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A Study of Allergy Skin Tests with Korean Pollen Extracts

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In Korea, there are two descrite pollen seasons: a tree pollen season and a weed pollen season. In order to investigate the incidence of sensitization to the major pollens and to evaluate its clinical significance in respriatory allergic disease, skin prick tests were performed using 7 species of Korean pollen extracts (K-P extracts) and specific IgE was measured by the Phadebas radioallergosorbent test (RAST) in patients with positive skin prick tests. Of the 317 patients with respiratory allergic diseases 73 patients (23.0%) were skin prick test positive to one or more K-P extracts and the positive reactions to individual pollens were as follows: 14.2% (45/317) positive to sagebrush, 10.4% to ragweed, 5.0% to grass, 4.1% to oak, 3.8% to alder, 1.9% to poplar, and 0% to pine. The 30-39yr old group manifested the highest skin test positivity, 36.7%. There was no difference in the skin reactivity according to the patient's sex and the kinds of allergic disease. Also there was no relationship between birth season and skin test positivity. The agreement between the results of the skin prick test with K-P and commercially prepared Bencard's pollen extracts (B-P extracts) was good. There were good correlations between the strongly positive skin prick tests and a posivite RAST and also between a netative skin prick test and a negative RAST. From this study, it can be concluded that overall sensitization rate of respiratory allergy patients to K-P exstracts was 23.0%, and that weed pollens such as sagebrush and ragweed were major pollens.

Key words: Skin prick tests, Korean pollen extract (K-P extracts), radioallergosorbent test (RAST).

Pollens, house dust-dust mites, and mold spores are the major aeroallergens which cause respiratory allergic disease. In 1819 John Bostock (Hansel 1936) described the importance of pollens as a cause of human diseasse and in 1873 Blackely (Hansel 1936) introduced the first skin prick test with a pollen extract for the diagnosis of hay fever. Noon (1911) noted that pollens could act as etiologic allergens of respiratory allergic diaease, and named the disease pollinosis.

In Korea there have been several reports of allergic skin tests in the patients with bronchial asthma and allergy rhinitis (Kang 1973; Whang *et al.* 1974; Hong *et al.* 1982). Thirty to 50% of the patients with respiratory allergic diseases tested posive to commercially produced pollen extracts (Bencard Co. England, Torii Co. Japan). Hong *et al.*(1981) and Min *et al.* (1982) emphasized the importance of sagebrush and ragweed in Korea as causative pollens in pollinosis from late summer to early autumn.

Surveys of airborn pollens (Joo 1965; Kim 1967; Min 1984; Hong *et al.* 1986), indicated that there are two discrete pollen seasons in Korea, one for tree pollens from Mrch to May, the other for weed pollens from mid August to late September. Hong *et al.* (1986) reported that the main airborn pollens during tree pollen season were alder, oak, poplar, and pine and that the primary pollens during weed pollen season were sagebrush, ragweed and hop Japanese.

The purpose of this study was to investigate the incidence of sensitization to 7 species of K-P extracts in patients with respiratory allergic diseases. At the same time skin prick tests with B-P extracts were performed and the results of skin prick tests of the former were compared to those of the later to demonstrate the reliability of K-P extracts. To evaluate the clinical significance of the pollens as causative allergens, allergen specific IgE measurements on Phadebas RAST were performed on patients with a posive skin test.

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MATERIALS AND METHODS

Patients

317 patients (male 148, female 169) with respiratory allergies who visited the Allergy Clinic of Severance Hospital, Yonsei University College of Medicine from November 1984 to July 1986 were included in the study group. The subjects ranged in age from 5 to 82 years with mean age 34.9 years. There were 144 cases of bronchial asthma, 120 cases of allergy rhinitis and 53 cases of both.

Also, data from 63 patients with allergic rhinitis and/or bronchial asthma who had undergone K-P skin prick tests and RAST prior to the study period, was included because there were few RAST cases during the study period to allow comparison of the results of skin tests and RAST.

Materials

Korean pollen extracts: Tree pollens including pine (Pinus spp.), oak (Quercus spp.), poplar (Populus spp.), and alder (Alnus spp.), weed pollens including sagebrush (Artemisia spp.) and ragweed (Ambrosia spp.) and grass pollen such as Zoysia japonica were collected during their respective pollen seasons.

Pollens were defatted with ethylether, dried and extracted with modified Cocca's solution (Phillips 1967, sodium chloride 9 gm, phenol crystal 4gm, sodium bicarbonate 2.5 gm in 1000 ml of distilled water) in 1:10 W/V at room temperature for 72 hours. The supernatant was dialysed through a cellulose membrane against an adequate amount of 0.4% phenol-0.9% saline and mixed with the same volume of sterile glycerine (K-P extract for skin test, 1:20 W/V).

Methods

Skin prick tests with K-P extracts and Bencard's pollen extracts (B-P extract) were performed simultaneously on subjects with respiratory allergy diseases who visited the Allergy Clinic during the study period. The B-P extracts which are made in England and used world wide in skin prick tests were pine (pine-Austra, Pinus nigra), oak (oak-common, Quercus spp.), poplar (poplar-Populus spp.), alder (alder, Alnus spp.), sagebrush (wormwood, Artemisia absinthium), ragweed (ragweed, Ambrosia elatior), and grass (Bermuda grass, Cynodon dactylon). A drop of each pollen extract was placed at least 3cm apart on the patients' backs. The skin under each droplet was pricked with a disposable 26 G needle. The results were interpreted 15 min after the prick. Glycerine-buffered saline was used as a negative control and Bencard's histamin solution as a positive control. When there was no skin reaction or no difference from the negative control, it was read as 0 or negative, erythema with diameter smaller than 21mm was +1, erythema with a diameter larger than 21mm and a wheal smaller than 3mm was +2, definite erythema and wheal sized 3 to 5mm in diameter but without a pseudopode was +3, and a wheal larger than 5mm in diameter or with a visible pseudopode was +4. All patients with a reaction of +2 or greater were included in the positive group.

Radioallergosorbant test (RAST)

Allergen specific IgE was measured by the Phadebas RAST method in the positive skin test patients with paper discs of Quercus alba (t7), Populus deltoides (t14), Alder incana (t2), Artemisia absinthium (w5), Ambrosia elatior (w1), and Cynodon dactylon (g2).

RESULTS

Table 1 shows the skin reactivity to 7 K-P extracts. Seventy three of the 317 cases (23.0%) were positive to the prick test to one or more of the K-P extracts. Forty five of the 317 cases (14.2%) tested positive to sagebrush, 10.4% to ragweed and 0% to pine.

Table 2 lists skin sensitivity to K-P extract in each

Table 1. Results of skin tests with Korean pollen and Bencard's allergenic extracts

(n=317)

Pollens	Korean		Bencard's	
	No. of positive cases	%	No. of positive cases	%
Pine	0	0	6	1.9
Oak	13	4.1	17	5.4
Alder	12	3.8	13	4.1
Poplar	6	1.9	16	5.0
Ragweed	33	10.4	28	8.8
Sagebrush	45	14.2	53	16.7
Grass*	16	5.0	14	4.4

Grass*: Korean : Zoysia japonica
Bencard's: Cynodon dactylon

Table 2. Incidence of sensitization to Korean allergenic extracts according to age

Age	No. of Cases	No. of positive cases	Incidence of Sensitization	(%)
-14	35	3	3/35	(8.5)
15-29	80	20	20/80	(25.0)
30-39	79	29	29/79	(36.7)
40-49	56	12	12/56	(21.4)
50-59	42	6	6/42	(14.2)
60-69	22	3	3/22	(13.6)
70-	3	0	0/3	(0)
Total	317	73	73/317	(23.0)

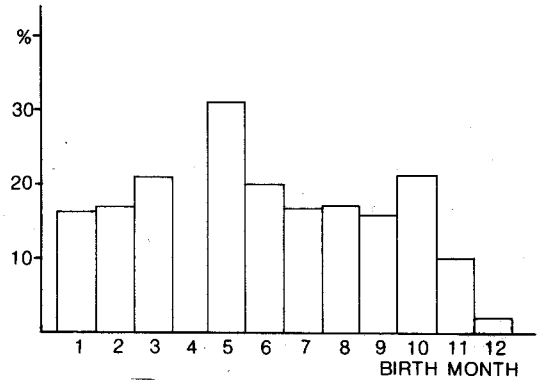

Fig. 1. *Incidence of sensitization to Korean weed pollen extracts according to the birth month*

Table 3. Incidence of sensitization to Korean allergenic extracts according to diseases

No. of positive cases / Pollen	AR (n=120)	(%)	BA (n=144)	(%)	AR + BA (n=53)	(%)
	30	(25)	31	(21.5)	12	(22.6)
Pine	0	0	0	0	0	0
Oak	8	6.6	4	2.7	1	1.8
Alder	7	5.8	3	2.0	2	3.7
Poplar	3	2.5	2	1.3	1	1.9
Ragweed	13	10.8	12	8.3	8	15.0
Sagebrush	18	15.0	20	13.8	7	13.2
Grass*	8	6.6	5	3.4	3	5.6

Grass*: Korean: Zoysia japonica
AR: Allergy Rhinitis
BA: Bronchial Asthma

Table 4. Agreement between skin tests with Korean and Bencard's allergenic extracts (%)

Pollen	Korean (−) & Bencard (−) / Bencard (−)	Korean (+) & Bencard (+) / Bencard (+)	Agreement
Pine	308/308 (100.0)	1/14 (7.1)	309/322 (95.9)
Oak	347/354 (98.0)	9/26 (34.6)	356/380 (93.6)
Alder	358/364 (98.3)	8/16 (50.0)	366/380 (96.3)
Poplar	297/303 (98.0)	4/19 (21.0)	301/322 (93.4)
Ragweed	298/327 (91.1)	33/53 (62.2)	331/380 (87.1)
Sagebrush	284/301 (94.3)	51/79 (64.5)	335/380 (88.1)
Grass*	345/362 (95.3)	9/18 (50.0)	354/380 (93.1)

Grass*: Korean: Zoysia japonica
Bencard's: Cynodon dactylon

$$\text{Agreement (\%)} = \frac{\{\text{Korean (−) \& Bencard (−)}\} + \{\text{Korean (+) \& Bencard (+)}\}}{\text{Total No. of cases}} \times 100$$

age group. The 30-39 year age group recorded the highest positivity (36.7% of 79 cases). There was no difference in skin reactivity between male and female (The data was not shown).

To evaluate the effects of birth seasons on the incidence of sensitization to pollens we analyzed skin test positivity to ragweed and sagebrush. There was no statistical significance between birth season and skin reactivity (Fig. 1).

Also, there was no difference in the rate of skin sensitization and sensitized pollen among the allergy rhinitis, bronchilal asthma, and the combined group (Table 3).

Table 4 presents the overall agreement of skin prick tests between K-P extracts and B-P extracts. There was good agreement of skin tests between the sources of pollen extracts. Agreement was calculated by dividing the number of cases which showed the same results in skin test with both pollen extracts by the total number of cases.

Table 5 dipicts the correlation between RAST and the skin tests with Korean sagebrush pollen, while Table 6 shows the correlation between RAST and the skin tests with Korean ragweed pollen.

Table 7 shows RAST negative probability in negative skin tests, and RAST positive probability in positive skin test performed with K-P extracts and B-P extracts. Good agreement between skin tests with pollen extracts and RAST is noted. The RAST negative probability was calculated by dividing the RAST negative cases by those cases with negative skin tests and the RAST positive probability was calculated by

Table 5. Agreement between RAST and skin tests with Korean allergenic extracts of *sagebrush*

RAST class	Skin Test					Total	Agreement %
	−	+	++	+++	++++		
0	38	29	25	3	4	99	67.7
1	3	1	4	3	1	12	66.7
2	3	0	1	1	7	12	75.0
3	0	0	0	1	2	3	100.0
4	0	0	0	0	4	4	100.0
Total	44	30	30	8	18	130	
Agreement	38/44	29/30	5/30	5/8	14/18		91/30
%	86.4	96.6	16.7	62.5	77.8		70.0

$$\text{Agreement}^{*}\ (\%) = \frac{\text{No. of cases in which the results of skin test and RAST are same}}{\text{Total No. of cases in which both skin test and RAST are done}} \times 100$$

Table 6. Agreement between RAST and skin tests with Korean allergenic extracts of *ragweed*

RAST class	Skin Test					Total	Agreement %
	−	+	++	+++	++++		
0	17	25	25	4	5	76	55.3
1	0	3	2	4	1	11	63.6
2	2	3	3	4	2	14	64.3
3	0	0	0	0	3	3	100.0
4	0	0	0	0	1	1	100.0
Total	20	31	30	12	12	105	
Agreement	17/20	25/31	5/30	8/12	7/12		62/105
%	85.0	80.6	16.6	66.7	58.3		59.0

$$\text{Agreement}^{*}\ (\%) = \frac{\text{No. of cases in which the results of skin test and RAST are same}}{\text{Total No. of cases in which both skin test and RAST are done}} \times 100$$

Table 7. The probabilities of RAST according to skin tests with Korean and Bencard's allergenic extracts.

Pollen	Korean		Bencard's	
	− probability	+ probability	− probability	+ probability
Oak (n=16)	66.6	42.8	85.7	55.5
Alder (n=13)	100.0	16.6	100.0	16.6
Poplar (n=19)	84.6	16.6	83.3	14.2
Rayweed (n=105)	82.4	37.0	81.6	41.4
Sagebrush (n=130)	90.5	42.8	89.2	36.9
Grass* (n=42)	81.4	33.3	86.6	50.0

Grass*:Korean : Zoysia japonica
Bencard's: Cynodon dactylon

$$-\text{ probability }(\%) = \frac{\text{cases with negative RAST}}{\text{cases with negative skin test}} \times 100$$

$$+\text{ probability }(\%) = \frac{\text{cases with positive RAST}}{\text{cases with positive skin test}} \times 100$$

dividing the RAST positive cases by those cases with positive skin tests.

DISCUSSION

The establishment of a diagnosis of respiratory allergic diseases is a multifactorial process involving the assessment and integration of the patient's personal and familial history, symptoms and seasonal variation, environment, occupation as well as specific diagnostic procedures. When pollinosis is suspected, it is especially essential to investigate the species of pollinating plants, the pollen season, and the pollen counts in the patient's residential district (Wayne 1977).

The kinds of pollens which are significant causative aeroallergens differ from area to area. For example in the United States ragweed is the most important pollen, while in Japan it is the pollen of Cryptomeria japonica (Hiroshi *et al.* 1983), grasses in Europe and birch in Alaska and Sweden (Anderson 1984). In Korea spring tree pollens contribute the majority of annual pollen distribution and grass pollen was detected from late April to the end of September, but the count was very low (Hong *et al.* 1986).

In order to evaluate the importance of Korean aeroallergenic pollens as a causative allergens in respiratory allergic diseases, the incidence of sensitization to 7 kinds of pollens including pine, oak, alder, poplar, sagebrush, ragweed, and Zoysia japonica, was investicated. Zoysia japonica is widely used in lawns and golf courses.

To determine the causative allergens, allergy skin tests, provocative tests, RAST, Prausnitz-Kuestner reactions and histamine release tests were used. Allergen extract skin tests are the safest and most available screening tests, while RAST is a widely used method for the measurement of specific IgE and correlated well with the provocative test, a confirmative diagnostic tool of allergic diseases (Bruce *et al.* 1974). There is good correlation between a strongly positive skin prick test and positive RAST and also between negative prick test and a negative RAST (Aas and Johansson 1971; Selter *et al.* 1985). We studied the correlation between the degree of skin reactivity and RAST with regard to sagebrush and ragweed pollens as they produced higher skin test positivity than other pollens. Although the correlation coefficients were not high, there was good correlation between a strong positive skin test and a positive RAST, and also between a negative skin test and negative RAST.

In this study the overall sensitization rate to K-P extracts was 23.0%, in which 73 of 317 patients showed positive skin reactions to one or more species of pollen. In comparison with Kang's study in which he observed a skin test positivity of 44% in 115 respiratory allergy patients (Kang 1973) and Hong *et al's* study in which the skin test positivity to tree and grass pollen was 38% in respiratory allergy patients (Hong *et al.* 1982), the sensitization rate of this study was low. Since there was a similar sensitization rate in skin tests with B-P extracts performed at the same time (Table 1), the possibility that the K-P extracts themselves were unreliable could be excluded. In order to determine

if there was a problem in the study period, skin tests were performed on 42 patients who visited the clinic during weed pollen season in September 1986. An overall sensitization rate of 38.5% was recorded. Since skin test positivity to a certain pollen increases in a specific pollen season, the study period from November 1984 to July 1986 may have contributed to the low skin test positivity results, because it omitted the weed pollen season of 1986.

The incidence of sensitization to sagebrush was the highest, 14.2%, and to ragweed, the next, 10.4%. Sixteen patients (5.0%) tested positive to grass pollen, Zoysia japonica. RAST positivity with Cynodon dactylon was 33.3% in patients with a positive prick test to Zoysia japonica. Thus an allergenic cross reactivity between Zoysia japonica and Cynodon dactylon was suggeted. All patients were negative to pine pollen, and this supports the finding that the allergenicity of pine pollen is not significant (Harris and German 1985).

Skin test positivity by age has been reportd to be greatest during the third decade and to abruptly decrease after the sixth decade (Barbee *et al.*, 1976; Curran & Goldman 1961). In this study the highest skin test positivity was observed in the forth dacade, and to decrease to 14.2% in the sixth decade, but there is a need for further study. Decrement of skin reactivity by age is thought to be due to a decrease in total serum IgE as well as a decrease in reactivity to histamin (Loeffler *et al.* 1973).

Patients with allergic rhinitis are known to manifest a higher skin reactivity to pollens than those with bronchial asthma (Hong *et al.* 1982). No significant difference in the sensitization rate by disease was found.

It is still controversal as to whether or not exposure to a given pollen within one month after birth increases skin reactivity to that pollen (Aurelia *et al.* 1982). The effect of birth season on sensitization rates to weed pollens was evaluated, but no relationships were noted.

The results of this study emphasize the importance of weed pollens—sagebrush and ragweed—as causative allergens of respiratory allergic diseases in Korea. The clinical significance of hop Japanese pollen, which is dispersed in the air during weed pollen season will be evaluated.

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