

Studies of bronchial asthma induced by chironomid midges (Diptera) around a hypereutrophic lake in Japan

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There have been few reports on hypersensitivity to chironomid midges in bronchial asthmatic patients around the area of hypereutrophic natural lakes, which have been notorious as an environmental hazard due to the massive occurrence of adult chironomids during several periods of the year. Our study investigated IgE antibodies to chironomid midges in bronchial asthmatic patients around the Lake Suwa area in comparison with those of the Matsumoto area (control area). A total of 123 adult patients with bronchial asthma were investigated by measurement of IgE antibodies with extracts of three chironomid midges (*Chironomus yoshimatsui*, *C. plumosus*, and *Tokunagayusurika akamusi*), mite, and silkworm. In addition, in the allergy testing, 12 common inhalant antigens were used. Of 123 adult patients with bronchial asthma, 65 (52.8%) produced positive allergy tests. Thirty-three (50.8%) were positive to mite, 28 (43.1%) to silkworm, 11 (16.9%) to *C. yoshimatsui*, eight (12.3%) to *C. plumosus*, and three (4.6%) to *T. akamusi*. We compared our results with the previous reports. The number of positive tests to silkworm in the Lake Suwa area was higher than in other areas. However, *C. plumosus* and *T. akamusi* showed a lower number of positive reactions. We considered the lower positive number of *C. plumosus* and *T. akamusi* tests in the Lake Suwa area to be related to the advanced age of patients in this area. The chironomids are an important cause of asthma, together with mite and silkworm, in the Lake Suwa area, but affect old asthmatic patients less than asthmatic children.

Key words: bronchial asthma; chironomid midges; IgE antibodies; Lake Suwa area; positive rate.

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The nonbiting chironomid midges (Diptera) include various species whose larvae develop in bodies of fresh water, and which play an important role in the transmission of nutrients along the food chain. Because they do not usually cause damage or transmit diseases, they have been neglected by entomologic research (1). However, in recent years, many researchers have found that chironomid particles are a strong allergen, one of the important inhalant antigens causing asthma (2–6). Using the

ELISA inhibition test to detect antigens of chironomids in house dust, Kimura et al. (7) found that the amount of antigen was nearly half that of the house-dust mite genus, *Dermatophagoides*. They also reported that they could detect the antigen in the soil and the air.

The larvae of various chironomid species live in Lake Suwa. *Chironomus plumosus* (Linnaeus) and *Tokunagayusurika akamusi* (Tokunaga) are especially abundant. Many researchers have reported on the role of these chironomid larvae in the lake's material cycle (8, 9). On the other hand, studies on adult chironomid midges have revealed little about these species. Aspects of adult midge behavior, including dispersal, patterns of abundance, diel eclosion periodicity, and resting behavior have been

Abbreviations: RAST=radioallergosorbent test; D.p.=*Dermatophagoides pteronyssinus*; B.m.=*Bombyx mori*; C.y.=*Chironomus yoshimatsui*; C.p.=*Chironomus plumosus*; T.a.=*Tokunagayusurika akamusi*.

reported by Hirabayashi (10–12) and Hirabayashi et al. (13). Yamagishi & Fukuhara (8) reported that adult midges of *C. plumosus* appeared three times and *T. akamusi* one time during the year. According to Hirabayashi (10), in this area, enormous numbers of adults are attracted by light to houses and other buildings, and massive accumulations of living and dead bodies have been observed during the year. Moreover, he pointed out that the dead bodies accumulate on the ground, break up, and may be inhaled.

So far, there have been few reports of hypersensitivity to chironomid midges in the area of hypereutrophic natural lakes, such as Lake Suwa, in Japan. Moreover, in the Lake Suwa area, clinical studies of bronchial asthma induced by chironomid midges have been neglected, although the area is notorious for the environmental hazard due to the massive occurrence of adult chironomids during several periods of the year. The purpose of our study was to look for IgE antibodies to chironomid midges in bronchial asthmatic patients in the Lake Suwa area in comparison with those of the Matsumoto area (control area).

Material and methods

Study site

Lake Suwa is a tectonic lake located in the central highlands of Honshu at an altitude of 759 m above sea level (36°03' N, 138°05' E at the center of the lake). The surface area of the lake is 13.3 km², and its maximum depth is 6.5 m. A single stream, the Tenryu River, drains the lake into the Pacific Ocean. Lake Suwa is a well-known hypereutrophic lake in Japan and is surrounded by several municipalities, i.e., Okaya city, Shimosuwa town, and Suwa city, with a total population of 200 000 (Fig. 1). Many resort hotels and business establishments in this area are severely affected by the dense swarms of adult chironomid midges that emerge from the lake. There are large populations of larval chironomids (*C. plumosus* and *T. akamusi*). In recent years, Hirabayashi et al. (14, 15) have attempted to control these adult midges by artificial light.

The Matsumoto area was the control area, located about 50 km from the Lake Suwa area. Situated at the foot of the Japanese Northern Alps, Matsumoto has developed as a highlands city, with a total population of 220 000. There are no big lakes and the city is almost surrounded by mountains. Until now, people living in Matsumoto City have not complained of massive swarms of chironomid midges.

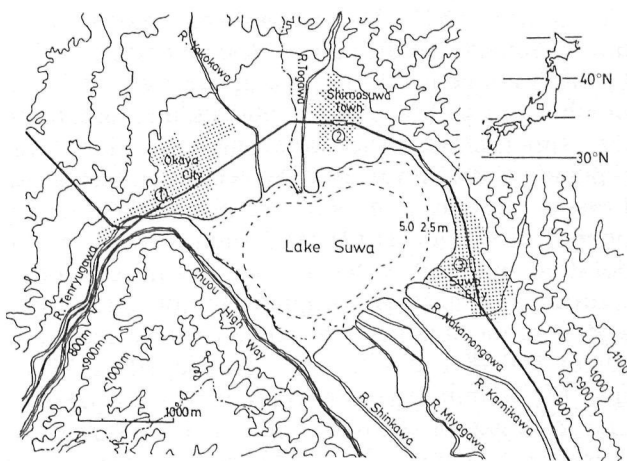


Fig. 1. Map of Lake Suwa area. Dotted areas are congested.

Patient sera

The sera were collected from early September to mid-November 1993. During this period, the adult midges of *C. plumosus* and *T. akamusi* emerge from the lake, and 123 sera were randomly collected from bronchial asthmatic patients who live in two different areas; the Lake Suwa area (62 sera were collected from four hospitals) and the Matsumoto area (61 sera were collected from one hospital). We defined the criteria for bronchial asthma as follows: chronic chest illness causing recurrent episodes of wheezing, breathlessness, chest tightness, and coughing. These symptoms are usually associated with widespread, but variable, airflow limitation that is at least partly reversible, either spontaneously or with treatment (heart disease excluded). Blood samples were obtained by venous puncture and maintained at room temperature for 1–3 h. Sera were separated and stored at –30°C until use.

Inhalant antibody tests

The presence of specific IgE antibodies in the sera was tested by the following procedure, with the Phadezyme RAST test kits (Phadiatop, Pharmacia, Sweden), which contain the extracts of 12 allergens (Japanese cedar, white birch, orchard grass, Bermuda grass [*Cynodon dactylon*], ragweed, mugwort, *Candida*, *Alternaria*, mites [*Dermatophagoides pteronyssinus* and *D. farinae*], cat dander, and dog dander). If Phadiatop produced a positive reaction, we assumed that the bronchial asthma had an external cause.

Measurement of IgE antibodies for insects

Extract of adult midges of *T. akamusi*. Adult midges of *T. akamusi* were collected in late October on the shore of Lake Suwa. They were lyophilized and

stored at -20°C . The lyophilized midges were homogenized. Defatting was done by adding ether to the homogenized material at the ratio of 30 ml of ether to 1 g of lyophilized midges, and discarding the supernatant. The defatting procedure was repeated three times, and the sediment was dried. Coca's solution (5 g NaCl, 2.75 g NaHCO_3 , 4 g phenol, 1000 ml H_2O) was then added to the defatted material at the ratio of 20 ml of Coca's solution to 1 g of the original lyophilized midges, and next the mixture was stirred with a magnetic stirrer for 48 h at 4°C , and centrifuged at 15 000 rpm for 40 min. The supernatant was dialyzed by four changes of distilled water over 2 days, and lyophilized again. The lyophilized material was stored at -30°C until use. The final solution contained 4.4 mg ml^{-1} protein when examined by Lowry's method. This extract of adult midges of *T. akamusi* was used for the CAP System.

CAP System. We used the CAP System (Pharmacia, Sweden), an assay system for measuring allergen-specific IgE antibodies (16) (Fig. 2), with extracts of four allergens: two chironomid midges (*Chironomus yoshimatsui* and *T. akamusi*), mite (*D. pteronyssinus*), and silkworm (*Bombyx mori*). Three allergens (C.y., D.p., and B.m.) were commercially available from Pharmacia, Sweden. The CAP System results were expressed in U_A per ml as estimated by comparison with IgE myeloma standards available from the World Health Organization; values less than 0.35: CAP System score = 0; 0.35–0.69: I; 0.70–3.49: II; 3.50–17.4: III; 17.5–49.9:

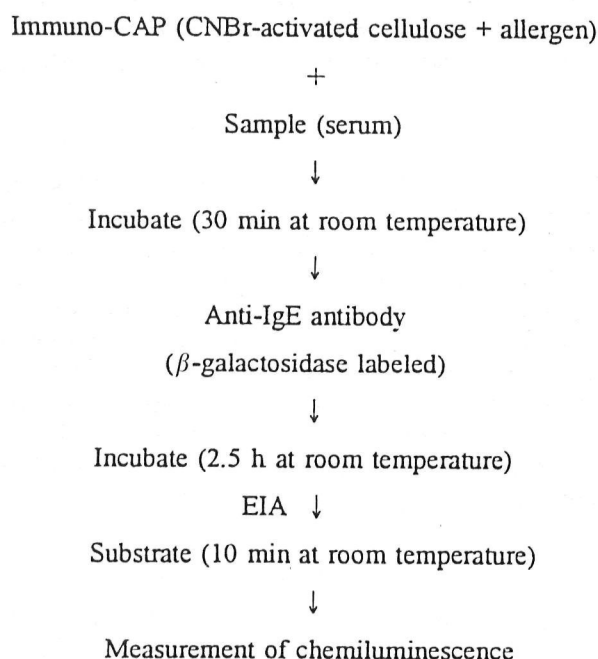


Fig. 2. Method of CAP System.

IV; 50.0–99.9: V; more than 100: VI. CAP System scores of I and over were defined as positive.

AlaSTAT. We measured IgE specific to *C. plumosus* with AlaSTAT (DPC, USA), a new assay system for the measurement of allergen-specific IgE antibodies (17) (Fig. 3). The AlaSTAT results were expressed in IU per ml, as estimated by comparison with commercially available reference standards; values less than 0.35: AlaSTAT score = 0; 0.35–1.49: I; 1.50–2.99: II; 3.00–14.9: III; more than 15.0: IV. AlaSTAT scores of I and over were defined as positive.

Results

Characteristics of patient group

Table 1 and Fig. 4 show the characteristics of patient groups, i.e., age and sex, in the Lake Suwa and Matsumoto areas. The patient mean age was 60.5 years (range: 17–94 years) (Fig. 4). The mean patient age in the Lake Suwa area was 62.8 ± 18.1 and in the Matsumoto area 58.1 ± 15.9 years. There were 57 men (46.3%) and 66 women (53.7%) (Table 1). The difference in characteristics was statistically nonsignificant between the Lake Suwa area and the Matsumoto area patient groups.

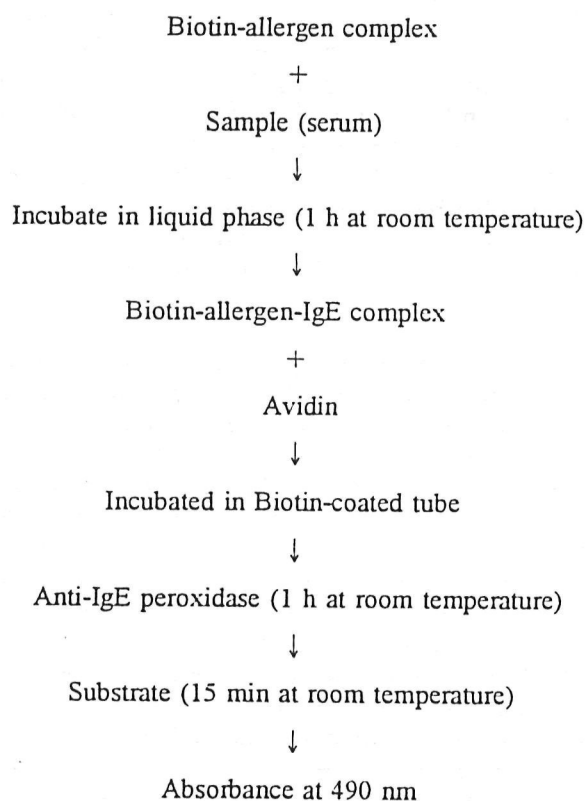


Fig. 3. Method of AlaSTAT system.

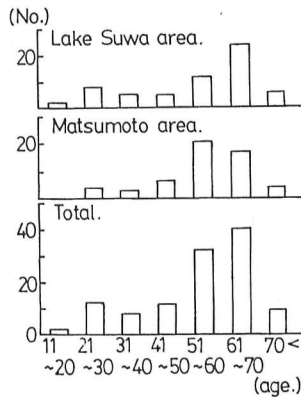


Fig. 4. Age distribution of bronchial asthmatic patients.

Inhalant allergy tests (Phadiatop)

Twelve common inhalant antigens were tested with Phadiatop. Table 2 shows the results of Phadiatop with the extracts of 12 allergens. Fifty-two (42.3%) of the 123 patients had positive reactions, whereas 71 patients had negative results. Comparison of the positive rate in the Lake Suwa area with that in the Matsumoto area revealed a statistically nonsignificant difference (chi-square test, $P < 0.05$); Lake Suwa area: 25 patients (40.3%) were positive; Matsumoto area: 27 patients (44.3%) were positive.

Measurement of IgE antibodies with insects

Table 3 shows the pattern of measurement of IgE antibody positivity to the extracts of five arthropods in 123 bronchial asthmatic adults. The patient numbers and percentages of positive reactions to the antigens of D.p., B.m., C.y., C.p., and T.a. were 33 (26.8%), 28 (22.8%), 11 (8.9%), eight (6.5%), and three (2.4%), respectively. There were 73 bronchial asthmatic adults who showed negative reactions to all five arthropods. On the other hand, of 50 bronchial asthmatic adults who showed positive reactions to any of the extracts of five arthropods, one (2.0%) was positive to all five antigens, 18 (14.6%) only to D.p., nine (7.3%) only to B.m.,

Table 1. Bronchial asthmatic patients by sex, age, and area distribution

	Male (%)	Female (%)	Total (%)	Mean age (years) $\pm$ SD
Lake Suwa area	32 (51.6)	30 (48.4)	62 (100)	62.8 $\pm$ 18.1
Matsumoto area	25 (41.0)	36 (59.0)	61 (100)	58.1 $\pm$ 15.9
Total	57 (46.3)	66 (53.7)	123 (100)	60.5 $\pm$ 18.7

Table 2. Phadiatop positive rate in atopic test with extracts of 12 allergens

	Patients n (%)	Phadiatop	
		Positive (%)	Negative (%)
Lake Suwa area	62 (100)	25 (40.3)	37 (59.7)
Matsumoto area	61 (100)	27 (44.3)	34 (55.7)
Total	123 (100)	52 (42.3)	71 (57.5)

and seven (5.7%) to both D.p. and B.m. Of 123 asthmatic patients, 90 were negative to D.p. Of 90 patients, 13 (14.4%) were positive to one of four species of insects (B.m., C.y., C.p., and T.a.) in the Lake Suwa area, while only four (4.4%) were positive in the Matsumoto area.

Fig. 5 shows the relationship between the CAP/AlaSTAT score and the number of patients who were positive to each allergen in both areas. Four patients in the Lake Suwa area and six patients in the Matsumoto area had a high score (IV or higher) to the extracts of D.p. On the contrary, patients in both areas did not show a high score to the extracts of chironomid midges.

Table 4 shows the result of cosensitization of the allergens among the extracts of five arthropods. This percentage was obtained from the number of bronchial asthmatic adults who had positive reactions to both allergens divided by the number positive to either of the two allergens. For example, for C.y. and B.m., of 29 bronchial asthmatic adults who showed positive reactions to either of the two allergens (C.y. or B.m.), 10 were positive to both

Table 3. Pattern of measurement of IgE antibody positivity to extracts of five arthropods in 123 bronchial asthmatic adults

Pattern of measurement of IgE antibody positivity																Positive no. (%)
D.p.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	33 (26.8)
B.m.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	28 (22.8)
C.y.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	11 (8.9)
C.p.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	8 (6.5)
T.a.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	3 (2.4)
Lake Suwa area	1	1	0	1	0	0	4	0	3	1	9	7	1	1	33	62
Matsumoto area	0	1	1	1	1	1	3	1	0	0	9	2	0	1	40	61
Total	1	2	1	2	1	1	7	1	3	1	18	9	1	2	73	123

D.p.: *Dermatophagoides pteronyssinus*; B.m.: *Bombyx mori*; C.y.: *Chironomus yoshimatsui*; C.p.: *Chironomus plumosus*; T.a.: *Tokunagayusurika akamusi*.

VI				—			VI	1			—		
V	4			—			V	2			—		
IV							IV	3					
III	5	2					III	3	2	1			
II	3	8	3			1	II	3	4	2			1
I	4	8	4	4			I	5	4	1	4		1
Score	D. p.	B. m.	C. y.	C. p.	T. a.		Score	D. p.	B. m.	C. y.	C. p.	T. a.	

Lake Suwa area Matsumoto area

Fig. 5. Relationship between CAP/AlaSTAT score and number of patients who were positive to each allergen in Lake Suwa area (left) and Matsumoto area (right).

Table 4. Cosensitization of allergens among extracts of five arthropods

	D.p.	B.m.	C.y.	C.p.	T.a.
D.p.	—	29.8	15.8	10.8	9.1
B.m.		—	34.5	20.0	6.9
C.y.			—	26.7	16.7
C.p.				—	10.0
T.a.					—

D.p.: *Dermatophagoides pteronyssinus*; B.m.: *Bombyx mori*; C.y.: *Chironomus yoshimatsui*; C.p.: *Chironomus plumosus*; T.a.: *Tokunagayusurika akamusi*.

allergens (C.y. and B.m.) (Table 3); that is, the ratio was 34.5%. Likewise, B.m. and D.p. were 29.8%, while C.p. and C.y. were 26.7%.

Of 65 adult patients with bronchial asthma having an external cause who were positive either by the measurement of IgE antibodies to the 12 common inhalant antigens or the extracts of five arthropods (D.p., B.m., C.y., C.p., and T.a.), 33 (50.8%), were positive to D.p., 28 (43.1%) to B.m., 11 (16.9%) to C.y., eight (12.3%) to C.p., and three (4.6%) to T.a. (Table 5). In comparison of the positive rate in the Lake Suwa area with that in the Matsumoto area, for D.p., C.p., and T.a., the rates were statistically nonsignificant. However, the positive rate of B.m. and C.y. in the Lake Suwa area was significantly higher (Student's *t*-test, $P < 0.05$) than in the Matsumoto area; i.e., Lake Suwa area:

B.m.: 18 patients (54.5%) positive; C.y.: seven (21.2%) patients positive; Matsumoto area: B.m. 10 patients (31.3%) positive; C.y.: four (12.5%) patients positive.

Discussion

Chironomids induce environmental allergies in exposed populations in Egypt, Italy, Sweden, the UK, the USA, and Japan (reviewed by Cranston [18]). Epidemiologic estimates were obtained from a Sudanese study in which a community living beside the Nile, and seasonally exposed to midge nuisance from *Cladotanytarsus lewisi*, were compared with a community living 8 km away from the midge source, upwind of the prevailing winds. In the exposed community, the prevalence of asthma among more than 5000 subjects surveyed was 4.9%, in contrast to 3.2% among more than 2500 subjects in the control area (19). According to Kagen et al. (20), 45% of atopic subjects in Wisconsin heavily exposed to *C. plumosus* had immunologic reactions to the midge. In Sweden, Eriksson et al. (21) tested 2368 consecutive adult patients reporting to a clinic with asthma and or rhinitis: 26% of atopics and 4% of nonatopics (14% of patients) had a positive skin prick test to chironomid extract from fish food. RAST on a subsample of 100 consecutive sera gave an 8% response in atopics; 4% overall. The authors concluded that chironomids might be allergens of clinical importance in Sweden.

In Japan, according to Ito et al. (22), RAST with extracts of *T. akamusi* larvae and adults was done in randomly selected asthmatic patients in the metropolitan area of Tokyo, and 32.4% of 105 asthmatic patients were positive to either extract. This positive rate in the asthmatic patients was second to the positive rate of RAST with mite antigen. The authors suggested that antigens from midges were the second most important allergen in the metropolitan area of Tokyo. Igarashi et al. (3) reported that 119 asthmatic children (aged 1–15 years, mean 8.3) were tested with extracts of selected species of nuisance chironomids (*Poly-pedilum kyotoense*, *C. yoshimatsui*, and *T. akamusi*; adults and larvae), and with house-dust mite

Table 5. Positive rate of bronchial asthma having external cause

Patient no.		D.p.	B.m.	C.y.	C.p.	T.a.	Total chironomid
Lake Suwa area (%)	33 (100)	16 (48.5)	18 (54.5)	7 (21.2)	4 (12.1)	1 (3.0)	9 (27.3)
Matsumoto area (%)	32 (100)	17 (53.1)	10 (31.3)	4 (12.5)	4 (12.5)	2 (6.32)	7 (21.9)
Total no. (%)	65 (100)	33 (50.8)	28 (43.1)	11 (16.9)	8 (12.3)	3 (4.6)	16 (24.6)

D.p.: *Dermatophagoides pteronyssinus*; B.m.: *Bombyx mori*; C.y.: *Chironomus yoshimatsui*; C.p.: *Chironomus plumosus*; T.a.: *Tokunagayusurika akamusi*; *: $P < 0.05$ (Student's *t*-test).

extracts in Toyama. Positive skin-test rates ranging between 7.6% and 23.4% were produced by different midge extracts, while 94.1% were positive for *D. farinae*, suggesting possible cross-reactivity.

There have been few reports on hypersensitivity to chironomid midges around the area of hyper-eutrophic natural lakes, where tremendous numbers of the midges emerge every year. Previous studies have reported hypersensitivity to chironomids in large cities such as Tokyo and in rice paddy areas such as Toyama, as already mentioned.

A wide variety of chironomid species has been described around Lake Suwa. In this study, three species of chironomid, *C. yoshimatsui*, *T. akamusi*, and *C. plumosus*, were studied. *C. yoshimatsui* is a species mainly breeding in sewage ditches, small streams, and the littoral zone of lakes. It emerges in all seasons except winter in Lake Suwa. *T. akamusi* is a large midge commonly and abundantly breeding in all the eutrophic lakes in Japan and China, together with the cosmopolitan giant midge *C. plumosus*, but the two differ remarkably in behavior. The adults of the former emerge only once a year from October to November, while the latter usually emerge three times a year, i.e., April, July, and September, in Lake Suwa (8). All midges swarm and die. The dead bodies break up and might be inhaled. According to Kimura et al. (7), the particles from the dead bodies of midges are detected in the air, soil, and house dust of the area around Lake Kojima (*T. akamusi* is a predominant species in this lake), with large seasonal and local variations. These particles are considered an important source of allergens, and usually become airborne after disturbance in rooms. We think this is also true in the Lake Suwa area.

The present investigation demonstrated that 42.3% of 123 consecutive adult patients reporting to hospitals with bronchial asthma (aged 17–94 years, mean 60.5) were positive to inhalant RAST (Table

2). Moreover, 65 adult patients (52.8%) having bronchial asthma with an external cause were positive either by inhalant RAST or the extracts of five arthropods (Table 5). In addition, 24.6% of atopics (16 subjects) had positive reactions to one of three species of adult chironomids (extracts) (Table 5). This positive rate was unexpectedly low compared to the positive rate of previous Japanese reports.

We compared our results with those of Ito et al. (23) in an attempt to elucidate further the characteristics of the Lake Suwa area (Table 6). The positive rate of B.m. in the Lake Suwa area was higher than in other places (except for Sagami-hara Hospital, Kinki University Hospital, and St Marianna University Hospital). B.m. was extracted from silk-worm wings and showed a higher positive rate than mite antigen in this area (Table 5). The Lake Suwa region is well known as a sericultural area. Many farming families have been raising silkworms for many years. Thus, it seems that B.m. has induced environmental allergies in exposed populations. The research on bronchial asthma related to sericulture has been summarized by Kobayashi (24). He found that the mature silkworm's urine and silkworm imago and pupa showed strong antigenicity in the asthmatic patients. On the other hand, C.y. almost always showed a lower rate in the Lake Suwa region than in other places (Table 6).

Moreover, it was unexpectedly shown that there was a statistically nonsignificant positive rate of C.p. and T.a. between the Lake Suwa and Matsumoto areas (Table 5). A lower positive rate was shown to both extracts in the Lake Suwa area than reported in other Japanese studies. van Kampen et al. (25) reported that 229 Japanese serum samples were tested for specific IgE antibodies against crude extract of T.a., which were measured in 13% of all the samples. According to Murakami et al. (26), the antigenicity of C.p. was stronger than that

Table 6. Positive rate of B.m. and C.y. in different areas

Hospital	Mean age (years) $\pm$ SD	Patient no.	B.m. Positive rate (%)	C.y. Positive rate (%)	Literature
Iwate Medical University	34.3 $\pm$ 15.0	56	53.6	20.7	Ito et al. (23) (1995)
Tokyo University	37.4 $\pm$ 17.1	53	45.3	50.9	Ito et al. (23) (1995)
St Marianna	39.8 $\pm$ 16.0	51	58.8	49.0	Ito et al. (23) (1995)
Sagami-hara	43.2 $\pm$ 17.8	50	62.0	38.0	Ito et al. (23) (1995)
Nagoya University	59.8 $\pm$ 13.5	23	34.8	4.3	Ito et al. (23) (1995)
Kyoto University	45.6 $\pm$ 17.8	153	52.3	37.9	Ito et al. (23) (1995)
Kinki University	40.0 $\pm$ 14.5	50	56.0	36.0	Ito et al. (23) (1995)
Okayama University	40.7 $\pm$ 17.8	195	49.7	38.5	Ito et al. (23) (1995)
Minami Fukuoka	23.0 $\pm$ 16.5	39	23.1	20.5	Ito et al. (23) (1995)
Lake Suwa area	62.8 $\pm$ 18.1	33	54.5	21.2	Present study
Matsumoto area	58.1 $\pm$ 15.9	32	31.3	12.5	Present study
Total	60.5 $\pm$ 18.7	65	43.1	16.9	Present study

B.m.: *Bombyx mori*; C.y.: *Chironomus yoshimatsui*.

of T.a. In this survey, the positive rates of C.p. and T.a. were 12.3% and 4.6%, respectively (Table 5). Thus, on this point, the present study agrees with the results of Murakami et al. (26). However, the positive rates were very low. We cannot completely explain this result with only our study data. On the other hand, in the case of C.y., the difference was statistically significant between the Lake Suwa and Matsumoto areas (Table 5). *C. yoshimatsui* is one of the nuisance species and emerges in all seasons except winter from the littoral zone of Lake Suwa. These dead bodies break up and might be one of the important inhalant antigens causing asthma.

When we compared our data with those of other Japanese reports, the age breakdown of patients proved important. Many previous Japanese reports showed a high positive rate against C.p., C.y., and T.a. in an investigation conducted on asthmatic children (most were aged 1–15 years [26–28]). Ito et al. (23) reported that the positive rates of B.m. and C.y. increased in proportion to patient age until a maximum of about 60 years. On the contrary, beyond age 60, the positive rate decreased gradually. In the present study, the relation to patient age was higher than in most other Japanese reports; therefore, the positive rate was lower than in other reports. Considering the circumstances mentioned above, the older asthmatic patients (60 years or over) showed a relatively low positive rate against C.p., C.y., and T.a.

In previous studies with human and rabbit sera, and partially with monoclonal antibodies, a wide range of cross-reactivity among different Chironomidae species was found (29). This study also investigated the cosensitization of antigens between the extracts of three chironomid species (Table 4). Kawai & Konishi (30) detected strong allergenic cross-reactivities among eight species of larvae belonging to three closely related genera. Murakami et al. (26) suggested the presence of cross-reactivity of the antigens between the extracts of four chironomid species of adult midges, C.p., C.y., T.a., and P.k. (*P. kyotoense* [Tokunaga]). However, in our results, 26.7% of C.y.-positive and 10.0% of T.a.-positive patients were positive to C.p. (Table 4). From these data, we cannot show clearly the presence of cosensitization between the extracts of these chironomid species, C.p., C.y., and T.a., in the old asthmatic patients. In addition, many previous studies reported that insect-positive patients were almost always mite-positive, and that there were few patients (less than 5%) who were only insect-positive (31, 32). However, in our study, of 90 patients who were negative to D.p., 13 (14.4%) were positive only to insects in the Lake Suwa area (Table 3). This finding was characteristic of the Lake Suwa area.

The results of our research on the relationship between old bronchial asthmatic patients and the chironomid midges suggest that the chironomids are an important cause of asthma, along with mite and silkworm, in the Lake Suwa area. However, they affected old asthmatic patients less than asthmatic children.

Further follow-up studies, such as prick tests, intradermal tests, and provocation tests, should be conducted for old patients who showed a high positive rate.

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