characteristics of the subjects

All the 57 subjects were included in this study. Among them, 26 were male, accounting for 45.6%, and the mean age was 9.3 years. Among allergic diseases (multiple responses possible), allergic rhinitis was the most common ($n = 56$, 98.2%), followed by allergic conjunctivitis ($n = 32$, 56.1%), atopic dermatitis ($n = 24$, 42.1%), bronchial asthma ($n = 16$, 28.1%), food allergy ($n = 14$, 24.6%), and oral allergy syndrome ($n = 7$, 12.3%) (Table 1).

Distribution of pollen collected in 2017

The most commonly collected pollen during one year from January 1, 2017 to December 31, 2017 was pine (20,858 grains/m³), followed by alder (4,078 grains/m³), oak (4,064 grains/m³), juniper (3,058 grains/m³), Japanese hop (1,918 grains/m³), beech (1,316 grains/m³), ginkgo (740 grains/m³), orchard grass (222 grains/m³), birch

(148 grains/m³), and ragweed (100 grains/m³) (Table 2). The monthly distribution of each pollen is shown in Fig. 1. Most of the tree pollen was collected in spring.

Assessment of sensitization using the ImmunoCap blood testing

The most common allergen found in the test was house dust mite. 47 out of 56 subjects were sensitized to house dust mites, with the highest sensitization rate of 83.9%. The sensitization rates to birch and alder were 35.1% and 35.1% (20 out of 57), respectively, that to orchard grass was 21.1% (12 out of 57), that to Japanese cedar was 19.3% (11 out of 57), that to juniper was 17.5% (10 out of 57), that to Japanese hop was 10.9% (6 out of 55), and that to pine and rag-

weed was 10.5% and 10.5% (6 out of 57), respectively. The sensitization to cypress was 8.2% (4 out of 49), and the sensitization rates to peach and tomato were 21.4% (6 out of 28) and 14.9% (7 out of 47), respectively (Table 3) (Fig. 2).

Birch pollen component test results

Birch pollen component testing was performed in 27 out of 57 subjects, of whom, 10 were sensitized to birch, and 11 were sensitized to alder. Bet v 1 (PR-10) showed a high correlation with birch ($r = 0.942$), alder ($r = 0.906$), and peach ($r = 0.857$) ($P < 0.01$). Bet v 2 (Profilin) showed a statistically significant correlation with all tree pollen except for cypress, orchard grass, and weeds. Bet v 4 (Polcalcin) showed no correlation

Table 1. Demographic data of the patients

Characteristic	Value
Total number	57
Male:Female	26:31
Age(Mean±SD)	9.3 ± 2.89
Age(yr), n(%)	
<5	5(8.8)
6-10	33(57.9)
11-15	17(29.8)
>16	2(3.5)
Disease, n(%)	
Allergic rhinitis	56(98.2)
Allergic conjunctivitis	32(56.1)
Atopic dermatitis	24(42.1)
Bronchial asthma	16(28.1)
Food allergy	14(24.6)
Oral allergy syndrome	7(12.3)

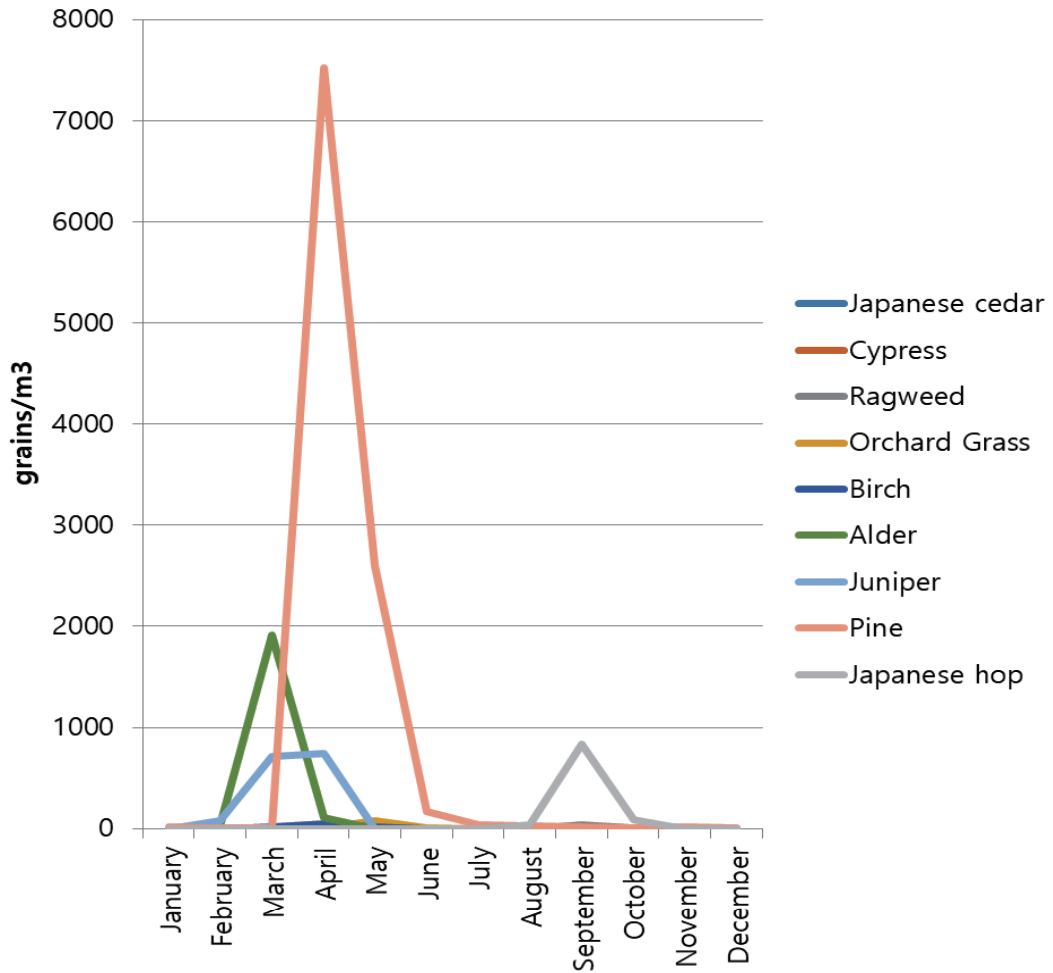


Fig. 1. Monthly pollen in Busan (2017)

Table 2. Total count of tree pollens in Busan, 2017

Pollen	grain/m ³
Pine	20,858
Alder	4,078
Oak	4,064
Juniper	3,058
Beech	1,316
Ginko	740
Birch	148
Japanese cedar	0

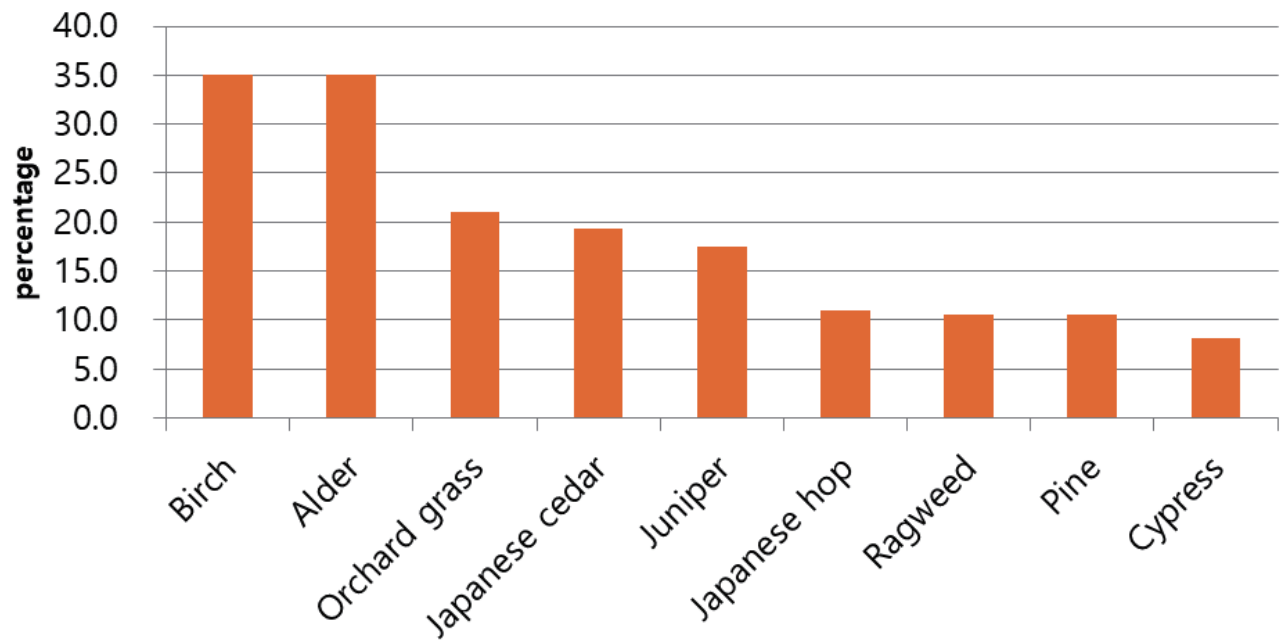


Fig. 2. The sensitization rates of the patients

Table 3. Serum specific Ig E by Immunocap

	Sensitized patients/ total patients	Sensitization rate (%)
Pine	6/57	10.5
Alder	20/57	35.1
Juniper	10/57	17.5
Japanese hop	6/55	10.9
Ragweed	6/57	10.5
Birch	20/57	35.1
Japanese cedar	11/57	19.3
Cypress	4/49	8.2
Orchard grass	12/57	21.1
HDM*	47/56	83.9
Peach	6/28	21.4
Tomato	7/47	14.9

Oak, Beech and Ginko were not investigated.

*HDM: house dust mite

because no patient was sensitized. Bet v 6 also showed statistically significant correlation with all pollen except for cypress ($P < 0.01$).

Correlation between pollen

Considering the tree pollen in Busan in 2017, alder among the Fagales and pine among the Pinales were the most dominant pollen types, followed by juniper pollen. The actual sensitization rates observed through the ImmunoCap testing were high for birch and Japanese cedar, which appears to be due to cross-reactivity between trees belonging to the same order. The correlation coefficient between alder and birch was $r = 0.994$, $P < 0.001$, showing a significant correlation. The correlation coefficient between juniper and Japanese cedar was $r = 0.879$ and $P < 0.001$, also showing a high correlation (Fig. 3).

Oral allergy syndrome

Out of the 57 subjects, 7 presented with oral allergy syndrome symptoms. The mean age was 10.6 (SD \pm 3.64) years, higher than the mean age

of all subjects. The causative foods included peach (n = 4), peanut (n = 3), apple (n = 2), nuts (n = 1), tomato (n = 1), kiwi (n = 1), and sesame seeds (n = 1) (multiple responses possible). Pollen sensitization tests showed that 5 subjects were sensitized to birch and alder, 4 were sensitized to juniper, and 2 were sensitized to pine. Of 7 subjects, 3 underwent allergen component testing simultaneously. The mean value of Bet v 1 was 67.3 kUA/L, the mean value of Bet v 2 was 3.42 kUA/L, the mean value of Bet v 4 was 0 kUA/Lm, and the mean value of Bet v 6 was 2.29 kUA/L, indicating that the mean value of Bet v 1 was significantly higher.

DISCUSSION

The results of this study showed that in terms of tree pollen in Busan in 2017, pine pollen was the dominant, followed by alder, oak, juniper, beech, ginkgo, and birch. These results are similar to those of a study regarding the nationwide

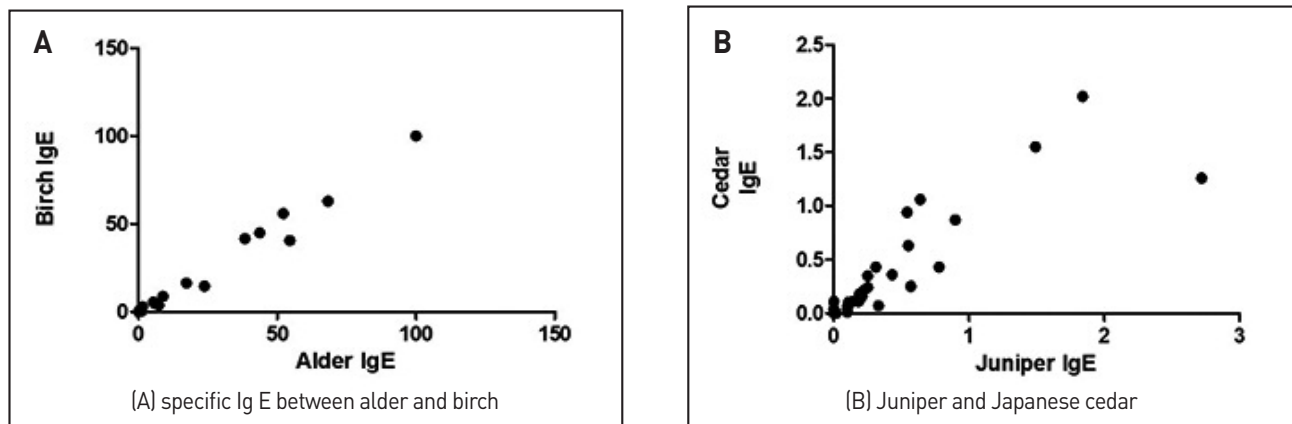


Fig. 3. The correlation of specific Ig E between alder and birch (A), juniper and Japanese cedar (B)

distribution of pollen for 11 years starting in 1997, reporting that the major tree pollen comprised pine, oak, alder, and birch, but were slightly different from its reporting on birch and juniper.²⁰ A study by Sung et al., regarding airborne pollen in Busan in 1998 - 2012 also reported that the major tree pollen in Busan were, in descending order, pine, alder, oak and juniper, and that in skin prick tests in children, the sensitization rates to birch (15.1%), oak (14.7%) and juniper (14.1%) among the Fagales were detected, but the Pinales except for pine were not tested.¹³

In this study, sensitization to pollen was identified with serum specific IgE using ImmunoCap assays. The findings showed that birch and alder had the highest sensitization rate, with 35.1% for each, while the collected pollen counts were, in descending order, of pine, alder and oak. Although birch pollen was not much collected, the sensitization rate to birch was found to be high. This is thought to be due to the cross-reactivity of the sensitization to alder and oak, as both belong to the same Fagales together with birch.²¹ In addition to the Fagales, Japanese cedar among the Pinales showed the highest sensitization rate, with 19.3%, followed by juniper (17.5%), pine (10.5%) and cypress (8.2%). In view of the fact that Japanese cedar pollen was rarely found in the actual pollen collection, it is inferred that a high sensitization rate to Japanese cedar was due to cross-reactivity with juniper. However, the sensitization rate to Japanese cedar was higher than that to juniper; further studies regarding this issue are thus needed. The allergen Jun a 1 from

juniper is known to have a high degree of homology with Japanese cedar and cypress, and cross-reactivity between juniper, Japanese cedar and cypress has been reported.^{22,23}

In this study, the correlation between juniper and Japanese cedar was found to be $r = 0.879$ ($P = 0.000$), and between juniper and cypress was found to be $r = 0.120$ ($P = 0.412$). In light of this, allergens from juniper are highly likely to have components that cross-react only with Japanese cedar, and further studies regarding this are needed.

Pine pollen was the most abundant tree pollen, accounting for 60.9% of the total. However, the actual sensitization rate to pine was only 10.5% ($n = 6$), which was similar to the results of a previous skin prick testing performed in this hospital.¹³ In general, pine pollen is known not to cause allergic diseases due to its low antigenicity.²⁴

Bet v 1 (PR-10) is a major allergen from oak and is homologous to Aln g 1 (alder), Car b 1 (hornbeam), Cas s 1 (chestnut tree), Cor a 1 (hazel), Fag s 1 (European beech), Ost c 1 (hop hornbeam), and Que a 1 (oak). Bet v 1 (PR-10) is known to have high cross-reactivity in oak allergies and is known to be highly associated with oral allergy syndrome.^{25,26} The results of this study also showed that children sensitized to PR-10 proteins were more likely to present with oral allergy syndrome.

Bet v 2 (Profilin) is known as a pan-allergen,^{27,28} and it is positive in 10-20% of patients with pollen allergy.²⁸ In this study, Bet v 2 showed a statistically significant correlation with most

trees, grasses and weeds except for cypress, indicating a pan-allergen pattern. However, it was shown to be less correlated with oral allergy syndrome.

Bet v 4 (Polcalcin) is also a pan-allergen, and the sensitization rate has been reported to be less than 10%.²⁹ In this study, none of the subjects was sensitized to Bet v 4, and its clinical significance in South Korea is thought to be small.

Bet v 6 is an isoflavone reductase, a minor allergen formerly called Bet v 5, which is involved in the defense function of plants. It shows cross-sensitization in about 32% of birch allergies, and is known to be clinically significant in oral allergy syndrome.³⁰ In this study, Bet v 6 was correlated with most tree pollen except for cypress, but the correlation coefficient was low, and it also had a low correlation with oral allergy syndrome.

Oral allergy syndrome was observed in 7 (12.3%) out of all 57 children. In serum specific IgE tests, 5 out of 7 children were co-sensitized to birch and alder, and 4 were sensitized to juniper. One child showed no sensitization to any of the allergens in the tests, and it was doubted whether the child had oral allergy syndrome. Of 7 children with oral allergy syndrome, 3 underwent allergen component testing. The mean value of Bet v 1 was significantly high, with 67.3 kUA/L. Similarly, a study by Choi et al. also found that only Bet v 1 tested positive in Korean children with oral allergy syndrome,²⁶ which was similar to the results of this study. It is inferred that oral allergy syndrome symptoms in Korean children are highly likely to be associated with

Bet v 1.

This study has a limitation in that although allergen component testing was performed, the number of subjects undergoing the testing was small. In particular, birch component testing was performed in this study, but only 10 children sensitized to birch underwent the testing. Therefore, the test results were of low statistical significance.

Another limitation is that ImmunoCap testing was not performed in all 57 children and could not be performed for some allergens. In view of the fact that under the current health insurance scheme, ImmunoCap testing can be performed for a maximum of 12 kinds of allergens. When skin prick testing is feasible, for a maximum of 6 kinds of allergens testing is possible. Therefore it could not be performed for more kinds of allergens. In such a situation, extensive allergen testing could not be performed for all collected species. Further studies regarding this are needed in the future.

In conclusion, alder and juniper are the clinically important tree pollen for children with allergic rhinitis and patients with asthmas in Busan. These pollens cause sensitization to birch and Japanese cedar by cross-reaction. Considering these points, it is thought that more appropriate testing items can be selected in allergy testing in Busan.

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