Allergol Immunopathol (Madr). 2020;48(1):90–94

Allergologia et immunopathologia

Sociedad Española de Inmunología Clínica, Alergología y Asma Pediátrica

www.elsevier.es/ai

ORIGINAL ARTICLE

The level of allergens in dust samples collected from selected schools in Shiraz, Iran and its asthma-risk implications

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Received 20 March 2019; accepted 10 May 2019
Available online 30 August 2019

KEYWORDS
Dust mites;
Allergens;
Schools;
Iran;
Asthma

Abstract
Background: Both home and school are important places where children are exposed to various indoor allergens. This study aimed to identify the profile of indoor allergens in schools and its impact on asthma development.
Methods: A total of 104 classrooms from 52 schools were selected for dust collection during the fall of 2017. The levels of indoor allergens including dust mite (Der f1, Der p1), cat (Fel d1), cockroach (Bla g1) and mouse (Mus m1) were measured by enzyme linked immunosorbent assay (ELISA). The diagnosis of asthma was made in all students of the selected classes by the allergist. The collected data were analyzed using SPSS version 21.0.
Results: Out of 2816 students in the selected classes, 180 students were involved with asthma. Students were mostly exposed to Bla g1 (83.1%), followed by Der f1 (51.5%), Mus m 1 (45.5%), Der p1 (8.9%) and Fel d1 (7.9%) in the dust collected from 101 classrooms. Although levels of all studied allergens in the settled dust of the classrooms were low, there was a relationship between Fel d1 in the classroom dust and development of asthma.
Conclusion: This study showed considerable levels of cockroach allergens in schools. Exposure to cat allergen in our schools played an important role in asthma development; further school-based investigations require evaluating the role of classroom allergen on asthma development.

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https://doi.org/10.1016/j.aller.2019.05.005

Introduction

Allergic asthma is the most common type of asthma which triggers by various indoor allergens such as cat, cockroach, mouse, and mites.[1,2] Indoor dust allergen levels in the homes of patients with childhood asthma have been determined in our previous works. Our results showed all the studied allergens were detectable in all homes but the concentrations of allergens were below the threshold level for sensitization.[3] While students are exposed to indoor school allergens for a prolonged period, they might be sensitized during this period.[4–6]

Cats are among the most common causes of allergy, and its major allergen is Felis domesticus (Fel d1). Fel d1 is produced in the skin and salivary glands of cats.[7] Feral cat allergen has been found in US and Swedish schools with the highest concentration.[8,9] Published data have shown that animal allergens can be present in environments where no animals reside; these allergens might be transported into classrooms by clothes and shoes.[10,11]

Cockroaches are ubiquitous and highly allergenic in inner-city areas, exposure to their allergens (Bla g1, Bla g2) is suggested as an important factor in asthma. The sources of cockroach allergens are saliva, feces and shedding their body parts.[12] Although mouse allergens (Mus m1) are found in hair, dander and urine, urine is the biggest source of their allergen. Exposure and sensitization to mice and related allergens (Mus m1) are a strong predictor of severe asthma in young children.[13] Infestation with cockroach and mouse allergens have been detected in US schools with low-income and inner-city populations.[14,15]

House dust mites (HDMs) are small creatures that feed mainly on the dead scales of skin. HDMs including *Dermatophagoides farina* (Der f1) and *Dermatophagoides pteronyssinus* (Der p1) are the most important sources of indoor allergens in the development of asthma.[16] A recent meta-analysis study concluded dust mites should be considered as allergenic components in 35% of patients with allergy in Iran.[17] In schools, researchers show that dust mite allergens are found in high levels in many classrooms.[18,19]

Childhood asthma is increasing and exposure to indoor allergens in schools is suspected as a possible cause. Few studies have centered on the association between asthma and allergen levels in classrooms.[20,21]

This study aimed to identify the profile of indoor allergens in the elementary and high schools in Shiraz, southwestern Iran. The relationship between the indoor classroom dust allergens and childhood asthma was also investigated.

Materials and methods

The study protocol was approved by the Ethics Committee of Shiraz University of Medical Sciences (IR.SUMS.REC.1396.S33). Six primary schools and seven high schools were selected from each of the four educational areas in Shiraz by stratification. General information about each school, such as surface area of the school, age of school building, number of students in each class, total number of students in school, the number of classrooms and finding cockroaches during the previous year in the school was taken from the school principal.

Study subjects

The diagnosis of asthma was made according to Global Initiative for Asthma (GINA) Guidelines 2007 by an allergist in all students of the selected classes.[22] Permission for examining students was issued by the department of education in Shiraz in coordination with the principal of each school.

Sample collection

Two classrooms were selected from each school and dust samples were collected from the classrooms which were not cleaned for one week prior to sampling by a 20,000 W vacuum cleaner equipped with a Dust stream collector containing a cylindrical plastic filter (Indoor Biotechnologies, Cardiff, UK). Sampling was done at the entrance, in the center and in front of the windows in each classroom. During the fall of 2017, 48 dust samples were collected from the elementary schools (with students in the age of 6–11 years old) and 56 from the high schools (with students in the age of 12–17 years old).

Laboratory tests

After removing the filter containing the dust sample, they were kept in small zip-lock bags at 4 °C. Extraction of dust samples was started by sieving through a 345 μm mesh to remove large particles and then adding 2.0 ml PBS-T (0.05% Tween 20 in phosphate buffered saline, pH = 7.4) to take a sample weighing 100 mg (50 mg/mL). The amount of dust in mg was multiplied by 20 to give the appropriate volume of buffer in μL. The other process was mixing on a shaker, centrifuging 20 min at 2500 rpm and removing carefully of the supernatant. The supernatant was stored at −20 °C in a freezer with a sample code for future analysis of allergen concentrations.

The concentration of allergens was quantified by commercial enzyme linked immunosorbent assay (ELISA) kits (Indoor Biotechnologies, Charlottesville, USA) according to the manufacturer's instructions. To calculate the allergen concentrations per gram of dust, the standard curve was plotted from certain dilutions of each pure allergen. The concentration of allergens was expressed as ng/g of dust. The minimum detection limit of each ELISA kit was 0.7 ng/mL for Der p1, 0.8 ng/mL for Bla g1 and Fel d1 as well as 0.2 ng/mL for Der f1 and Mus m1. Information about the concentration of house dust allergens in patients' home with childhood asthma in Shiraz was adopted from our previous work.[3]

Statistical analysis

Descriptive statistics (mean ± standard deviation, frequency) were used to describe the basic features of the data in the study. Pearson Correlation was done for assessing the association between classroom dust allergens with characteristic of school and with percentage of children with asthma. Independent sample *t*-test was applied for comparing the means. All statistical analyses were done with SPSS

Table 1 Characteristics of the studied school.

Characteristic	Minimum	Maximum	Mean	SD
Space of school (m^2)	1900	20000	5551.5	4366.5
Age of school building (years)	2	54	21.9	14.7
Number of classrooms in school	8	50	16.0	10.3
Number of students in each school	64	700	355.8	155.4
Number of students with asthma in each classroom	1	5	1.8	1

Table 2 The mean concentration of five studied indoor allergens in classroom dusts.

Indoor allergens	Mean (ng/g)	SD	Minimum (ng/g)	Maximum (ng/g)
Cat (Fel d1)	0.2	0.91	0.0	7.3
Cockroach (Bla g1)	41.3	13.8	1.4	300.0
Mite (Der f1)	1.3	1.5	0.0	7.9
Mite (Der p1)	0.4	1.3	0.0	5.6
Mouse (Mus m1)	3.2	8.7	0.0	40

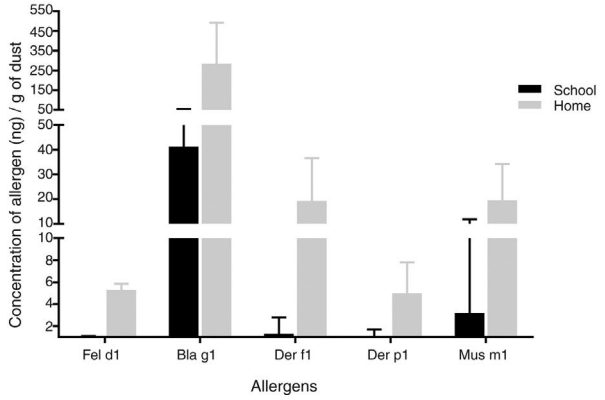

Figure 1 Concentration of classroom dust allergens in the selected schools in comparison to house dust allergens in Shiraz.

version 22 (SPSS Inc., Chicago, IL, USA), and $p \leq 0.05$ was considered statistically significant.

Results

A total of 104 dust samples from 52 schools were collected. Three dust samples were excluded because of insufficient quantity for allergen extraction. Characteristics of the studied school are shown in Table 1. Out of 101 dust samples which were analyzed: 46 were from classrooms in elementary schools and the remaining 55 were from high schools. Out of 2816 (1014 girls, 1802 boys) students in the selected classes, 180 students were involved with asthma. The concentration of the five studied indoor allergens including Fel d1, Bla g1, Der f1, Der p1 and Mus m1 in classroom dusts is shown in Table 2.

The most common allergens in collected samples were Bla g 1 (83.1%), Der f1 (51.5%), Mus m1 (45.5%), Der p1 (8.9%), and Fel d1 (7.9%). There was a positive correlation between finding cockroach in schools and Bla g1 allergen level in classrooms ($p = 0.03$).

Comparison of the mean content of five indoor allergens in school dust and home dust of children involved with asthma in Shiraz[3] is shown in Fig. 1. The results of the independent *t*-test showed the levels of all studied allergens in schools were significantly less than in homes in Shiraz ($p < 0.001$).

The level of classroom dust allergen showed no difference between primary schools and high schools. Table 3 shows a positive relationship between the number of classrooms in a school and Der f1 level ($p = 0.02$).

The effect of indoor allergen level on developing asthma in students is shown in Table 4; there was a negative relation between the number of students with asthma and Der p1 ($p = 0.04$). Exposure to Fel d1 in school was associated with increased prevalence of asthma ($p = 0.05$).

Discussion

Children spend a large amount of their time in schools where they might be exposed to high levels of perennial allergens including cat, cockroach, mite and mouse. Our study showed that cockroach allergen was the prevalent detected allergen in 83% of the schools with a mean concentration of 41 ng/g. In Malaysia, cockroach allergens was found in 59% of the classrooms with a mean level of 500 ng/g.[23] Detectable levels of cockroach allergen were found in all schools with a median level of 5.5 ng/g in Texas,[24] while the low rate of 0.7% cockroach exposure was reported in elementary schools in the Northeastern USA.[9] It seems that the prevalence of cockroach allergen in schools is widely dependent on geographic location and the weather and the high rate of cockroach allergen in our study could be attributed to the warm weather in this area.

Dust mites (Der p1 and Der f1) were detected in about 60% of our school samples; however, Der f1 was more common than Der p1. In two other studies in schools, mite allergens were found in 46.5% in the Northeastern USA and in all studied schools in Malaysia with a low level.[9,23] Dust mite tends to grow in ambient humidity, moreover, students' activities such as jumping and exercise can increase indoor air humidity levels. Furthermore, seats and books can also be important reservoirs for dust mite allergens in the

Table 3 Correlation between school characteristics and indoor dust allergen levels.

School characteristics	R & p-value	Fel d1	Bla g 1	Der f1	Der p1	Mus m 1
Space of school	R	0.16	−0.04	0.18	−0.12	−0.12
	p-value	0.15	0.74	0.93	0.29	0.27
Age of school building	R	−0.14	−0.16	−0.19	0.01	−0.14
	p-value	0.20	0.16	0.86	0.91	0.20
Number of classrooms in school	R	−0.04	0.20	0.25	−0.03	−0.08
	p-value	0.69	0.06	0.02*	0.80	0.46
Number of students in each school	R	0.11	0.08	0.05	0.007	0.15
	p-value	0.31	0.48	0.64	0.95	0.19

* $p < 0.05$; R: Pearson Correlation.

Table 4 Correlation between school dust allergen levels and prevalence of asthma in the studied schools.

	Allergens in classroom dust					
	Fel d 1	Bla g 1	Der f1	Der p1	Mus m 1	
Percentage of asthmatic students	R	0.21	−0.07	0.11	−0.22	−0.20
	p-value	0.05*	0.55	0.32	0.04*	0.07

* $p < 0.05$; R: Pearson Correlation.

classrooms. The mean concentration of Der f1 was more common than Der p1 in our schools, which is consistent with our report from homes in this area.[3] Nevertheless, the concentration of none of the studied allergens exceeded the allergic sensitization threshold (>200 ng/g).[24]

This study showed mouse allergen in 45.5% of the classrooms with a mean of 3.2 ng/g. Sheehan et al. found mouse allergen in 99.5% of studied schools with a median concentration of 900 ng/g in the Northeastern USA.[9] Feral cats are abundant in Shiraz and can be considered as a reason for decreasing the number of mice.[25] Matsui et al. reported that exposure to more than 500 ng/g of mouse allergen can induce asthma symptoms.[26]

Cat allergens were detected in 7.9% of our studied school samples, with ranges from 0 to 7.3 ng/g. This rate is less than those reported from the Northeastern USA with 94.8%,[9] France with 57%,[27] and Poland with 31.8.[28] Threshold levels of cat allergen, Fel d1, for asthma symptoms were reported $\geq$1–8 μg/g,[29] which is much higher than we found in our studied schools.

The results of this study showed lower levels of indoor allergens in schools than in homes in our area. This finding is consistent with the distribution of indoor allergens in Singapore.[30] It is noteworthy that the concentration of all studied allergens in schools and homes were less than the threshold concentrations of allergic sensitization in this area.

The concentration of Der f1 was significantly higher in schools with an extra number of classrooms. More classrooms are usually related with more student activities and poor ventilation which can influence indoor air humidity levels.

There was a negative association between Der p1 and asthma in our study; we have no explanation to this protective role of Der p1 which could be due to a chance finding. Students who were exposed to cat allergens showed increased prevalence of asthma in our study. A recent study revealed exposure to mouse allergen in schools was associated with increased asthma symptoms and decreased lung function in students in Boston.[9] The levels of all studied allergens in the settled dust of our classrooms were lower than that needed for allergic symptoms; therefore, it appears that sensitization may occur at much lower concentrations of these allergens in schools. Future school-based environmental interventions require evaluating the role of school-specific indoor allergen exposures on asthma development.

Multicenter studies could help to determine the various common allergens in schools of other cities of Iran, and we could not consider sensitization to allergens in students by skin-prick test.

This study showed considerable levels of cockroach allergens in schools and this knowledge could be used to minimize allergen in classrooms. Exposure to cat allergens played a role in induction of asthma; further research should be done to examine the relationship between allergen exposure in schools and induction of asthma.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgements

This work was supported by a grant from Shiraz University of Medical Sciences (grant number 10883). We thank the department of education and the principal of each school for their cooperation in dust collection.

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