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House Dust Mite Sensitisation as a Risk Factor for Exacerbation of Asthma in the Fall

HH ***Kim***, SJ ***Choi***, S ***Won*** , HS ***Kim***, YH ***Chun***, JS ***Yoon***, JT ***Kim***

Abstract ***Purpose:*** Exposure and sensitisation to house dust mites (HDMs) are known to aggravate asthma. We aimed to determine whether sensitisation to HDM acts as a risk factor for asthma exacerbation in fall and we also investigated whether asthma exacerbation in the fall had any distinctive features by comparing various cytokines and chemokines with other seasons. ***Method:*** We measured the levels of total immunoglobulin E (IgE), HDM-specific IgE (sIgE), and various cytokines and chemokines in those who visited the emergency department for acute asthma exacerbation and classified them according to the season. ***Findings:*** HDM-sIgE levels were higher in those presenting with asthma exacerbation in the fall. There was no difference in the levels of cytokines and chemokines between the fall and the other seasons. ***Conclusion:*** HDM sensitisation could be a risk factor for asthma exacerbation in the fall.

Key words Asthma; Child; Cytokines; House dust mite; Seasons

Introduction

Acute asthma exacerbation is a reversible state of bronchoconstriction and decreased airflow induced by a trigger that aggravates the inflammation of the airways. In the northern hemisphere with a temperate climate, fall is the most common season for acute asthma exacerbation and various aggravating factors such as respiratory infections, climatic stimuli, and allergen exposures have been identified.[1-5] Previous studies found that house dust mite (HDM) exposure can cause airway hyperresponsiveness and exacerbate asthma.[6,7] A seasonal variation exists in the indoor HDM concentration which is the highest in the fall, which correlates with the incidence of asthma exacerbation.[8-10]

Until now, many studies have investigated the effect of HDM exposure on incidence of acute asthma exacerbation but few have addressed the issue whether HDM sensitisation acts as a risk factor for asthma exacerbation stratified by season. In our study, we aimed to determine whether sensitisation to HDM acts as a risk factor for asthma exacerbation in fall. We also investigated whether asthma exacerbation in the fall had any distinctive features by comparing various cytokines and chemokines with other seasons.

Department of Pediatrics, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea

HH **Kim** (金顯熙)* *MD, PhD*
S **Won** (元雪美) *MS*
HS **Kim** (金煥洙) *MD, PhD*
YH **Chun** (全倫弘) *MD, PhD*
JS **Yoon** (尹鍾緖) *MD, PhD*
JT **Kim** (金振宅) *MD, PhD*

Department of Pediatrics, Asan Medical Center Children's Hospital, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

SJ **Choi** (崔乘準)* *MD*

**HH Kim and SJ Choi contributed equally to this work.*

Correspondence to: Dr HS **Kim**

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Methods

Setting

We enrolled children aged 3 to 14 years who visited the emergency department (ED) of Seoul St. Mary's Hospital

due to acute asthma exacerbation. The diagnosis of asthma was based on history of recurrent wheezing or cough without a cold in the preceding 12 months with evidence of bronchial hyperresponsiveness upon methacholine challenge (PC_{20} ≤8 mg/mL) or at least 12% reversibility of FEV_1 after inhalation of a short-acting bronchodilator. With younger children (under 5 years of age) or when such evidence was not readily available: diagnosis was based on a history of three or more episodes of at least two of the following – persistent day time or night time cough, physician-diagnosed non-febrile wheezing, recurrent episodes of shortness of breath or exercise-induced shortness of breath and cough. Exclusion criteria were (i) age younger than 3 years; (ii) life-threatening exacerbation attack (FEV_1% predicted) <30% or a condition necessitating intubation owing to organ failure or disordered consciousness); (iii) history of admission to intensive care unit (ICU) due to severe acute exacerbations; (iv) major comorbidities of the heart or lungs, such as congenital heart disease, viral myocarditis, bronchiectasis and severe pulmonary infection; and (v) allergy to any component of drugs.

The children were classified into different severity groups based on initial findings upon visiting ED (Table 1).[11] All children were treated in accordance with the standardised treatment protocol, and residual sera from blood sampled during the course of treatment were utilised to measure white blood cell count (WBC), absolute neutrophil count (ANC), eosinophil count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), total IgE, *Dermatophagoides farinae*-specific IgE (Der f-sIgE), *Dermatophagoides pteronyssinus*-specific IgE (Der p-sIgE), eosinophil cationic protein (ECP), cytokine, and chemokine (e.g., interleukin [IL]-1β, -2, -5, -6, -8, granulocyte-colony stimulating factor [G-CSF], interferon [IFN]-γ, and induced protein [IP]-10) levels. Peripheral blood samples were obtained within 24 hours of presentation, and plasma were stored at -80°C. The study was approved by the Institutional Review Board of Seoul St. Mary's Hospital (protocol no: KC13TISI0075) and informed consent was obtained from the patients and their parents.

Study Population

A total of 223 children between 3 to 14 years of age visited the ED due to acute asthma exacerbation January 2008 to December 2013. Blood samples were collected from 108 children during the course of treatment. Of these, 53 children were excluded due to their parents' refusal to participate or failure to obtain residual serum and 55 children were enrolled in the study (Figure 1). Patients were then divided into 2 groups – those who visited the ED in the fall

Table 1 Severity scale of asthma exacerbation

Mild	Moderate	Severe	Respiratory arrest imminent
Breathlessness		All 3 positive:	
	1. While walking	1. While talking	1. While at rest
	2. Can lie down	2. Difficulty feeding	2. Sits upright due to dyspnoea
		3. Difficulty lying down	
Talks in	Sentences	Phrases	Words
Alertness	Calm	Agitated due to respiratory distress	Drowsy or confused
RR >30/min AND HR >120 beats/min	No	Yes	
Chest retraction	No	Yes	Paradoxical thoraco-abdominal movement
Wheezing	Moderate, often only end-expiratory	Loud	Absence of wheeze
SpO_2 (room air)	>95%	91-95%	<90%
PaO_2 (room air)	>75 mmHg	60-75 mmHg	<60 mmHg
$PaCO_2$ (room air)	<45 mmHg	–	>45 mmHg

HR, heart rate; $PaCO_2$, arterial carbon dioxide tension; PaO_2, arterial oxygen tension; RR, respiratory rate; SpO_2, arterial oxygen saturation measured with a pulse oximeter

Normal rates of breathing in awaking children: <2 months: <60/min; 2-12 months: <50/min; 1-5 years old: <40/min; 6-8 years old: <30/min. Normal pulse rates in awaking children: 2-12 months: <160/min; 1-2 years old: <120/min; 2-8 years old: <110/min. PEF: peak expiratory flow; PaO_2: partial pressure of oxygen in arterial blood; SaO_2: saturation of O_2 in artery.

(fall group) versus those who visited in seasons other than the fall ("other season" group).

Measurement of Serum Total IgE, HDM-sIgE, ECP, Cytokine, and Chemokine Levels

Total IgE, HDM-sIgE, and ECP levels were measured using a fluoroenzyme immunoassay kit (Phadia AB; Uppsala, Sweden). Cytokine and chemokine levels were measured using the Bio-Plex Pro Human Cytokine Assay (Bio-Rad Laboratories, Inc.; Hercules, California, USA).

Statistical Analysis

All analyses were conducted using the SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Comparison of acute childhood asthma exacerbation in the fall (from September to November) versus other seasons (from December to August) was tested using the Chi-square statistics, Fisher's exact test, student's t-test, Wilcoxon rank-sum test, and logistic regression. For all analyses, P value <0.05 was considered statistically significant.

Results

Participant Characteristics

A total of 55 children (35 boys and 20 girls) participated in the study. The children's age ranged from 3 to 14 years and their mean age was 6.0±2.8 years. When categorised according to asthma exacerbation severity, 19 children were included in the mild group, 35 in the moderate, and 1 in the severe group (Table 2).

Clinical Manifestation and Laboratory Findings in the Fall and "Other Season" Groups

There was no significant difference in the rate of a previous diagnosis of asthma or the presence of fever at the ED. More children with asthma of moderate severity were included in the "other season" than the fall group (P<0.05). There was no statistically significant difference in total WBC, ANC, ESR, or CRP between the 2 groups (Table 3).

Allergy-related Markers in the Fall and "Other Season" Groups

There was no significant difference in the mean eosinophil counts or the number of children who presented with eosinophilia (>3% of total WBC) between the 2 groups. However, more than half of children presented with eosinophilia in both groups. Similarly, the number of children sensitised to Der f and Der p and the mean levels of HDM-sIgE were significantly higher in the fall group. After univariate analysis, Der f-sIgE and Der p-sIgE were significant and after multivariate analysis, adjusting for total IgE, Der f-sIgE and Der p-sIgE remained as significant risk factors for exacerbation in fall. Although ECP levels did not show a statistically significant difference between the fall and "other season" groups, both groups showed levels exceeding 16 μg/L (Table 4).

Cytokines and Chemokine Levels in the Fall and "Other Season" Groups

Cytokines (IL-1β, -2, -5, -6, -8, G-CSF, and IFN-γ) and chemokine (IP-10) levels were not significantly different between the two groups (Table 5).

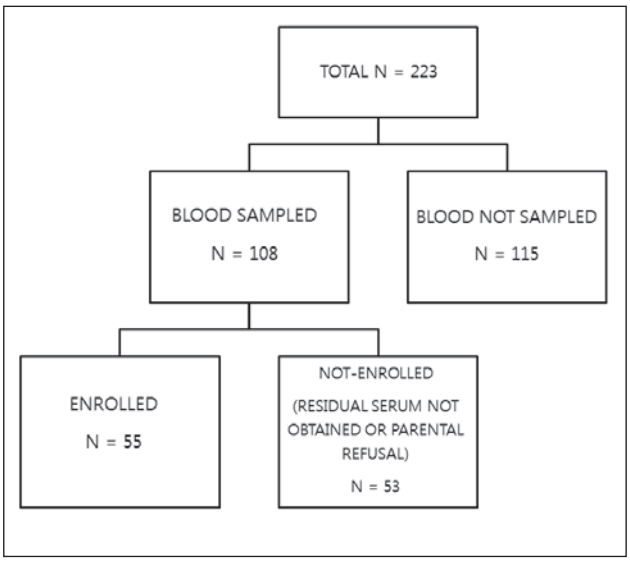

Figure 1 Flow chart of the study population. A total of 223 patients visited the emergency room during the study period. Among them, the blood of 108 patients was sampled. Of the 108, 53 were excluded, leaving 55 final study participants.

Table 2 Participant characteristics (n=55)

Characteristics	Value
Sex	
Male	35 (63.6)
Female	20 (36.4)
Age (years)	6.0±2.8
Severity of asthma exacerbation	
Mild	19 (34.5)
Moderate	35 (63.6)
Severe	1 (1.8)

Data are presented as n (%) or as means ± standard deviation.

Discussion

We found that more children visit ED with acute asthma exacerbation in fall as previously known. Our laboratory findings showed that more children were sensitised to HDM and the mean level of HDM-sIgE was significantly higher in children of the fall group. More than half of the children presented with eosinophilia with higher ECP levels exceeding the reference value in both groups. There was no significant difference in levels of cytokines or chemokine between the two groups.

The fact that eosinophilia and elevated ECP levels were observed in children with acute asthma exacerbation was in concordance with previous studies.[12,13] A study showed that 40% of patients hospitalised for asthma exacerbation had eosinophilia.[12] This was similar to our study in which we found that 50% of the patients had eosinophilia.

Many studies found that exposure to HDMs aggravates asthma and cause airway hyperresponsiveness.[7,14] Indoor HDM allergen concentration is known to vary depending on the seasons, and its rise correlates with airway hyperresponsiveness.[7,15] Similar findings have been observed in Korea.[9,10] For the first time in literature, our study suggests that a causal relationship exists between HDM sensitisation and asthma exacerbation in the fall and findings were significant after adjusting for total IgE.

Table 3 Clinical manifestation and laboratory findings in the fall and "other season" groups

	Fall (n = 31)	**Other season (n = 24)**	**P value**
Sex			
Male	18 (58.1)	17 (70.8)	0.329
Female	13 (41.9)	7 (29.2)	
Age (years)	6.3±0.5	5.5±0.6	0.238
Fever			
No	27 (87.1)	21 (87.5)	0.999
Yes	4 (12.9)	3 (12.5)	
Severity of asthma exacerbation			
Mild	16 (51.6)	3 (12.5)	0.003
Moderate	14 (45.2)	21 (87.5)	
Severe	1 (3.2)	0 (0.0)	
Total WBC (cells x 10^4)	12,523.9±770.5	11,642.6±1,061	0.233
<4,000	–	–	0.304
4,000-12,000	13 (46.4)	14 (60.9)	
>12,000	15 (53.6)	9 (39.1)	
ANC (cells x 10^4)	8,933.8±816.9	8,084.8±1,124.8	0.268
<1,500	1 (3.6)	0 (0.0)	0.999
≥1,500	27 (96.4)	23 (100.0)	
ESR (mm/h)	10.7±1.4	19±3.5	0.098
≤20	23 (82.1)	14 (63.6)	0.139
>20	5 (17.9)	8 (36.4)	
CRP (mg/L)	0.5±0.1	0.7±0.2	0.957
≤1	26 (86.7)	18 (78.3)	0.478
>1	4 (13.3)	5 (21.7)	

Data are presented as n (%) or as means ± SEMs.

ANC, absolute neutrophil count; CRP, c-reactive protein; ESR, erythrocyte sedimentation rate; WBC, white blood cell count

P value refers to the difference between the fall and "other season" groups and was calculate by the Chi-square statistics, Fisher's exact test, student's t-test, or Wilcoxon rank-sum test.

Table 4 Allergy-related markers in the fall and "other season" groups

	Fall (n=31)	Other season (n=24)	OR (95%CI)	P value	Adjusted OR* (95%CI)	P value
Eosinophil (%)						
<1	7 (25.0)	5 (21.7)	1.20 (0.25-5.84)	0.821		
1-3	7 (25.0)	6 (26.1)	ref			
>3	14 (50.0)	12 (52.2)	1.00 (0.26-3.80)	>0.999		
Total IgE (kU/L)	780.2±118.4	385.8±139.7	2.25 (0.99-5.13)	0.053		
Der f s-IgE (kU/L)						
<0.35	2 (6.5)	10 (41.7)	ref		ref	
≥0.35	29 (93.5)	14 (58.3)	10.36 (2.00-53.75)	0.005	7.68 (1.41-41.81)	0.018
Der p s-IgE (kU/L)						
<0.35	2 (6.5)	10 (41.7)	ref		ref	
≥0.35	29 (93.5)	14 (58.3)	10.36 (2.00-53.75)	0.005	7.68 (1.41-41.81)	0.018
ECP (μg/L)						
<2.3	1 (3.3)	0 (0.0)		0.986		
2.3-16	4 (13.3)	3 (13.0)	ref			
>16	25 (83.3)	20 (87.0)	0.94 (0.19-4.68)	0.937		

*Adjusted for total IgE

Data are presented as N (%) or as means ± SEMs and median (range).

CI, confidence interval; OR, odds ratio; Der f-sIgE, *Dermatophagoides farinae*-specific immunoglobulin E; Der p-sIgE, *Dermatophagoides pteronyssinus*-specific immunoglobulin E; ECP, eosinophil cationic protein; IgE, immunoglobulin E

P value refers to the difference between the fall and "other season" groups and was calculate by the Chi-square statistics, Fisher's exact test, student's t-test, or Wilcoxon rank-sum test.

Table 5 Serum levels of cytokines and chemokine

	Fall (n=31)	Other season (n=24)	P value
IL-1β (pg/mL)	3.6±0.9; 1.7 (0.6-20.7)	5.0±1.5; 2.3 (0.7-35.6)	0.137
IL-2 (pg/mL)	7.1±1.5; 4 (0-25.4)	6.5±1.7; 3.3 (0-34.3)	0.497
IL-5 (pg/mL)	7.1±1.0; 4.9 (1.2-22.8)	7.8±1.5; 6 (0.9-29.3)	0.899
IL-6 (pg/mL)	23.1±3.1; 19.5 (4.3-82.7)	20.6±2.8; 19.7 (4.7-52.4)	0.738
IL-8 (pg/mL)	129±34; 45.5 (7.8-777.3)	83.6±37.6; 24.1 (5.4-770.0)	0.119
G-CSF (pg/mL)	24.1±3.3; 17.4 (0.7-77.3)	27.3±5.9; 21.3 (1.7-136)	0.932
IFN-γ (pg/mL)	46.2±13.4; 20.6 (0-348.5)	31.5±.8.4; 20.9 (0-178.6)	0.534
IP-10 (pg/mL)	1310.2±168.3; 993.6 (217.2-3860.4)	1242.5±153.2; 1029.5 (230.5-3236.1)	0.869

Data are presented as as means ± SEMs; median (range).

G-CSF, granulocyte-colony stimulating factor; IFN-γ, interferon gamma; IL, interleukin; IP-10, inducible protein-10

P value refers to the difference between the fall and "other season" groups and was calculate by the Chi-square statistics, Fisher's exact test, student's t-test, or Wilcoxon rank-sum test.

We examined various cytokines and chemokine reflecting Th1, Th2 type inflammation, proinflammatory cytokines but there was no difference in levels of cytokines and chemokine in those who with asthma exacerbation stratified by season. A previous study reported that VEGF, TNF-α and IL-1β was significantly higher in those who experience asthma exacerbation[16] and another animal study found that IL-33 drives activation of alveolar macrophages and airway inflammation acute exacerbation of asthma.[17] Therefore we could conclude that cytokines play an important role in asthma exacerbation but not different according to season.

The strong point of our study lies in the fact that we could elucidate the causal relationship between HDM-sIgE levels and asthma exacerbation in the fall by collecting serum samples when the patients presented with symptoms of asthma exacerbation. This distinguishes our study from prior studies that utilised data and samples of selected patients with a past history of asthma irrespective of the presence of symptoms.[2,7]

The main limitation of our study is the incomplete collection of serum samples. Approximately half of those who visited the ED because of asthma exacerbation did not participate in the study because of their parents' refusal. In order to determine whether the study population was representative of the total population, we compared the subject characteristics between the study population and those who didn't participate and we found that there was no significant difference. Therefore we could presume that the study population was representative of the total population. We also assessed the power of the size of the study population. The post hoc power calculation based on our actual data of Table 4 revealed that an overall sample size of 55 subjects achieves 88% power at a 0.05 significance level to detect an effect size (Cohen's W) of 0.4228 using Chi-Square test. (computed as Cohen's W=square root[chi-square/N]); corresponding to a Cohen's W of 0.50, i.e., a medium effect size). Secondly, we did not include other triggers in our study. For instance, respiratory virus (typically rhinovirus) infection is an important trigger for asthma exacerbation in the fall.[1,18] Because most of the children visiting the ED were discharged after appropriate treatment, and since the respiratory virus polymerase chain reaction results were only reported after several days, the cost-effectiveness of this test was judged to be low. Hence, we could not analyse the association of asthma exacerbation in the fall with factors, such as tobacco smoke exposure, pollution levels, and seasonal climatic changes other than HDM exposure. Thirdly, we only measured a limited number of serum markers. Recent studies identified novel serum markers associated with asthma development and exacerbation.[19,20] However, in this study, we could not analyse markers mentioned in the recent literature. Fourthly, most of the patients included were mild or moderate asthma exacerbation. During the study period, there was only one patient with severe exacerbation and there were no patients with severe asthma exacerbation needing ICU care. Finally, we did not perform any follow-up studies of the markers. Therefore, it was not possible for us to differentiate whether the seasonal variability of serum markers was merely a transient phenomenon due to asthma exacerbation.

In conclusion, children with high HDM-sIgE levels can be regarded as a high-risk group for acute asthma exacerbation in the fall, and measurement of HDM-sIgE can help in applying individual therapy to prevent acute exacerbation of asthma.

Further study by analysing various serum markers by season, level of pollution, seasonal climatic changes and viral etiologies will be needed to determine the pathogenesis of seasonal variation of asthma exacerbation.

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Declaration of Interest

We declare that we have no conflict of interests.

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