

The prevalence of allergic disease and IgE antibodies to house dust mite in schoolchildren in Taiwan

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Summary

The prevalence of positive specific IgE antibodies to house dust mites (*Dermatophagoides pteronyssinus*; *D. farinae*) was determined by enzyme-linked immunosorbent assay (ELISA) in 5097 (61%) volunteers of 8345 schoolchildren aged between 7 and 14 yr from two government schools. All of them filled out a questionnaire concerning allergic symptoms. Among them, 412 (8.1%) children showed a positive reaction to at least one of the two mite allergens, the range varying between 5.6 and 11.2% according to the child's age. Boys had higher prevalence of positive mite specific IgE than girls (9.8% vs. 6.4%, $P < 0.01$), with the overall male to female ratio 1.5:1. The prevalence of bronchial asthma in boys and girls was 5.3% and 3.3% respectively. The positive mite specific IgE antibody in children with asthma and allergic rhinitis was 52% (103 of 198) and 28.7% (193 of 673) respectively. The mean levels of mite specific IgE were not significantly related to the age of onset and severity of asthmatic symptoms ($P > 0.1$), but were significantly different among subjects with current and past asthma ($P < 0.001$). It is suggested that the mite-specific IgE may play a role in the pathogenesis of bronchial asthma in children.

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Introduction

Skin test has been used traditionally in the diagnosis of clinical allergic disease. The development of the laboratory tests for specific IgE has been a great advance for studies of IgE-mediated disease. The sandwich type enzyme-conjugated immunoassay has been widely used to detect specific IgE as an adjunct or substitute for skin tests in the routine diagnosis of specific allergens responsible for IgE-mediated diseases [1,2].

Several authors have reported the prevalence of allergen skin-test reactivity in various communities [3–7]. Godfrey and Griffiths [3] reported a positive skin-test rate of 33.7% to *Dermatophagoides pteronyssinus* and grass pollen allergens.

The allergen exposure related to geographical differences would appear to affect the overall reaction rate. The house dust mites, *D. pteronyssinus* and *D. farinae* were identified as the main important causative allergens for

perennial rhinitis and extrinsic asthma in childhood. In our previous studies using an enzyme-linked immunosorbent assay (ELISA), as many as 93% of asthmatic children admitted to our hospital had positive skin tests and specific IgE to *D. pteronyssinus* and *D. farinae* [8,9]. The purpose of the study was to determine the prevalence of diagnosed allergic disease from questionnaire data and to determine the prevalence of specific IgE to house dust mites by ELISA.

Materials and methods

The questionnaires were distributed to children of two compulsory schools in the Taipei area in 1987. Children between 7 and 12 yr from Shih-Pai elementary school and children between 13 and 14 yr from Min-Deh junior high school were studied. Of a total 8345 children, 5097 (2503 boys and 2594 girls) children were permitted by their parents to do mite-specific IgE tests in this study. According to the questionnaire data, we interpreted the symptoms reported and randomly selected 500 normal

(non-allergic) children from equally distributed age-group as a control group. The serum sample was obtained from the fingertip of 5097 children, and stored at -20°C for immunological study.

The questionnaire was distributed to the children by the school nurse. It consisted of questions about allergic symptoms, including the occurrence of bronchial asthma, allergic rhinitis and atopic eczema which were diagnosed earlier by a physician. In this report asthma was defined as a history of disease that had been diagnosed by a physician in the past. Current asthma was defined as a history of recurrent symptoms more than three times a year in the 12 months up to the study and past asthma was defined as no symptoms in the previous 12 months. The questionnaire included questions on asthma symptoms and illnesses, allergic disease, household environmental exposures, and parents' history.

Mites extract

D. pteronyssinus and *D. farinae* were purchased from Allergon (Allergon Inc., Carthage, U.S.A.). The extraction and the purification of the allergens were performed according to the method of Nakada *et al.* [10]. One gram of mite was mixed with 100 ml cold phosphate-buffered saline (PBS; pH 7.4) and stirred for 48 hr at 4°C . Then the mixture was centrifuged for 1 hr at 12000 r.p.m. The supernatant was filtrated with a $0.45\text{-}\mu\text{m}$ filter membrane (Millipore Co., Bedford, U.S.A.) and dialysed against cold deionized water for 48 hr and then lyophilized before use. The protein concentration of lyophilized extract was measured by a protein assay kit (Bio-Rad Lab., Richmond, U.S.A.).

IgE (enzyme linked immunosorbent assay)

According to our previous report [8], the ELISA was used to study the prevalence of positive specific IgE antibody to house dust mite allergen. In brief, microtitre plates (Costar, Cambridge, U.S.A.) were coated with approximately 100 ng (50 μl) per well of *D. farinae* or *D. pteronyssinus* extract in 0.1 M sodium carbonate buffer (pH 9.6) overnight at 4°C . The plates were washed four times with PBS containing 0.05% Tween 20 (PBS-Tween). Fifty microlitres of 1:5 diluted sera in aliquots of 0.1% bovine serum album (BSA) in PBS (BSA-PBS) were added to the wells and incubated for 16 hr at room temperature. After washes, 50 μl of diluted peroxidase-conjugated, rabbit anti-human IgE (Dako, Denmark) in 0.1% BSA-PBS were added and incubated at 37°C for 3 hr. The plate was washed and then bound enzyme-labelled antibody was detected by the addition of 50 μl aliquots of 2,2'-azinodi(3-ethylbenzthiazoline sulphonic acid) (ABTS

Table 1. Number in sample and response rate at each age group in schoolchildren

Age (yr)	No. receiving questionnaire		No. attending for ELISA	
	Male	Female	Male	Female
7	547	568	336	307
8	561	503	339	327
9	534	512	341	319
10	521	506	347	401
11	493	490	281	299
12	499	429	331	262
13	540	522	322	349
14	578	542	206	330
Total	4273	4072	2503 (58.6%)	2503 (63.7%)
Mean			(61.1%)	

68 mg/100 ml in 0.1 N citrate buffer, pH 4.2) containing 0.02% H_2O_2 for 30 min. The absorbance at 414 nm was read with a Multiskan spectrophotometer (Flow Lab, Renfrewshire, U.K.). Each assay was carried out in triplicate. The positive mite-specific IgE in allergic children was compared with that in the normal controls.

Statistical methods

The prevalence of allergic disease and positive rate of mite-specific IgE antibody was analysed by age and sex. Chi-square tests were used to determine statistical significance between categories. The Scheffé method was performed in the analysis of the relationship between mite-specific IgE levels and frequencies of the wheezing, age of onset of syndromes and status of asthma (current or past).

Results

A total of 5097 of 8345 children (61.1%) agreed to have mite-specific IgE levels measured (Table 1). An estimation of the normal upper limits (the mean value plus two standard deviations) from 500 non-allergic children with the ELISA was $0.27 (0.188 \pm 0.041)$ for *D. pteronyssinus* and $0.26 (0.177 \pm 0.039)$ for *D. farinae*, and any value beyond these limits was regarded as a positive ELISA. According to this standard, the *D. pteronyssinus*- and *D. farinae*-specific IgE among non-allergic schoolchildren within the normal limits were 97.5% and 97.4%, respectively. The average prevalence of positive ELISA in

Table 2. The positive rate of mite-specific IgE antibody in 5097 schoolchildren

Age (yr)	No. of tests performed		No. of positive ELISA (%)							
			Dp + Df		Dp		Df		Total	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
7	336	307	13 (2.8)	5	10 (2.0)	3	4 (0.8)	1	27 (5.6)	9
8	339	327	7 (2.3)	8	12 (2.7)	6	3 (1.0)	4	22 (6.0)	18
9	341	319	19 (3.5)	4	10 (3.2)	11	10 (2.6)	7	39 (9.2)	22
10	347	401	10 (2.0)	5	15 (3.2)	9	17 (3.7)	11	42 (9.0)	25
11	281	299	10 (2.6)	5	10 (3.1)	8	15 (5.5)	17	35 (11.2)	30
12	331	262	12 (3.2)	7	1 (0.1)	0	18 (7.6)	8	31 (7.8)	15
13	322	349	9 (2.7)	9	18 (4.2)	10	9 (2.1)	5	36 (8.9)	24
14	206	330	3 (2.4)	10	4 (1.7)	5	7 (2.8)	8	14 (6.9)	23
Total (%)	2503	2594	83 (3.3)	53 (2.0)	80 (3.2)	52 (2.0)	83 (3.3)	61 (2.4)	246 (9.8)	166 (6.4)
Mean (%)			(2.7)		(2.6)		(2.8)		(8.1)	

Dp, *D. pteronyssinus*; Df, *D. farinae*.

Table 3. Prevalence of bronchial asthma, allergic rhinitis and eczema in schoolchildren

Disease	Percentage prevalence (children with disease/children surveyed)		
	Male	Female	Both
Bronchial asthma	5.3 (226/4273)	3.3 (133/4072)	4.3 (359/8345)
Allergic rhinitis	11.6 (497/4273)	7.1 (291/4072)	9.4 (788/8345)
Eczema	6.0 (256/4273)	6.0 (245/4074)	6.0 (501/8345)

the whole group was 8.1%, the range varying between 5.6 and 11.2% according to the child's age (Table 2). The prevalence of positive mite-specific IgE in boys was higher than in girls (9.8 vs. 6.4; $P < 0.01$), with the overall male to female ratio being 1.5:1 (Table 2).

Analysis of schoolchildren with allergic disease, according to the questionnaires, showed that the prevalence rates of allergic disease in schoolchildren were 4.3% for asthma, 9.4% for allergic rhinitis and 6.0% for eczema (Table 3). The boys had a higher prevalence of asthma (5.3% vs. 3.3%) and allergic rhinitis (11.6% vs. 7.1%) than the girls (Table 3). Among those children with allergic disease, specific IgE antibody levels against house dust mite were assayed in 198 children with asthma, 673 with allergic rhinitis and 428 with eczema.

The allergic symptoms and positive mite-specific IgE are shown in Tables 4, 5 and 6. The positive specific IgE antibody to mite in children with bronchial asthma was found to be 52% (103/198), and in children with allergic rhinitis and eczema was 28.7% (193/673) and 16.8% (72/428), respectively.

The relationships between mean levels of mite-specific IgE and the frequency and age of onset of clinical symptoms of asthma are shown in Figs 1 and 2. Mean levels of IgE were not significantly related to either the number of episodes of wheezing per year or to the age of onset of symptoms ($P > 0.1$).

Table 4. The positive rate of specific IgE antibody to *D. pteronyssinus* and/or *D. farinae* in 198 asthmatic children

Allergen	Percentage rate (no. positive/no. tested)		
	Male	Female	Total
<i>D. pteronyssinus</i> + <i>D. farinae</i>	27.0 (34/126)	30.6 (22/72)	28.3 (56/198)
<i>D. pteronyssinus</i>	11.1 (14/126)	11.1 (8/72)	11.1 (22/198)
<i>D. farinae</i>	11.1 (14/126)	8.7 (11/72)	12.6 (25/198)
Total	49.2 (62/126)	56.9 (41/72)	52.0 (103/198)

Table 5. The positive rate of specific IgE antibody to *D. pteronyssinus* and/or *D. farinae* in 673 children with allergic rhinitis

Allergen	Percentage rate (no. positive/no. tested)		
	Male	Female	Total
<i>D. pteronyssinus</i> + <i>D. farinae</i>	12.6 (52/414)	10.4 (27/259)	11.7 (79/673)
<i>D. pteronyssinus</i>	8.2 (34/414)	9.7 (25/259)	8.8 (59/673)
<i>D. farinae</i>	6.5 (27/414)	10.8 (28/259)	8.2 (55/673)
Total	27.3 (113/414)	30.9 (80/259)	28.7 (193/673)

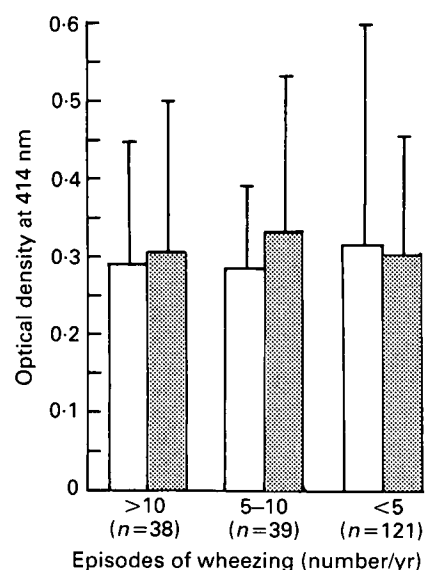
Preliminary data obtained from subjects with current and past asthma showed that the mean levels of specific IgE to *D. pteronyssinus* and *D. farinae* were significant different in these two categories (Fig. 3).

Discussion

House dust mite allergens are the main aetiological agents of asthma in children in Taiwan [8,9]. Skin tests and *in-vitro* specific IgE tests are two major approaches for diagnosis of the specific allergens responsible for IgE-mediated diseases. We reported a good correlation

Table 6. The positive rate of specific IgE antibody to *D. pteronyssinus* and/or *D. farinae* in 428 children with eczema

Allergen	Percentage rate (no. positive/no. tested)		
	Male	Female	Total
<i>D. pteronyssinus</i> + <i>D. farinae</i>	7.4 (16/217)	4.7 (10/211)	6.1 (26/428)
<i>D. pteronyssinus</i>	6.0 (13/217)	6.2 (13/211)	6.0 (26/428)
<i>D. farinae</i>	6.0 (13/217)	3.3 (7/211)	4.7 (20/428)
Total	19.4 (42/217)	14.2 (30/211)	16.8 (72/428)

**Fig. 1.** Mean level of mite-specific IgE antibody in children with different frequencies of asthma. (□) *D. pteronyssinus* and (■) *D. farinae*.

between skin test and ELISA in the detection of the specific IgE antibody to mite [8]. We therefore used the ELISA to measure specific IgE antibody to mite allergen and to determine the prevalence of mite allergy in schoolchildren.

The overall positive rate of mite-specific IgE in the population of schoolchildren in our series was 8.1%. The previous studies have shown the prevalence of positive skin tests in various normal populations to range between

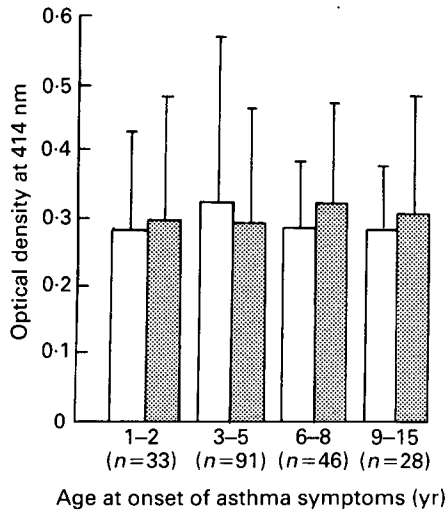


Fig. 2. Mean levels of mite-specific IgE antibody in children with different ages of the onset of asthma symptoms. (□) *D. pteronyssinus* and (▨) *D. farinae*.

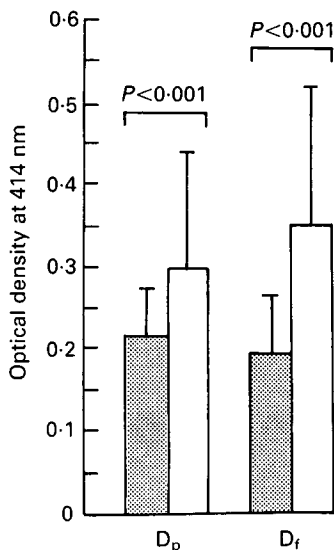


Fig. 3. Mean levels of mite-specific IgE antibody in current ($n = 80$; □) and past ($n = 198$; ▨) asthma.

9 and 50% [3-7,10]. The differences in the methods of study, the age of the population, the types of allergens and criteria of positive reaction may cause a difference in the prevalence rate [11-13]. Genetic factors and environmental effects also caused IgE variation [14].

A previous questionnaire study showed an increase in the prevalence of asthma from 1.30% in 1974 to 5.07% in 1985 in schoolchildren of the Taipei area [15]. In this report it was estimated that 4.3% of the children had a

physician's diagnosis of current asthma. Asthma was reported more frequently in boys than in girls. It is believed that asthma has increased in frequency over the past 10 years.

Skin-test reactivity has been shown to correlate well with total IgE and specific serum IgE levels [16,17]. Age has been shown to affect the immediate hypersensitivity reaction, peak reactivity occurring in subjects aged between 12 and 24 yr [18]. In this study of children aged between 7 and 14 yr, the proportion with abnormal levels of mite-specific IgE ranged between 5.6 and 11.2%. In children with allergic diseases there was a high percentage of positive specific IgE antibody to mite with the highest rate in cases of bronchial asthma. Since house dust mite was a dominant agent it was suggested that the exposure to house dust mite allergen may play a role in the development of allergic reaction, especially in bronchial asthma.

Studies have shown that male subjects have more significant skin test reactivity than female subjects [18-20]. Similar findings were obtained in our study in which boys had a higher prevalence of positive mite-specific IgE than girls.

Our survey revealed that the prevalence of allergic rhinitis was 9.4% and that of eczema dermatitis 6.0%. In other series, they were 8-12% and 2-15%, respectively [20-23].

The age of onset may not predict the course of childhood asthma, while the frequency of wheezing may be a more useful predictor [24]. In our study, mean levels of mite-specific IgE were not significantly related to the age of onset of asthma or to the frequency of attacks of wheezing, but significant differences occurred in subjects according to whether they had a current or past history of asthma. It is suggested that the mite-specific IgE may play a role in the pathogenesis of bronchial asthma in relation to natural history of asthma.

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