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Skin reactivity and specific IgE antibody to two nonbiting midges in Korean respiratory allergy patients

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To evaluate the significance of chironomid as a respiratory allergen, we performed skin prick tests with Chironomus plumosus (CP) and Tokunagayusurika akamusi (TA) extracts on 475 respiratory allergy patients, and their specific IgE antibodies were detected by enzyme-linked immunosorbent assay (ELISA) in 106 positive reactors to skin prick test and 30 negative controls. Ninety-seven (20.4%) showed more than 2+ of allergen to histamine ratio to CP and 98 (20.6%) to TA on skin prick test. Seventy-one (73.2%) of 97 positive reactors had increased specific IgE to CP, and 34 (34.7%) of 98 positive reactors, to TA. CP-specific IgE was detected in 14 (14.4%) non-atopic asthmatics and 6 (6.2%) non-allergic rhinitis patients. TA-specific IgE was detected in 17 (17.4%) non-atopic asthmatics and 6 (6.1%) non-allergic rhinitis patients. No association was noted between skin reactivity to Dermatophagoides farinae and the prevalence of specific IgE to CP or TA ($p > 0.05$). The correlation between total IgE level and specific IgE level to CP and TA was poor ($r = 0.07, 0.04$). ELISA inhibition test suggested specificity of IgE binding and cross-allergenicity between CP and TA.

It is suggested that CP and TA can induce IgE-mediated reaction in exposed patients and should be considered as important causative allergens in respiratory allergy patients in Korea.

Key Words : *Chironomus plumosus, Tokunagayusurika akamusi, skin reactivity, specific IgE antibody.*

INTRODUCTION

Non-biting midges of the family Chironomidae are one of the most common insects. Their larvae breed in almost all types of land waters, such as lakes, ponds, swamps, rivers, sewage ditches and rice paddies. More than two thousand species have so far been identified from various regions of the world.

Since a species of Cladotanytarsus lewisi had been reported as a common cause of bronchial asthma among the people residing around man-made lakes in northern Sudan (Lewis, 1956 ; Kay et al., 1978 ; Gad El Rab and Kay, 1980 ; Gad El Rab et al., 1980), several investigators have reported that various kinds of Chironomidae could be significant causative allergens in the United States (Kagen et al., 1984), Japan (Ito et al., 1986 ; Kino et al., 1987) and Germany (Baur, 1980).

In Korea, Park et al. (1991) have reported two cases of asthmatic patients who showed significant bronchoconstrictions on Chironomus plumosus (CP) and Tokunagayusurika akamusi (TA)-bronchoprovocation test. Lee (1987) has reported that more than 30 species of Chironomidae were

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observed in the air of Seoul.

In order to evaluate the significance of two common Chironomid midges, CP and TA, as repiratory allergens in this country, we studied their skin reactivity and the prevalences of specific IgE antibody to these two midges.

MATERIALS AND METHODS

Extract of Chironomus plumosus (CP) and Tokunagayusurika akamusi (TA)

The lyophilized extracts of CP and TA extracts were donated by professor G. Murakami, Toyama University, Japan. 1 : 40 w/v extract was used for the skin prick test. The protein content measured by Lowry method (1951) was 3.3mg per 1ml of 1 : 40 w/v CP extract and 2.2mg per 1ml of 1 : 40 w/v TA extract. These tow lyophilized extracts were dissolved in carbonate buffer (pH 9.6) and used for the antigens in enzyme linked immunosorbent assay.

Subjects

The study subjects were 475 respiratory allergy patients who visited the Allergy Clinic of the National Medical Center in Seoul. We performed skin prick tests with 1 : 40 w/v of CP and TA extracts, and 60 common inhalant allergens. CP and TA-specific IgE antibodies were detected in postive reactors to CP and/or TA (>2 of allergen to histamine ratio) on skin prick tests. Thirty patients who showed all negative responses on skin prick tests were included as negative controls. The sera of all subjects were kept frozen at -20℃ and assayed simultaneously.

Allergy skin test

Skin prick tests with 60 common inhalant allergens (Bencard Allergy Unit, Brentford, Middlesex, England), 1 : 40 w/v of CP and TA extracts and histamine (1 mg/ml, Bencard Allergy Unit) were performed on the backs of the patients simultaneously. The reactions were read 15 minutes later. The wheal size of each antigen (A) and histamine (H) was measured by the maximum diameter (A_1 and H_1) and vertical length at the midpoint of the maximal length (A_2 and H_2). Skin reactivity was expressed as the ratio of the wheal size of the antigen to that of histamine $\langle (A_1+A_2)^2/(H_1+H_2)^2$: A/H ratio$\rangle$ according to the criteria of the Scandinavian Society of Allergology, 1972 (Table 1).

Enzyme linked immunosorbent assay (ELISA)

The optimal concentrations (10ug/ml of CP and TA extract measured by Lowry method) which were decided in the preliminary experiment, of antigens were coated in each well of a microtiter plate (Dynatech, U.S.A.) overnight at 4℃ After washing three times with 0.5% Tween phosphate buffered saline (PBS-T), 250ul of 10% newborn calf serum (Sigma Chemical Co., U.S.A.) were incubated for 1 hour at room temperature to block the remaining binding sites. 100ul of each patients' serum was incubated for 2 hours and washed again. Then 100ul of 1 : 1000 w/v biotin-labelled goat anti-human IgE antibody (Sigma Chemical Co., U.S.A.) was incubated for 2 hours at room temperature. After washing again, to 100ul of 1 : 1000 w/v streptavidin-peroxidase (Sigma Chemical Co., U.S.A.) was incubated for 30 minutes, and then added 100ul of ABTS (2, 2'-azido-ethyl benzthiazoline sulfonic acid in citrate phosphate buffer) for 10minutes and stopped with 2 N sodium azide. The absorbance value was read at 410nm by an automated microplate reader. All assays were performed simultaneously in triplicate.

Table 1. Interpretation of the allergy skin test results

Grading	Skin reactivity	
	AH ratio	Erythema(mm)
1+	0.1 −1	<21
2+	0.1 −1	>21
3+	1 −2	>21
4+	2 −3	>21
5+	3 −4	>21

(Criteria of the Scandinavian Society of Allergology, 1972)

Enzyme-linked immunosorbent assay (ELISA) inhibition test

To observe the specificity of IgE binding and the allergenic relationship between CP and TA, ELISA inhibition test was performed. Sera of 10 patients who showed high absorbance values of specific IgE antibody to CP and/or TA in ELISA were pooled. Of this pooled sere, 100ul of sera pool were incubated with 100ul of four concentrations (1.0 to 100ug) of CP or TA extracts as inhibitors for 1 hour at room temperature. Control samples were incubated with PBS instead of inhibitors. Then incubated sera pool was incubated again in ELISA plate. Following procedures were same as the above ELISA. All procedures were performed in triplicate.

The inhibitions of the specific IgE binding were expressed as the following :

$$100-\frac{\text{absorbance value with inhibitors}}{\text{absorbance value of control samples}}$$

RESULTS

Skin reactivity to Chironomus plumosus (CP) and Tokunagayusurika akamusi (TA)

Table 2 shows the skin reactivity to CP in the study subjects. Ninety-seven (20.4%) showed more than 2+ of A/H ratio to CP on skin prick tests. Seventy-four (76%) of them showed 2+ and 3+. Twenty-three (23%) of them showed 4+ and 5+. Seven (23.3%) rhinitis and 14 (29.8%) asthmatic patients showed positive responses only to CP.

Table 3 shows skin reactivity to TA in the study subjects. Ninety-eight (20.6%) patients showed more than 2+ of A/H ratio to TA on skin prick tests. Six (18.8%) rhinitis and 16 (34.0%) asthmatic patients showed positive responses only to TA on skin prick test.

Detection of specific IgE antibody to Chironomus Plumosus (CP) and Tokunagayusurika akamusi (TA)

Fig. 1 shows specific IgE antibodies to CP in the study subjects and negative controls. A positive cut-off value was decided as 0.06 which was derived from mean and two folds standard deviation of absorbance values of 30 negative controls. Seventy-one (73.2%) among 97 positive reactors had high CP-specific IgE antibodies.

Fig. 2 shows specific IgE antibodies to TA in the study subjects and negative controls. A positive cut-off value was decided as 0.075 which was derived from mean and two folds standard deviation of absorbance values of negative controls. Thirty-four (34.7%) among 98 positive reactors had high TA-specific IgE antibodies.

Table 2. The skin reactivity to Chironomus plumosus (CP) in study subjects

Diagnosis	Skin Reactivity(A/H Ratio)			
	2	3	4	5
Rhinitis	12(4)	12(3)	2(0)	4(0)
Asthma	18(6)	21(3)	1(1)	7(4)
Rhinitis+Asthma	2	6	5	3
Chronic Urticaria	2	1	1	0
Total(%)	34(35)	40(41)	9(9)	14(14)

( )indicates number of patients who showed only a response to CP and/or Tokunagayusurika akamusi on skin prick test.

Table 3. The skin reactivity to Tokunagayusurika akamusi(TA) in study subjects

Diagnosis	Skin Reactivity(A/H Ratio)			
	2	3	4	5
Rhinitis	11(4)	10(1)	6(0)	5(1)
Asthma	15(5)	17(7)	9(2)	6(2)
Rhinitis+Asthma	3	6	1	6
Chronic Urticaria	3	0	0	0
Total(%)	32(33)	33(34)	16(16)	17(17)

( )indicates number of patients who showed only a positive response to TA and/or Chironomus plumosus on skin prick test.

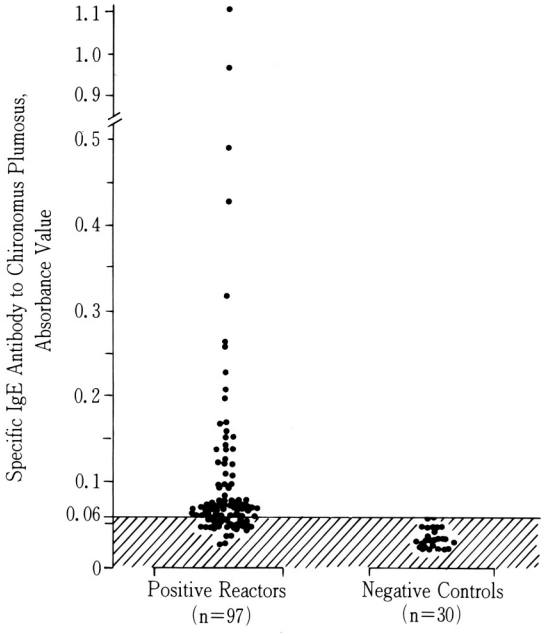

Fig. 1. Specific IgE antibody to Chironomus plumosus in 97 positive reactors on skin prick test and 30 negative controls.

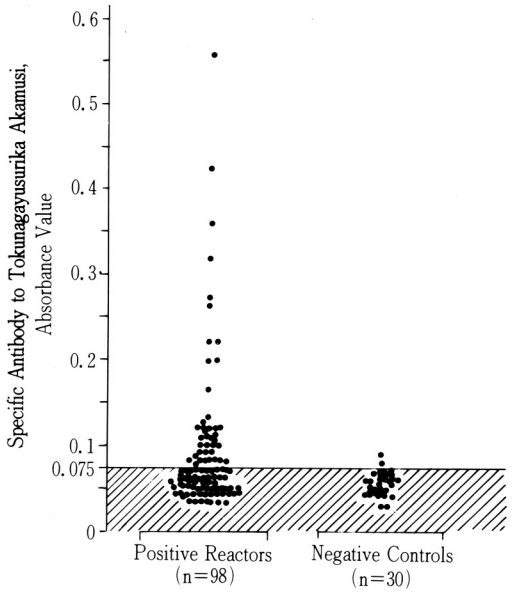

Fig. 2. Specific IgE antibody to Tokunagayusurika akamusi in 98 positive reactors on skin prick test and 30 negative controls.

Prevalence of specific IgE to Chironomus plumosus and Tokunagayusurika akamusi

Table 4 shows the prevalence of specific IgE to CP. In the 2+ group, 23 (68%) patients had specific IgE antibodies, in the 3+ group, 30 (75%) had. In the 4+ group, 8 (89%) had specific IgE antibodies and in the 5+ group, 10 (71%) had them.

Table 4. The prevalence of specific IgE antibody to Chironomus plumosus (CP) according to its skin reactivity

Diagnosis	Skin Reactivity(A/H Ratio)			
	2	3	4	5
Rhinitis	6	8	2	2
Asthma	14	16	1	6
Rhinitis+Asthma	3	5	5	1
Chronic Urticaria	0	1	0	0
Total(%)	23/34(68)	30/40(75)	8/9(89)	10/14(71)

Table 5. The prevalence of specific IgE antibody to Tokunagayusurika akamusi (TA) according to its skin reactivity

Diagnosis	Skin Reactivity(A/H Ratio)			
	2	3	4	5
Rhinitis	9	3	1	2
Asthma	8	9	4	1
Rhinitis+Asthma	2	4	1	3
Chronic Urticaria	0	0	0	0
Total(%)	19/32(59)	16/33(70)	6/16(38)	6/17(35)

Table 5 shows the prevalence of specific IgE to TA in the study subjects. In the 2+ group, 19 (59%) had specific IgE antibodies and in the 3+ group, 16 (70%) had them. In the 4+ group, 6 (38%) had specific IgE antibodies and in the 5+ group, 6 (35%) had them.

Correlation between specific IgE to Chironomus plumosus (CP) and specific IgE to Tokunagayusurika akamusi (TA)

Fig. 3 shows the correlation of specific IgE level between CP and TA. The correlation coefficient was 0.25.

Correlation between total IgE level and specific IgE to Chironomus plumosus (CP) and Tokunagayusurika akamusi (TA)

Fig. 4 shows the correlation between total IgE level and specific IgE to CP. The correlation coefficient was 0.07.

Fig. 5 shows the correlation between total IgE level and specific IgE to TA. The correlation coeffi-

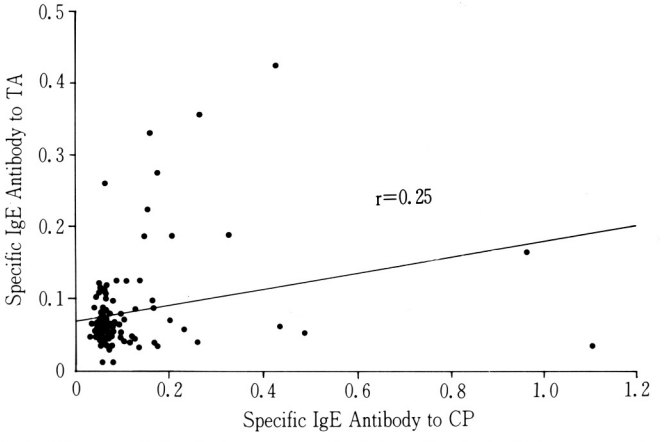

Fig. 3. The correlation between specific IgE antibody to Chironomus plumosus (CP) and specific IgE antibody to Tokunagayusurika akamusi (TA).

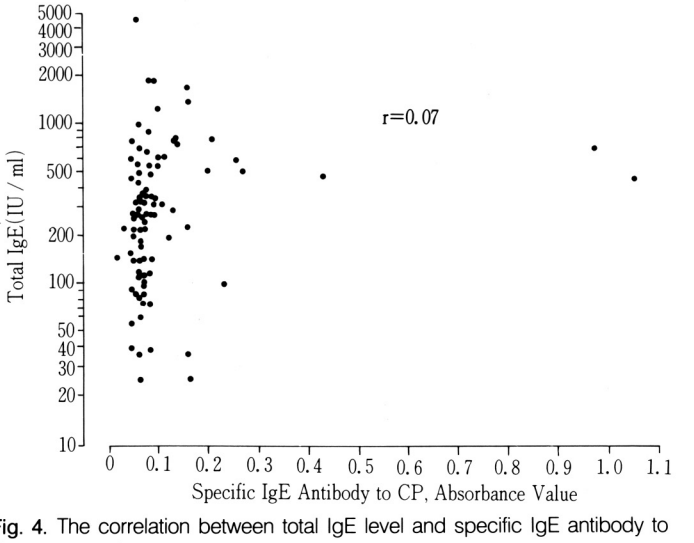

Fig. 4. The correlation between total IgE level and specific IgE antibody to Chironomus plumosus.

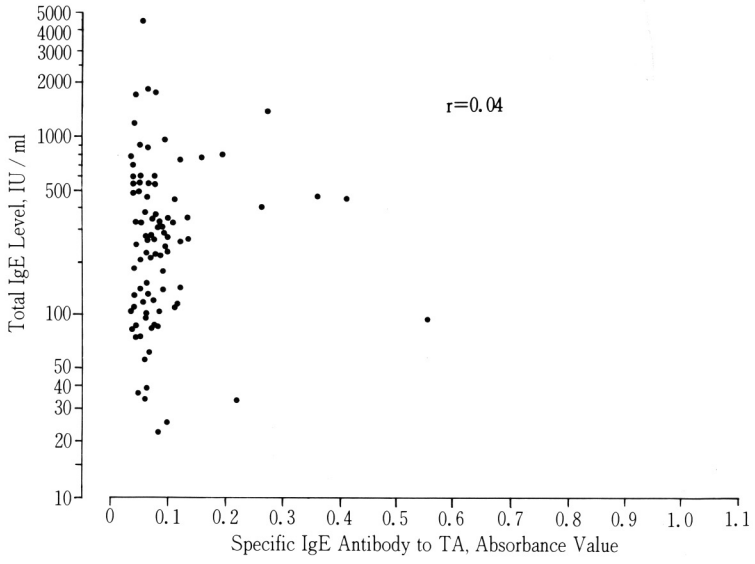

Fig. 5. The correlation between total IgE level and specific IgE antibody to Tokunagayusurika akamusi.

cient was 0.04.

ELISA inhibition test

Fig. 6 reveals the CP-ELISA inhibition test results with serial addition of CP and TA extracts. Significant inhibitions with a dose response parttern were noted with addition of CP extracts. With addition of 100ug of TA extracts, more than 50% inhibition was noted.

Fig. 7 reveals the TA-ELISA inhibition test with serial addition of CP and TA extracts. With TA extracts, significant inhibitions were noted. Also, significant inhibitions were noted with addition of 50ug and 100ug of CP extracts.

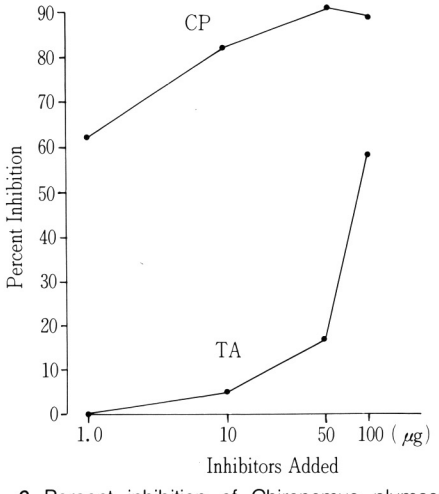

Fig. 6. Percent inhibition of Chironomus plumosus(CP) specific IgE binding with serial addition of CP and Tokunagayusurika akamusi(TA) extracts.

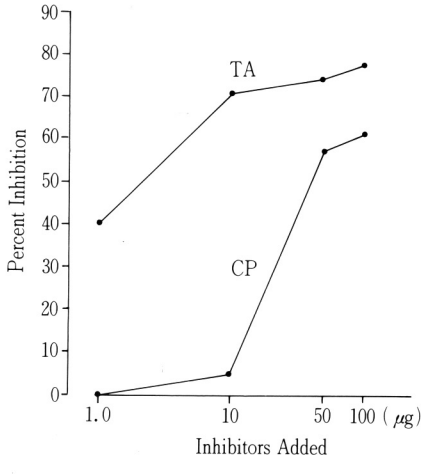

Fig. 7. Percent inhibition of Tokunagayusurika akamusi(TA) specific IgE binding with serial addition of TA and Chironomus plumosus(CP) extracts.

DISCUSSION

This study reveals that 20.4% and 20.6% of study subjects show positive responses to CP and TA on skin prick test. Among them, the prevalence of specific IgE to CP (73.2%) was higher than that of TA (34.7%). There were no significant differences in skin reactivity according to different allergic diseases. Appreciable numbers of patients showed positive responses only to CP and/or TA on skin prick tests. These results suggest that these two midges should be considered as important respiratory allergens in our country and be included in the antigen lists of skin prick tests.

The Chironomidae are one of the largest insect families and more than two thousand species have been identified. In Sudan, Cladotanytarsus lewisi was reported to be the most prevalent one (Gad El Rab and Kay, 1980 ; Gad El Rab et al., 1980). In Japan (Ito et al., 1986 ; Igarashi, 1987 ; Yamashita et al., 1987 ; Kimura et al., 1990) which has a similar environment to our country, four species, Tokunagayusurika akamusi, Chironomus yoshimatsui, Chironomus plumosus, Polypedlum kyotense were reported as prevalent species. In the Korean environment, Lee (1988) reported 23 genera, 39 species of Chironomus midges all over the country. Among them, Chironomus flaviplumus and Chironomus kitensis were the most prevalent ones. Chironomus plumosus, Polypedlum and Tokunagayusurika akamusi were also prevalent (Lee : personal communication). Therefore, we have chosen CP and TA as study materials. In this study, the prevalence of skin prick test and specific IgE antibody to these two midges were high. We can speculate that these two midges are important causative inhalant allergens in Korea.

The pathogenetic mechansim by CP and TA might be a IgE-mediated reaction. Cho et al. (1993) reported 15 cases of bronchial asthma with/without rhinitis who showed positive responses on skin prick test and significant bronchoconstrictions on bronchoprovocation test with CP and/or TA. Among them, 13 (86.7%) had CP and/or TA-specific IgE antibody. But two (13.3%) of them had no specific IgE antibody in spite of positive responses on skin prick test. We also tried the detection of specific IgG_1 and IgG_4 to CP and TA by enzyme-linked immunosorbent assay (Cho et al., 1993). One case had high specific IgG_1 antibody to TA without specific IgE antibody. Therefore the possibility of other mechanisms of bronchoconstriction was suggested. Yamashita et al. (1987) reported that the prevalences of TA and Chironomus yoshimatsui-specific IgG_1 and IgG_4 antibodies in asthmatic patients were significantly higher than those of controls. They suggested that specific IgG might be involved in the development of their asthmatic symptoms. Recently, Park et al. (1993) reported the prevalences of specific IgG subclasses to CP in respiratory allergy patients. Specific IgG_1, G_2 and G_3 antibodies were detected in sera of control patients. There were no differences according to different allergic diseases. High specific IgG_4 antibody level was noted in two rhinitis patient and one asthma patient, who had no specific IgE antibody. It is suggested that CP and TA can induce IgE-mediated reaction. Further studies are needed to investigate other pathogenetic mechanisms.

The hemoglobins of Chironomids have been reported to be potent allergent (Baur et al., 1982). Baur et al. (1983) reported that there was cross-allergenicity among chironomid species and the common antigenic determinants were present in their hemoglobin component. Also, the partial cross-reactivity between Chironomus yoshimatsui and Tokunagayusurika akamusi has been reported. No cross-reactivity has been suggested with house dust mite and mosquito allergens (Yamashita et al., 1989). In this study, ELISA inhibition results revealed that significant inhibitions were noted reciprocally with additions of CP and TA extracts. Although the ELISA inhibition test can not completely evaluate cross-allergenicity, our results suggest that there may be some cross-reactivities between CP and TA. Further investigations are needed to clarify these allergenic relationships using crossed radioimmunoelectrophoresis.

In conclusion, these results suggest that Chironomus plumosus and Tokunagayusurika akamusi should be considered as important respiratory allergens in Korea.

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