Environmental Health and Preventive Medicine (2007.09) 12巻5号:187～192.

Comparative Study of Simple Semiquantitative Dust Mite Allergen Tests (簡易な半定量的ダニアレルゲン検査の比較研究)

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Comparative Study of Simple Semiquantitative Dust Mite Allergen Tests

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Running title: House Dust Mite Allergen Evaluation

Key words: house dust, mite allergen, sick building syndrome, allergy

Type of contribution: Original article

Number of tables: 4

Number of figures: 0

Abstract

Objective: Two simple, commercially available and semiquantitative dust mite allergen tests, namely, the Acarex test® and Mitey Checker®, were compared using 2 and 10 μg of Der 1 allergen per gram of dust, as evaluated by enzyme-linked immunosorbent assay (ELISA), to clarify which method is better suited for practical use.

Methods: Mite allergen exposure levels of 106 floor, bed and sofa surfaces were evaluated by the Acarex test®, Mitey Checker®, and ELISA. A template of 100 cm × 100 cm was placed on the same surfaces to identify the examined areas. A dust collection filter was attached to a vacuum cleaner, and the area in the template (1 m^2) was vacuumed. Then, to evaluate the other two tests, samples from the two other areas in the template (1 m^2) that neighbored each other and did not overlap were vacuumed.

Results: To predict Der 1 levels of 2 μg/g dust or higher, the sensitivity and specificity of the Acarex test® were 100% and 13.3%, and those of Mitey Checker® were 91.8% and 71.1%, respectively. To predict Der 1 levels of 10 μg/g dust or higher, the sensitivity and specificity of the Acarex test® were 50.0% and 96.2%, and those of Mitey Checker® were 85.7% and 79.5%, respectively. Compared with Der 1 <2.0, 2.0-9.9, ≥10.0 (μg/g dust), the percent agreement and kappa of the Acarex test® were 47.2% and 0.234, and those of Mitey Checker® were 70.0% and 0.505, respectively.

Conclusion: To evaluate mite allergen exposure level for practical use in Japanese living environments, Mitey Checker® is better than the Acarex test® because of its higher sensitivity and specificity.

Introduction

The prevalence of bronchial asthma is high in many countries (1). The Second International Workshop on Dust Mite Allergens and Asthma, under the auspices of the International Association of Allergology and Clinical Immunology and the World Health Organization, reported that exposure to 2 μg of Der 1 allergen per gram of dust is considered to increase the risk of sensitization and bronchial hypersensitivity and that exposure to 10 μg of Der 1 allergen per gram of dust is considered to increase the risk of acute attacks of asthma (2). Later, the Third International Workshop on Indoor Allergens and Asthma reported that symptom severity among allergic individuals is not closely correlated with the concentration of the Der 1 level in the house, thus, the notion that the exposure level increases the risk of acute attacks of asthma was disregarded (3). However, the increased risks of sensitization and bronchial hypersensitivity are still present at 2 μg of Der 1 allergen per gram of dust, as described in this report. An increased dust mite allergen exposure level during infancy is associated with a higher risk of sensitization in the presence of a positive parental history, but is protective for children of parents without a history of atopic disease (4). Meanwhile, a relationship between dust mite allergen exposure level in children's bedrooms and the development of asthma has not been found in young children (5). Thus, there have been some negative reports on the effects of dust mite allergen level on the development of asthma; however, mite allergen exposure definitely induces mite allergen

sensitization. Therefore, the measurement of environmental mite allergen exposure level is important.

According to the "Standard for School Environment and Hygiene" by the Japanese Ministry of Education, culture, Sports, Science and Technology, mite exposure levels must be evaluated in schools. In “Towards Healthy Air in Dwellings in Europe (THADE)”, the mite allergen has been described as one of the major determents of health in dwellings (6) . From these viewpoints, mite allergen exposure level has been widely measured to improve the safety of indoor environments.

The prevalence of sick building syndrome among residents living in newly built dwellings has been highlighted in Japan. In these dwellings, not only exposures to chemicals but also dampness relate to the occurrence of sick building symptoms (7,8). Moreover, home dampness has been associated with an increased level of sensitization to dust mites (9).

Sick building syndrome involves several well-defined building-related illnesses, including rhinitis, bronchial asthma, and hypersensitivity pneumonitis (10). The dust mite has been classified as a potentially important indoor air contaminant. Thus, to elucidate the causes of sick building syndrome, the determination of the presence of the dust mite is one of the measures to consider.

The dust mite allergen level is usually determined by enzyme-linked immunosorbent assay

(ELISA). ELISA kits, however, are relatively expensive and their use requires specialized techniques. Therefore, simple, commercially available tests that are valid and reliable are required.

In this study, we selected two simple, representative and commercially available simple semiquantitative tests used in Japan for evaluation. One is the Acarex test® (Allergopharma, Reinbek, Germany), which was recommended by the Second International Workshop for practical use (2); however, it has not been validated in Japan. The other is Mitey Checker® (Sumika Enviro-Science, Co., Ltd., Hyogo, Japan). The mite allergen level evaluated by Mitey Checker® was reported to correlate significantly with Der 2 allergen per square meter measured by ELISA (11). Although Der 1 has usually been considered as the gold standard for mite allergen exposure level evaluation (2,3), Yasueda et al. reported that the monoclonal-antibody-based ELISAs for Der p 2 and Der f 2, as well as those for Der p 1 and Der f 1, are useful for the assessment of mite allergen exposure levels (12). Because the Der 1 level is almost the same as the Der 2 level in Japanese dwellings (13), it is possible to determine Der 1 levels using Mitey Checker, which was originally designed for the determination of Der 2 level. Moreover, there are no reports that compare the two simple, commercially available and semiquantitative methods of the Acarex test® and Mitey Checker®.

In this study, we compared the Acarex test® and Mitey Checker®, using 2 and 10 μg of

Der 1 allergen per gram of dust, as evaluated by ELISA, to clarify which method is better suited for practical use, particularly in Japanese living environments.

Methods

Materials

Samples used for the evaluation of mite allergen exposure level were obtained from 56 dwellings of the authors, medical students and their associates, one hospital, and one college in Hokkaido, the northern most main island in Japan. Information on places from which samples were collected are summarized in Table 1.

Table 1

This study was conducted after receiving informed consent from all the residents and approved by the Institutional Ethical board for Epidemiological Studies of Asahikawa Medical College.

Sampling procedure and allergen analysis

Mite allergen levels were evaluated using the Acarex test®, Mitey Checker®, and ELISA. Each evaluated surface was not cleaned for at least 24 hours, and mattress and sofa covers were not removed. A template of 100 cm × 100 cm was placed on the surface of a floor, bed, or sofa in a room. A dust collection filter was attached to a vacuum cleaner tube, and the area in the template (1 m^2) was vacuumed for two minutes. Then, to evaluate the other two tests, samples form other two areas in the template (1 m^2) that neighbored each other and did not overlap – at least 30 cm apart – were vacuumed for two minutes. The sampling order of the three tests was randomly

determined. After the sampling, the filters now containing trapped dust were stored at -20°C in the plastic bag.

The Acarex test® is a colorimetric test designed to detect guanine, a metabolite of the purine metabolism of Arachnidae (14). Guanine is a major component of the excrement of house dust mites and can be found in house dust. The test was performed on each sample according to the manufacturer's instructions. Each house dust sample was mixed with the provided alkaline alcohol extraction liquid in a bag and a test strip was placed in the mixture for 60 seconds. Guanine in the mixture reacts with an aromatic diazonium compound. The colors of the detection area on the strip were considered as follows: red is strongly positive (A), deep pink is moderately positive (B), pale pink is slightly positive (C), and white is negative (D).

Mitey Checker® is a chromato-immunoassay using the anti-Der 2 monoclonal antibody specific for *Dermatophagoides* species. The test was performed on each sample according to the manufacturer's instructions. The house dust samples were mixed with 10 ml of 0.1 M phosphate buffer in the bag and the test strip was placed in the mixture for three seconds. During the 10-minute incubation, the Der 2 allergen absorbed into the test strip reacts with the antibody in the strip. As a result, a red line appears on the test strip. By comparing the allergen-induced line with the control red line on the test strip, the grades were determined as follows: thicker than the control line denotes strongly positive (++), almost the same thickness as the control line denotes

moderately positive (+), thinner than the control line denotes slightly positive (±), and no line denotes negative (-).

We requested Nichi Nichi Pharmaceutical Co., Ltd. to evaluate Der p1 and Der f1 levels using monoclonal-antibody-based colorimetric ELISA (Der p1 and Der f1 ELISA kits; Nichi Nichi Pharmaceutical Co., Ltd., Mie, Japan). The company was not notified of the results of either the Acarex test® or Mitey Checker®. The detection limit was 0.1 μg of allergen per gram of dust. The concentration of Der 1 allergen was calculated by summing the Der p1 and Der f1 concentrations. In cases in which the concentrations of Der p1 and Der f1 were below the detection limit, zero was assigned to the data for the calculation of Der 1 concentration.

Statistical analysis

To predict Der 1 levels of 2 or 10 μg/g dust or higher, the sensitivity and specificity of the Acarex test® and Mitey Checker® were calculated. By comparing Der 1 levels of 2 and 10 μg/g dust, the percent agreement and kappa statistics of the Acarex test® and Mitey Checker® were calculated.

Spearman's rank correlation test was used to analyze the associations of the results of the Acarex test® and Mitey Checker® with Der 1 levels (continuous variable) evaluated by ELISA.

All statistical analyses were conducted using SPSS software for Windows version 15.0

(SPSS Inc., Chicago, U.S.A.).

Results

Table 2 shows the Der p1, Der f1 and Der 1 levels measured by ELISA. Der f1 was predominant, and the median Der 1 level was 2.94 μg/g dust (range: ND -110.98).

Table 2

Table 3 shows the results of the Acarex test® in comparison with the Der 1 levels measured by ELISA. C or more was the cutoff point for the comparison with Der 1 ≥ 2.0 μg/g dust, and B or more was the cutoff point for the comparison with Der 10.0 ≥ μg/g dust. To predict Der 1 levels of 2 μg/g dust or higher, the sensitivity and specificity of "C" or more were 100% and 13.3%, respectively (Table 3-1). To predict Der 1 levels of 10 μg/g dust or higher of Der 1 level, the sensitivity and specificity of "B" or more were 50.0% and 96.2%, respectively (Table 3-2). Compared with Der 1 <2.0, 2.0-9.9, ≥10.0 (μg/g dust), the percent agreement and kappa of "D", "C" and "A or B" were 47.2% and 0.234, respectively (Table 3-3).

Table 3

Table 4 shows the results of Mitey Checker® in comparison with the Der 1 levels measured by ELISA. "±" or more was the cutoff point for the comparison with Der 1 ≥ 2.0 μg/g dust, and "+" or more was the cutoff point for the comparison with Der 10.0 ≥ μg/g dust. To predict Der 1 levels of 2 μg/g dust or higher, the sensitivity and specificity of "±" or more were 91.8% and 71.1%, respectively (Table 4-1). To predict Der 1 levels of 10 μg/g dust or higher, the sensitivity and specificity of "+" or more were 85.7% and 79.5%, respectively (Table 4-2). Compared with Der 1<2.0, 2.0-9.9, ≥10.0 (μg/g dust), the percent agreement and kappa of "-", "±" and "++ or +"

Table 4

were 70.0% and 0.505, respectively (Table 4-3).

The Spearman's rank correlation coefficient between the four grades of the Acarex test® and Der 1 level (μg/g dust) was 0.559 ($P<0.001$) and that between the four grades of Mitey Checker® and Der 1 level (μg/g dust) was 0.756 ($P<0.001$).

Discussion

The evaluation of the dust mite exposure levels of the residents who have symptoms related to the sick building syndrome is useful for developing countermeasures. As previously described, ELISA, which is typically used as the gold standard for evaluating dust mite allergen level, is relatively expensive and requires specialized techniques. Therefore, simple, commercially available tests that can be carried out by untrained residents or health workers are desired. Thus, the Acarex test® and Mitey Checker® were developed are supplied to meet those needs.

Good intraobserver reliabilities for the Acarex test® (15) and Mitey Checker® (11) were reported when the determination was performed by trained technicians. However, the determination depends on individual estimation, so we must be cautious about the problems that may arise during practical use. The Acarex test® involves comparing the test result color with a standard color sheet. The color comparison seems to be more influenced by individual estimation than the line width comparison. However, the Acarex test® has some advantages: the cost of the Acarex test® is as about half of that of Mitey Checker® and the test time of the Acarex test® is about one-third of that of Mitey Checker®.

To our knowledge, this is the first study in which both the Acarex test® and the Mitey Checker® were compared with Der 1 levels measured by ELISA at the same time for evaluating mite allergen exposure levels in Japanese residential buildings.

We selected the gold standard mite exposure levels of 2 and 10 μg Der 1 per gram of dust evaluated by ELISA. As previously described, in the Second and Third International Workshop (3), the exposure level (2 μg Der 1 per gram of dust) that increased sensitization risk had consistently been adopted. However, in the Third International Workshop, the notion that the exposure level (10 μg Der 1 per gram of dust at the Second International Workshop) increases the risk of acute asthma attacks was disregarded. However, there have been no criteria for relatively high-level exposures to house dust mites. Therefore, the former criterion of 10 μg Der 1 per gram of dust was applied in this study.

In epidemiological studies, the expression of Der 1 level in weight per weight of dust is considered to be adequate; however, in intervention studies, the expression of Der 1 level in weight per surface area is recommended (16). Therefore, we expressed the Del 1 level as weight per surface area.

In this study, the Spearman's rank correlation coefficient between the Acarex test® and the ELISA-determined Der 1 level (ng/m^2) was 0.559 ($P<0.001$) and that between the Mitey Checker® and the ELISA-determined Der 1 level (ng/m^2) was 0.805 ($P<0.001$). Thus, Mitey Checker® correlated beter with ELISA, which measures Der 1 levels in ng/m^2 than the Acarex test®.

Mitey Checker® is designed to detect Der 2. As previously mentioned, the Der 1 level is

almost the same as the Der 2 level in Japanese dwellings, and the data evaluated by Mitey Checker® significantly correlates with the Der 2 level ($\mu g/m^2$) measured by ELISA (Spearman's rank correlation coefficient: $r=0.83$, $p<0.01$) (11).

The Acarex test® is designed to detect guanine. A significant correlation between Acarex test® results and Der 1 level measured by ELISA has been reported (linear correlation: $p<0.0001$) (17). On the other hand, mites are not the only producers of insoluble guanine; spiders and birds – to a lesser extent – can also contribute to the presence of guanine in house dust (18). That may result in a weaker Acarex test® association.

Compared with the Acarex test®, Mitey Checker® showed sufficient sensitivity and specificity, and higher percentage of agreement with ELISA. Thus, Mitey Checker® seems to be better suited for evaluating mite allergen level. However, the Acarex test® is less expensive, and excluding exposures of 10 μg Der 1 per gram of dust or higher, the Acarex test® is preferable because of its higher specificity. Thus, we can choose the test in accordance with the intended use.

This study was performed in only the northern most main island of Japan, Hokkaido. There is a possibility that different results would have been obtained if dust samples from other areas of Japan had been used, because Japan shows quite a wide variety of climates and changes during the four seasons.

Dani Scan®（Asahi Food & Health Care, Ltd.）is another simple, commercially available

and semiquantitative dust mite allergen test. It has been reported that Mitey Checker® had a higher Spearman's rank correlation coefficient with ELISA than Dani Scan® (Mitey Checker®: 0.888, Dani Scan®: 0.391) (19). However, another study indicated that the Spearman's rank correlation coefficient of Dani Scan® with ELISA was almost the same as that of Mitey Checker® with ELISA (Mitey Checker®: 0.86, Dani Scan®: 0.83) (20). Therefore, further study is needed to compare Dani Scan® with the other methods in various environments.

It has been recommended that preventive measures for house dust mites should be carried out including fitting allergen-proof bedclothes, sheets, mattresses and pillowcases, washing bedding regularly, and reducing humidity (21). These countermeasures should be taken after the evaluating dust mite allergen level. These measures can be performed by residents easily. Dust mite allergen evaluation is also best performed by residents repeatedly for a more effective prevention of sick building syndrome induced by house dust.

In conclusion, to evaluate mite allergen exposure level for practical use in Japanese living environments, Mitey Checker® is better than the Acarex test® because of its higher sensitivity and specificity.

ACKMOLEDGMENT

This work was supported by a Health Science Research Grant from the Japan Ministry of Health, Labor and Welfare.

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Table 1 Information on places from which samples were collected（n=106)

	Number	%
Type of building		
Detached house	43	40.2
Multi-dwelling	59	55.1
Hospital	3	2.8
College	1	0.9
Building structure		
Wooden	70	65.4
Reinforced concrete	36	33.6
Type of surface		
Floor		
Wood or tile	37	34.6
Carpeted	49	45.8
Tatami mat	13	12.1
Bed mattress	6	5.6
Sofa	1	0.9

Table 2 Mite allergen levels evaluated by ELISA (n=106)

	Median	Min	25th percentile	75th percentile	Max
Der p1(μg/g dust)	ND	ND	ND	0.37	55.45
Der f1(μg/g dust)	2.15	ND	0.42	8.37	110.86
Der 1(μg/g dust)*	2.94	ND	0.55	11.16	110.98

*Der p1 + Der f1

Table 3 Results of Acarex test® in comparison with Der 1 levels evaluated by ELISA

3-1 Compared with Der 1 ≥2.0 (μg/g dust)

		Der 1 (μg/g dust)			Sensitivity*	Specificity*
		<2.0	≥2.0	Total		
Acarex test®	A	0	6	6		
	B	0	16	16		
	C	39	44	83	100%	13.3%
	D	6	0	1		
Total		45	61	106		

*Cut-off value: C or more

3-2 Compared with Der 1 ≥10.0 (μg/g dust)

		Der 1 (μg/g dust)			Sensitivity*	Specificity*
		<10.0	≥10.0	Total		
Acarex test®	A	0	1	1		
	B	3	13	16	46.4%	96.2%
	C	69	14	83		
	D	6	0	6		
Total		78	28	106		

*Cut-off value: B or more

3-3 Compared with Der 1 <2.0, 2.0-9.9, ≧10.0 (μg/g dust)

		Der 1 (μg/g dust)				Percent	Kappa
		≤2.0	2-9.9	≥10.0	Total	agreement	
Acarex test®	A or B	0	3	14	17	47.2%	0.234
	C	39	30	14	83		
	D	6	0	0	6		
Total		45	33	28	106		

Table 4 Results of Mitey Checker® in comparison with Der 1 levels evaluated by ELISA

4-1 Compared with Der 1 ≥2.0 (μg/g dust)

		Der 1 (μg/g dust)			Sensitivity*	Specificity*
		<2.0	≥2.0	Total		
Mitey	(++)	0	8	8		
Checker®	(+)	3	29	32		
	(±)	10	19	29	91.8%	71.1%
	(-)	32	5	37		
Total		45	61	106		

*Cut-off value: (±) or more

4-2 Compared with Der 1 ≥10.0 (μg/g dust)

		Der 1 (μg/g dust)			Sensitivity*	Specificity*
		<10.0	≥10.0	Total		
Mitey	(++)	1	7	8		
Checker®	(+)	15	17	32	85.70%	79.50%
	(±)	25	4	29		
	(-)	37	0	37		
Total		78	28	106		

*Cut-off value: (+) or more

4-3 Compared with Der 1 <2.0, 2.0-9.9, ≥10.0 (μg/g dust)

		Der 1 (μg/g dust)				Percent	Kappa
		≤2.0	2-9.9	≥10.0	Total	agreement	
Mitey	(++) or (+)	3	13	24	40	70.0%	0.505
Checker®	(±)	10	15	4	29		
	(-)	32	5	0	37		
Total		45	33	28	106		