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The relationships among *Dermatophagoides pteronyssinus* exposure, exhaled nitric oxide, and exhaled breath condensate pH levels in atopic asthmatic children

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Abstract

This study examined seasonal changes in indoor *Dermatophagoides pteronyssinus* 1 (*Der p* 1)/*Blattella germanica* 1 (*Bla g* 1) antigen concentrations in the homes of atopic asthmatic and atopic nonasthmatic children. Possible associations between environmental allergen exposure and levels of exhaled breath indices were also evaluated.

A total of 38 atopic children were recruited for this cross-sectional study: 22 were asthmatic and 16 were nonasthmatic. Home visits were conducted for indoor air and dust sampling each season. Exhaled nitric oxide (eNO)/spirometric measurements were taken and exhaled breath condensate (EBC) was collected after sampling of the domestic environment.

The highest *Der p* 1 concentrations were on the top of mattresses in the homes of recruited children. The floors of kitchens and living rooms had the highest *Bla g* 1 concentrations in the homes of atopic asthmatic children. A positive correlation was found between *Der p* 1 exposure of mattress, bedroom floor, and living room floor and eNO levels in the atopic asthmatic children. The *Der p* 1 concentrations on the surfaces of mattress and bedroom floor were positively related to high eNO levels in the atopic asthmatic children after adjusting for season. No association was found between *Der p* 1 exposure and EBC pH values in the recruited children.

A positive correlation was found between *Der p* 1 exposure and high eNO levels in atopic asthmatic children, especially in *Der p* 1 exposure of mattress and bedroom floor.

Abbreviations: *Der p* 1 = *Dermatophagoides pteronyssinus* 1, *Bla g* 1 = *Blattella germanica* 1, eNO = exhaled nitric oxide, EBC = exhaled breath condensate, CO_2 = carbon dioxide.

Keywords: allergen, asthma, *Bla g* 1, *Der p* 1, exhaled breath condensate, exhaled nitric oxide

1. Introduction

The prevalence of allergic respiratory diseases, especially in children, has gradually increased in highly urbanized areas in recent decades.[1,2] The prevalence of asthma in Taiwan is approximately 12%,[2] and 20.4% of preschool children have asthma symptoms.[3] Children under 19 years old account for 41.92% of asthmatic patients in the emergency room.[4]

Asthma develops from various genetic and environmental factors. Common household risk factors include house dust mite allergens[5] and cockroach allergens.[6,7] According to a previous study, 70% to 90% of patients with allergies are sensitive to house dust mite.[8] In Taiwan, 72.0% to 82.5% of asthmatic patients are sensitive to house dust mite allergens.[9] Asthma symptoms and exposure to house dust mite/cockroach allergens in children with atopic asthma are clearly related.[10,11]

The detectable percentage of (house dust mite) *Dermatophagoides pteronyssinus* 1 (*Der p* 1) allergen is 85.9% in households in the Unites States, with the highest detectable percentage found in living room upholstery, bedroom floors, and mattresses.[12] In Taiwan, the highest concentration of *Der p*1 is also found on the surfaces of mattresses in the homes of mite-allergic asthmatic patients.[13] House dust mite allergen exposure is not different between asthmatic and nonasthmatic children.[14] However, long-term variations in cockroach allergen concentration in the domestic environment have seldom been examined. In the United States, the highest and lowest concentrations of (cockroach) *Blattella germanica* 1 (*Bla g* 1) allergen are in living room floors and bedroom mattresses, respectively.[12] In Taiwan, a study reported that the geometric mean concentration of *Bla g* 1 from kitchen floors was 0.61 U/g (of dust).[13] According to previous studies, the levels of exhaled gas indices – exhaled nitric oxide (eNO) and exhaled breath condensate (EBC) pH – closely correspond to asthma symptoms.[15,16] Moreover, a positive

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association exists between *Der p* 1 concentrations in the homes and schools of asthmatic children and their eNO levels.[17] The degree of immunoglobulin E sensitization to *Der p* 1 shows a positive relationship with the amount and frequency of eNO.[18] Using eNO to guide treatment decisions may decrease asthma exacerbations.[19]

To our knowledge, few studies have investigated the seasonal variations of indoor cockroach *Bla g* 1 concentration in homes. Thus, this study examined seasonal changes in indoor *Der p* 1 and *Bla g* 1 concentrations (on living room, kitchen, and bedroom floors, and in mattresses) in the homes of atopic asthmatic and nonasthmatic children in northern Taiwan. In addition, possible associations with environmental allergen exposure in the homes were evaluated, based on the children's eNO levels and EBC pH values.

2. Methods

2.1. Study population

This cross-sectional study was conducted from February 2010 to May 2012. Thirty-eight children were recruited, including 22 atopic asthmatic and 16 atopic nonasthmatic children. Their mean age was 9.47 (standard deviation 1.97) years. All asthmatic children were recruited from the Department of Pediatrics at Chang Gung Children's Hospital. The children were diagnosed by a pediatrician based on the guidelines of the Global Initiative for Asthma[20] and the modified National Asthma Education and Prevention Program:[21] they had a hyper-reactive airway response to a bronchodilator inhalation test using methacholine. All recruited atopic asthmatic and nonasthmatic children were sensitive to *Der p* allergen, as determined through blood testing. Around 36.4% of the asthmatic children and 6.25% of the nonasthmatic children were sensitive to *Bla g* allergen. Approximately 77% of atopic asthmatic children used regular medication for disease control, including dry powder inhalers containing steroid and long-acting bronchodilator (fluticasone propionate and salmeterol; 70.6%) and oral drugs (antileukotrienes, steroids, and antihistamines). Some atopic asthmatic children did not use regular medication; they used a short-acting β_2 agonist when necessary. The atopic asthmatic children did not change their use of medication during the study period. Atopic nonasthmatic children (who had no history of asthma or pulmonary disease) were also recruited from the Department of Pediatrics of Chang Gung Children Hospital. The Institutional Review Board of Chang Gung Memorial Hospital approved the study protocol (99-3720B), and the children's parents provided written informed consent.

2.2. Air sampling

Home visits, convenient to each family's time schedule, were made for indoor air sampling once during each season. Indoor environmental measurements of air temperature, relative humidity, and carbon dioxide (CO_2) were determined by using a digital psychrometer (TSI, Inc., Shoreview, MN). Thereafter, air samplers were placed in the center of each living room for 2 consecutive hours. The sampling height was 1.0 to 1.2 m above the floor, approximating a child's breathing zone.

2.3. House dust collection and Der p 1/Bla g 1 allergens analysis

House dust sampling was performed at the same time as indoor air sampling. Dust samples were collected each season from 4 sites in the houses: the living room floor, kitchen floor, top surface of the child's mattress, and the bedroom floor next to the mattress. Additionally, a 1 m^2 area was vacuumed for 2 minutes at each sampling site using a vacuum cleaner fitted with a fresh glass-fiber filter (Shivn Feng, Inc., New Taipei City, Taiwan). Finally, collected dust samples were sieved through a 425 μm mesh screen to obtain fine dust samples.

A fine dust sample (5 mg) was agitated with 1 mL borate buffered saline buffer containing 0.1% Tween 20 (pH 8.0) for 1 hour to extract *Der p* 1/*Bla g* 1 allergens. *Der p* 1/*Bla g* 1 concentrations in dust samples were determined using a sandwich enzyme-linked immuno-sorbent assay (INDOOR Biotechnologies, Inc., Manchester, UK). Standard response curves were then generated using *Der p* 1 and *Bla g* 1 standards in the range of 0.05 to 250 ng/mL and 0.002 to 1 U/mL, respectively (correlation coefficients were 0.99 and 0.99 for *Der p* 1 and *Bla g* 1, respectively). Finally, a negative control with 1% albumin from bovine serum and phosphate buffer saline with Tween 20 (pH 7.4) was used with each assay.

2.4. Exhaled NO, EBC pH, and spirometric measurements

The eNO/spirometric measurements and EBC collection were taken within 1 week of the domestic environmental sampling. The eNO levels of all children were evaluated at an expiratory flow rate of 50 mL/s using a chemiluminescence analyzer (CLD 88 sp; ECO Physics, Dürnten, Switzerland), based on international standards.[22] The EBCs were then collected by using a noninvasive cooling device (EcoScreen, Jaeger Toennies, Hoechberg, Germany). Although sitting upright and wearing a nose clip, each subject breathed normally through a mouthpiece for 15 minutes for EBC sampling. Approximately 1 to 3 mL of EBC were collected and analyzed. All EBC samples were deaerated with argon at a flow rate of 350 mL/minute to remove CO_2. The EBC pH was then determined with a digital pH meter (UB-5; Denver Instruments, Denver, CO).

Forced expiratory volume in 1 second, forced vital capacity, maximum mid-expiratory flow, and peak expiratory flow rate were determined by using a spirometer (MIR Spirolab II, Pinyork, Japan). The eNO, EBC pH, and spirometry measurements were determined at the same frequency as the indoor air sampling.

2.5. Statistical analysis

All statistical analyses were performed with SPSS software (Version 21.0, SPSS, Inc., Chicago, IL). Figures were graphed with GraphPad Prism 6.0 software (GraphPad Software, Inc., San Diego, CA). Significance for all tests was set at $P < 0.05$. The necessary study sample size was calculated based on the assumed criteria in the association between *Der p* 1 and eNO level (as the primary outcome),[17] with a type I error of 5%, power of 90% (1 – type II error), and an estimated dropout rate of 10%. For data analysis, the 4 seasons were divided into hot and cold seasons, defined as follows: hot season (May–October) and cold season (November–April). Group differences in continuous variables were identified by using the 2-sample independent *t* test for normally distributed data and Kruskal–Wallis or Mann–Whitney *U* test for nonnormally distributed data. Group differences in categorical variables were identified by using the Chi-squared test. Additionally, the strength of correlation was evaluated with the Spearman test for nonnormally distributed data to determine the relationship between *Der p* 1 concentrations in dust samples in the homes and eNO/EBC pH levels in atopic asthmatic and

Table 1

Basic characteristics of study population.

	Atopic asthma group	Atopic nonasthma group	*P*
Sample size, n	22	16	
Gender, M/F, n	9/13	11/5	0.11
Age, year*	9.27 (2.21)	9.75 (1.61)	0.47
BMI, kg/m^2*	17.44 (3.15)	19.05 (3.86)	0.17
Pulmonary function, % predicted*			
FEV_1	92.03 (14.59)	91.02 (9.28)	0.81
FVC	89.09 (14.39)	88.14 (8.27)	0.81
FEV_1/FVC	101.21 (9.90)	102.91 (9.41)	0.60
Maximum expiratory flow	85.20 (26.13)	79.52 (18.19)	0.46
Peak expiratory flow	89.98 (13.98)	82.95 (11.55)	0.11
Exhaled breath indices*			
eNO, ppb	48.11 (31.82)	30.56 (14.60)	0.048
EBC pH	7.58 (0.45)	7.76 (0.34)	0.19
Environmental allergens†			
Der p 1, μg/g dust	1.33 (0.76–2.08)	0.71 (0.26–2.12)	0.21
Bla g 1, U/g dust	0.32 (0.23–0.54)	0.25 (0.13–0.45)	0.16

BMI = body mass index, FEV_1 = forced expiratory volume in 1 second, FVC = forced vital capacity, eNO = exhaled nitric oxide, EBC = exhaled breath condensate, *Der p* 1 = *Dermatophagoides pteronyssinus* 1, *Bla g* 1 = *Blattella germanica* 1.

* Data were presented as mean (SD).

† Median (25–75 percentiles).

nonasthmatic children. Additionally, in order to evaluate the influence of *Der p* 1 exposure on eNO level, logistic regression models were used to estimate the associations between *Der p* 1 concentration and high eNO level (>22 ppb) in the atopic asthmatic children after adjusting for season. The *Der p* 1 concentrations on the surfaces of mattress, bedroom floor, and living room floor were considered in the logistic regression models.

3. Results

The children recruited were between 6 and 13 years old. The atopic asthmatic and nonasthmatic children did not differ in sex, age, or body mass index, as shown in Table 1. The mean pulmonary function parameters were normal and did not differ significantly between atopic asthmatic and nonasthmatic children. The severity of asthma was mild intermittent. A significant difference in eNO level was found between the atopic asthma group (48.11 ppb) and atopic nonasthma group (30.56 ppb, $P = 0.048$). The eNO level was not associated with the dose of steroid ($r_s = -0.098$, $P = 0.565$) administered in the asthmatic children (data not shown). Furthermore, atopic asthmatic children did not differ from atopic nonasthmatic children in terms of the EBC pH value. The concentrations of environmental allergens (*Der p* 1 and *Bla g* 1) did not differ between the homes of asthmatic and those of nonasthmatic children.

Indoor air indices such as temperature, humidity, and CO_2 concentrations were measured in all of the homes. During the study period, median air temperatures were 19.87 to 29.80 °C (data not shown). Median relative humidity was 66.84% to 78.35%, and median CO_2 concentrations were 434.87 to 593.49 ppm. The homes of atopic asthmatic children did not differ from those of atopic nonasthmatic children in terms of air temperature, humidity, and CO_2 concentrations. Overall, 3.3% of the measured CO_2 concentrations did not fulfill the threshold requirement (1000 ppm) set by Taiwan's Environmental Protection Administration.

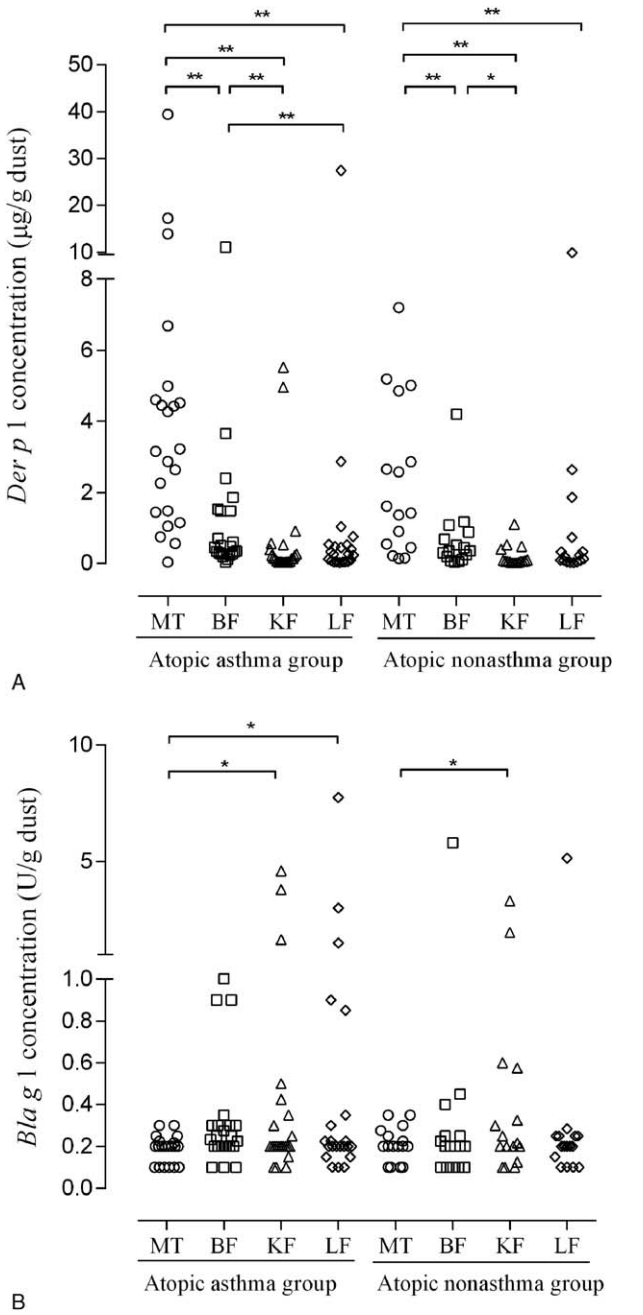

Figure 1. Concentration distributions of environmental allergens in homes of atopic asthmatic and atopic nonasthmatic children. (A) *Der p* 1 allergen, (B) *Bla g* 1 allergen. *$P < 0.05$; **$P < 0.01$. *Der p* 1 = *Dermatophagoides pteronyssinus* 1, *Bla g* 1 = *Blattella germanica* 1, MT = mattress, BF = bedroom floor, KF = kitchen floor, LF = living room floor.

The median *Der p* 1 concentration at the 4 sites in the homes of atopic asthmatic children (mattress, 2.71 μg/g; bedroom floor, 0.44 μg/g; kitchen floor, 0.08 μg/g; and living room floor, 0.20 μg/g of dust) did not differ from those in the homes of atopic nonasthmatic children (mattress, 2.09 μg/g; bedroom floor, 0.37 μg/g; kitchen floor, 0.08 μg/g; and living room floor, 0.17 μg/g of dust), shown in Fig. 1A. In the homes of atopic asthmatic children, the median *Der p* 1 concentration on the mattresses was significantly higher than that on the bedroom, kitchen, or living room floor (all $P < 0.01$). In the homes of atopic nonasthmatic children, the *Der p* 1 concentration was highest on

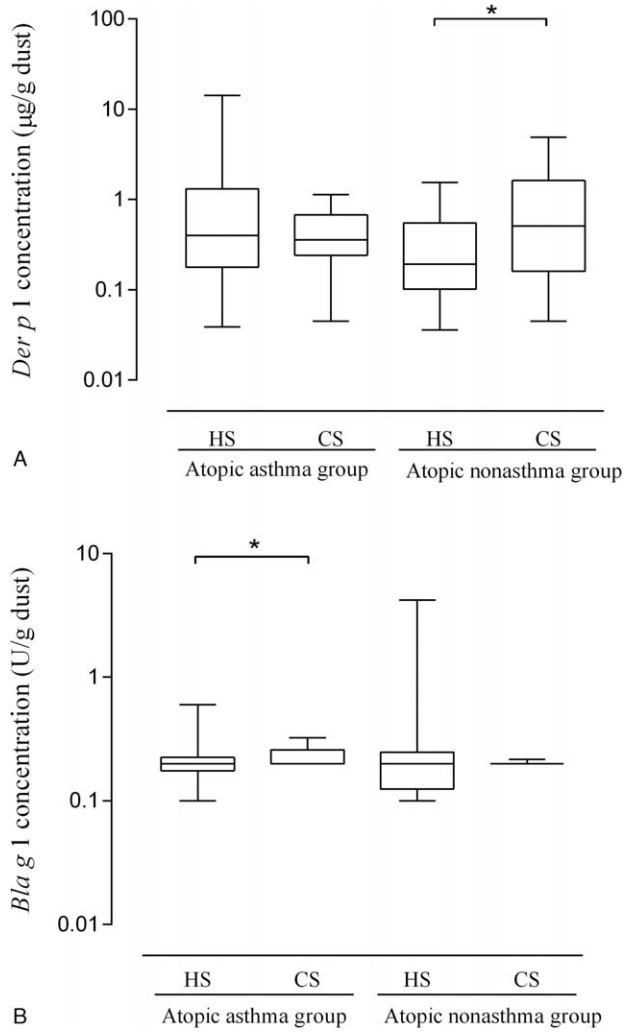

Figure 2. Seasonal variation of environmental allergens in dust samples of children's homes. (A) *Der p* 1 allergen, (B) *Bla g* 1 allergen. $^{*}P<0.05$. *Der p* 1 = *Dermatophagoides pteronyssinus* 1, *Bla g* 1 = *Blattella germanica* 1, HS = hot season, CS = cold season.

mattresses, compared with that on the bedroom, kitchen, and living room floor (all $P<0.01$). Moreover, in the homes of atopic asthmatic children, the median *Der p* 1 concentration on bedroom floors was significantly higher than that on the kitchen or living room floors (all $P<0.01$). In the homes of atopic nonasthmatic children, the *Der p* 1 concentration on bedroom floors differed from that on the kitchen room floors ($P=0.013$). Additionally, in the homes of atopic asthmatic children, the *Bla g* 1 concentration of the mattresses was significantly lower than that of the kitchen floor ($P=0.009$) and living room floor ($P=0.022$) (Fig. 1B). In the homes of atopic nonasthmatic children, *Bla g* 1 concentration of dust samples from mattresses was significantly lower than that of the kitchen floors ($P=0.031$). Furthermore, the *Bla g* 1 concentration in the homes of atopic asthmatic children was similar to that in the homes of atopic nonasthmatic children.

The homes of atopic asthmatic children did not differ from those of atopic nonasthmatic children in terms of *Der p* 1 concentration during hot or cold seasons (Fig. 2A). In addition, no seasonal change in *Der p* 1 concentration was found in the homes of atopic asthmatic children (0.38 μg/g during the hot season; 0.43 μg/g of dust during the cold season; $P=0.401$). However, *Der p* 1 concentrations in the homes of atopic nonasthmatic children differed significantly between the hot and cold seasons ($P=0.024$). A seasonal change in *Bla g* 1 concentrations was found in the homes of atopic asthmatic children ($P=0.037$), but not in the homes of atopic nonasthmatic children (Fig. 2B). Additionally, *Bla g* 1 concentrations in the homes of atopic asthmatic and nonasthmatic children did not differ during hot or cold seasons.

In this study, 20.39% of dust samples from the homes of atopic asthmatic children and 20.0% of samples from those of atopic nonasthmatic children contained over 2 μg/g of *Der p* 1 allergen (data not shown). Additionally, 6.73% of dust samples from the homes of atopic asthmatic children and 3.13% of dust samples from those of atopic nonasthmatic children contained over 2 U/g of *Bla g* 1 allergen. Over 8 U/g of *Bla g* 1 allergen was found in only 0.96% and 0% of dust samples from the homes of atopic asthmatic and nonasthmatic children, respectively. To evaluate the relationship between dominant exposure of *Der p* 1 and eNO/EBC pH levels for the atopic asthmatic and nonasthmatic children, the *Der p* 1 concentrations of mattress, bedroom floor, and living room floor were used for analysis in this study. Among atopic asthmatic children, positive correlations were found between eNO levels and *Der p* 1 exposure of mattress ($r_s=0.317$, $P=0.025$), bedroom floor ($r_s=0.368$, $P=0.007$), and living room floor ($r_s=0.277$, $P=0.047$), as shown in Table 2. However, no correlation was found between EBC pH values and *Der p* 1 exposure in homes of all recruited children. In the logistic models that estimated the associations between *Der p* 1 concentration and high eNO level, an important factor-season was adjusted. Model 1 shows that a risk of 4.057 (odds ratio, OR) (95% confidence interval [CI] of OR = 1.034–15.927, $P=.045$) was observed suggesting that *Der p* 1 concentration on the surface of mattress was related to high eNO level in the atopic asthmatic children (Table 3). Also, model 2 shows that the risk with high eNO level was increased up to 4.235-fold (95% CI of OR = 1.080–16.610, $P=0.038$) when the average *Der p* 1 concentration of mattress and bedroom floor was over the median (3.317 μg/g) of *Der p* 1 allergen in the homes of atopic

Table 2

Associations between *Der p* 1 exposure and eNO/EBC pH levels in the atopic asthmatic children and atopic nonasthmatic children.

	Atopic asthma group				Atopic nonasthma group			
	eNO level, ppb		EBC pH value		eNO level, ppb		EBC pH value	
Sampling locations	r_s	P	r_s	P	r_s	P	r_s	P
Mattress	0.317	0.025	0.224	0.160	−0.058	0.720	−0.067	0.704
Bedroom floor	0.368	0.007	−0.246	0.117	0.271	0.091	−0.037	0.835
Living room floor	0.277	0.047	−0.212	0.177	0.303	0.057	0.134	0.442

Der p 1 = *Dermatophagoides pteronyssinus* 1, eNO = exhaled nitric oxide, EBC = exhaled breath condensate.

Table 3

Adjusted ORs and 95% CIs for associations between *Der p* 1 exposure and eNO level in atopic asthmatic children.

	High eNO level (>22 ppb)		
Risk factors	**OR**	**95% CI of OR**	***P***
Model 1: mattress			
Season	1.030	0.269–3.937	0.966
Der p 1 concentration†	4.057	1.034–15.927	0.045
Model 2: mattress and bedroom floor			
Season	1.006	0.263–3.854	0.993
Der p 1 concentration‡	4.235	1.080–16.610	0.038
Model 3: mattress, bedroom floor, and living room floor			
Season	0.855	0.234–3.124	0.813
Der p 1 concentration‡	2.666	0.734–9.682	0.136

OR = odds ratio, CI = confidence interval, *Der p* 1 = *Dermatophagoides pteronyssinus* 1, eNO = exhaled nitric oxide.

† Baseline = the *Der p* 1 concentration was below the median (3.176 μg/g dust).

‡ Baseline = the average *Der p* 1 concentration was below the median (model 2: 3.317 μg/g dust, model 3: 1.321 μg/g dust).

asthmatic children. No association between the average *Der p* 1 concentration of mattress, bedroom floor, and living room floor and high eNO level in the atopic asthmatic children was found after adjusting for season. Additionally, owing to the small sample size (n = 9) of recruited children sensitized to *Bla g* allergen, the relationship between *Bla g* 1 exposure and eNO/EBC pH levels was not evaluated in this study.

4. Discussion

To our knowledge, this is the 1st study to evaluate the seasonal variation of *Der p* 1/*Bla g* 1 and the association between *Der p* 1 allergen exposure and exhaled breath indices (eNO/EBC pH) in Taiwan. In this study, the median *Der p* 1 concentrations were 1.33 and 0.71 μg/g of dust in the homes of atopic asthmatic and nonasthmatic children, respectively. The measured *Der p* 1 concentration was lower (1.53 μg/g of dust) than that reported in domestic households in the United States.[23] The highest *Der p* 1 concentration was found on top of the mattresses of atopic asthmatic and nonasthmatic children in this study. This finding closely resembles that of a previous study.[13] Additionally, the median *Der p* 1 concentrations in dust samples from kitchen and bedroom floors in the homes of atopic asthmatic children in this study were significantly lower than those from the kitchen floors (0.72 μg/g)[13] and on the bedroom floors (6.72 μg/g),[24] respectively, in the homes of atopic asthmatic children.

Furthermore, atopic asthmatic children and nonasthmatic children in this study did not differ in *Der p* 1 exposure. This similarity may be related to the fact that environmental control education is provided for asthmatic children and their families; this may contribute to efforts to decrease *Der p* 1 concentration in homes.[25] Previous studies have proposed a dust mite allergen concentration of 2 μg/g of dust to be the threshold of exposure required to develop allergic sensitization.[26,27] In this study, 20.39% and 20% of dust samples from homes of atopic asthmatic children and of atopic nonasthmatic children had *Der p* 1 concentrations above 2 μg/g of dust, respectively. The high *Der p* 1 concentration in dust samples of the homes of the recruited children suggests that the children's families should pay attention and increase their housekeeping practices to decrease house dust mite growth and *Der p* 1 concentration in their homes.

The median *Bla g* 1 concentration was 0.2 U/g of dust measured from 4 sites in the homes of atopic asthmatic children and of atopic nonasthmatic children. The highest geometric mean concentrations of *Bla g* 1 allergen were in dust samples from the kitchen (0.38 U/g) and living room floors (0.37 U/g) in the homes of atopic asthmatic children and from the kitchen floors (0.30 U/g) in the homes of atopic nonasthmatic children. The lowest geometric mean concentrations were on top of mattresses of atopic asthmatic children (0.20 U/g of dust) and nonasthmatic children (0.22 U/g of dust). These findings differ from those of a previous study.[12] The difference may be related to the building characteristics and lifestyles of the recruited children. The *Bla g* 1 concentrations from dust samples from the kitchen floor in the homes of atopic asthmatic children (geometric mean 0.38 U/g) and nonasthmatic children (geometric mean 0.30 U/g) in the study were lower than those in a study from central Taiwan (0.61 U/g)[13] and the US (1.38 U/g).[28] Previous studies have proposed a threshold *Bla g*1 concentration of 2 U/g of dust, above which allergic sensitization to cockroach allergen can occur.[29,30] Approximately 6.73% of dust samples in the homes of atopic asthmatic children in this study had *Bla g*1 concentrations above 2 U/g, slightly lower than those reported in different sites in a US study (13.4%).[28] Moreover, 3.13% of dust samples in the homes of atopic nonasthmatic children in this study had *Bla g* 1 concentrations above 2 U/g. The relationship between *Bla g* 1 exposure and asthma symptom in children warrants further study.

Taiwan is located in a subtropical region with a high mean air temperature and humidity, making it conducive for house dust mites and cockroaches to thrive.[12] In this study, a seasonal change was found in *Der p* 1 concentration in the homes of atopic nonasthmatic children. Additionally, a seasonal change was found in *Bla g* 1 concentration of the homes of atopic asthmatic children. This finding suggests that the homes of atopic asthmatic children should be cleaned more frequently during the hot season (May to October) to decrease *Bla g* 1 concentrations.

A previous study indicated that the optimal cutoff value of eNO for the diagnosis of asthma was 22 ppb.[31] In this study, approximately 71.63% of the atopic asthmatic children and 64.76% of the atopic nonasthmatic children have high levels of eNO (>22 ppb). This finding reveals that over half of these children have airway inflammation problems. These results may be confounded by exposure to other air pollutants[32–34] and environmental allergens[35] in homes. Moreover, this study demonstrates that *Der p* 1 exposure is positively associated with eNO level in atopic asthmatic children, similar to the findings of a previous study.[17] According to our results, after adjusting for the influence of season, *Der p* 1 exposure from the mattress and bedroom floor profoundly affects the health of atopic asthmatic children. We thus recommend cleaning the mattress and bedroom floor in homes of allergic children more diligently.

Although the pH value in EBC is an indicator of acute asthma exacerbation in asthmatic children,[15] this study found no association between *Der p* 1 exposure and EBC pH value in atopic asthmatic or nonasthmatic children. This finding may be explained by the mild intermittent severity of asthma of the recruited children. We recommend that future research should evaluate the relationship between *Der p* 1 exposure and exhaled breath indices (eNO and EBC pH) in moderate-to-severely asthmatic children. The interactions among environmental allergens, eNO/EBC pH levels, and medications used also warrant further study. Furthermore, the sample size of atopic asthmatic (n = 8) and nonasthmatic (n = 1) children sensitized to *Bla g* allergen in this study was very small. Thus, future research should examine the feasibility of increasing the sample size of atopic asthmatic and nonasthmatic children who are sensitive to

the *Bla g* allergen, in order to evaluate the relationship between *Bla g* 1 exposure and eNO/EBC pH levels.

In conclusion, the *Der p* 1 allergen exposure of the mattress and bedroom floor in homes was positively associated with high eNO levels in atopic asthmatic children. It suggests increase cleaning frequency, especially on the top surface of mattress and bedroom floor in homes of allergic children.

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