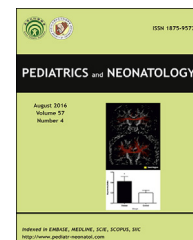


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Original Article

Prevalence of airway hyperresponsiveness and its seasonal variation in children with asthma

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Received May 21, 2017; received in revised form Nov 12, 2017; accepted Jan 2, 2018

Available online ■ ■ ■

Key Words

airway hyperresponsiveness;
childhood asthma;
methacholine

Background: Airway hyperresponsiveness (AHR) is a key feature of asthma and can be detected using various bronchoprovocation tests. In pediatric populations, the percentage of a positive methacholine challenge test (MCCTs) in children with asthma varies among studies, and some have reported seasonal variability. However, these studies have mostly been conducted in temperate regions.

This study evaluated the prevalence of AHR to methacholine and its seasonal variation in asthmatic children in Taiwan, a subtropical country.

Methods: A total of 276 children with asthma and their MCCT results were retrospectively reviewed. All were diagnosed with asthma and received asthma controllers regularly. They were assigned to four season groups depending in which season MCCTs were administered, with seasons categorized by the Central Weather Bureau of Taiwan. Subgroup analyses, including for sex, age, and atopy level, were compared for seasonal difference.

Results: The prevalence of methacholine hyperresponsiveness was 70.7% ($n = 195$), and the children who were younger and had higher total serum IgE were more sensitive to methacholine ($p = 0.019$ and $p < 0.005$, respectively). No significant difference in AHR prevalence among seasons was observed ($p = 0.480$). The percentage of borderline, mild, and moderate severity of MCCT results was almost equally distributed among the seasons. In subgroup analysis, the children with a higher IgE level (≥ 75 th percentile of all data) had a higher proportion of positive MCCTs in summer (88.6%, $p = 0.016$).

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<https://doi.org/10.1016/j.pedneo.2018.01.005>

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Please cite this article in press as: Huang S-J, et al., Prevalence of airway hyperresponsiveness and its seasonal variation in children with asthma, Pediatrics and Neonatology (2018), <https://doi.org/10.1016/j.pedneo.2018.01.005>

Conclusion: In total, 70% of the children with asthma in Taiwan had AHR to methacholine, which varied among seasons. Children with a higher total serum IgE level may be more seasonally dependent, particularly in summer.

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1. Introduction

Bronchial asthma is a chronic inflammatory respiratory disease with an increasing prevalence worldwide. The diagnosis of asthma is based on clinical history and presentation, and airway hyperresponsiveness (AHR) is a key feature of asthma. AHR can be detected using direct or indirect challenge tests. Methacholine acts directly on the airway smooth muscle receptor and has both high sensitivity and specificity compared with other bronchoprovocation tests in asthma diagnosis.¹ However, in the pediatric population, the percentage of a positive methacholine challenge test (MCCT) has varied among studies but has mostly been above 70%^{2,3} compared with those in adults.⁴

Viral infection, allergens, and other environmental events cause temporary airway inflammation and contribute to the short-term variation of AHR.⁵ Studies have reported seasonal variability of AHR in both methacholine and exercise challenge tests.^{6–9} Temperature, humidity, viral infection, and other causes could partially explain the seasonal difference.⁶ However, these studies have mostly been conducted in temperate regions, and their target population was patients who suspected that they had asthma. Because age, sex, and atopy have been proven to have an effect on AHR,^{10,11} whether these factors affect the seasonal variation of AHR must be ascertained.

We evaluated AHR in a pediatric population by reviewing MCCTs performed on Taiwanese children.

2. Methods

2.1. Study populations

All the patients enrolled in this study had received a diagnosis of asthma from clinical physicians in a tertiary medical center in Northern Taiwan. The patients had clinical symptoms and a disease course compatible with asthma and responded to bronchodilators. We retrospectively reviewed all their MCCTs performed during 2013–2015. All the patients received regular asthma controllers before MCCTs. Age, sex, duration since asthma diagnosis, the total serum IgE level, and the MCCT date were recorded. Only the result of the first test was considered in those who had several MCCT results. The patients who had a total serum IgE level of >77 KU/L were defined as having “serum atopy,” and the reference value is based on a cohort study published earlier by our institute.¹²

2.2. Seasonal difference

Because Taiwan is located in a subtropical area, the seasons are classified according to the definition of the Central Weather Bureau, Taiwan: March 1 to May 31 is spring, June 1 to August 31 is summer, September 1 to November 30 is fall, and December 1 to February 28 is winter. The average seasonal temperatures between 1981 and 2010 were 21.8 °C, 28.8 °C, 24.5 °C, and 16.8 °C for spring, summer, fall, and winter, respectively. The patients were divided into four groups according to MCCT date. Subgroup analysis included sex, age, and atopy level, which were compared for seasonal difference.

2.3. MCCT

MCCTs were performed according to the five-breath dosimeter protocol published by the American Thoracic Society in 1999.¹³ Prior to the MCCT, all the patients were instructed to stop using their short-acting B₂-agonist bronchodilators, leukotriene antagonist (LTA), and inhaled corticosteroid for 1 day. The MCCT was postponed for at least 2 weeks if the patient had an acute asthma exacerbation or respiratory tract infection. The patients were instructed to inhale the diluted methacholine nebulizer in a serial concentration (0.0625, 0.25, 1, 4, and 16 mg/mL) and to perform spirometry 5 min after each nebulization. When the FEV₁ fell by more than 20% from the baseline, we stopped further inhalation and calculated the methacholine concentration in accordance with the linear formula provided by the American Thoracic Society guidelines, termed “PC₂₀.” This represented AHR. If the FEV₁ did not substantially decrease despite the high concentration (PC₂₀ > 16 mg/mL), the result was negative. AHR was defined as borderline, mild, and moderate when PC₂₀ was between 4 and 16 mg/mL, between 1 and 4 mg/mL, and below 1 mg/mL, respectively.

2.4. Statistical analysis

Continuous variables were analyzed using the independent t test and one-way analysis of variance, whereas categorical variables were examined using the chi-square test and Fisher’s exact test. Statistical significance was defined as $p < 0.050$. All statistical analyses were performed using SPSS version 22.

This study was approved by the Institutional Review Board of Linkou Chang Gung Memorial Hospital, Taiwan (IRB No. 101-1138A3).

Table 1 Demographic features and AHR.

	Total (%)	AHR (%)	No AHR (%)	P
Total	276	195 (70.7%)	81 (29.4%)	
Male	173 (62.7%)	119 (68.8%)	54 (31.2%)	0.378
Current Age	7.45 ± 2.34	7.23 ± 2.33	7.96 ± 2.3	0.019
Serum Atopy	216 (78.3%)	154 (71.3%)	62 (28.7%)	0.660
Total IgE KU/L	478.92	559.62	282.02	<0.005
Duration of Asthma diagnosis (year)	2.06 ± 1.4	2.02 ± 1.4	2.16 ± 1.3	0.420
Medication ^a				
Montelukast	195 (70.7%)	142 (72.8%)	53 (27.2%)	0.369
ICS	12 (4.3%)	6 (50%)	6 (50%)	
Montelukast + ICS/LABA	18 (6.5%)	12 (66.7%)	6 (33.3%)	
ICS + LABA	51 (18.5%)	35 (68.6%)	16 (31.4%)	

AHR = airway hyperresponsiveness, defined as PC20 ≤ 16 mg/mL.

Serum atopy = total IgE > 77 kU/L.

^a Medication used at the time when the lung function test was performed.

3. Result

All MCCT results obtained from 276 children were reviewed. In total, 62% of the population was male ($n = 173$), and the average age was 7.45 years (Table 1). The average duration since asthma diagnosis was 2.06 (± 1.4) years, and the majority of the children (70.7%) received LTA as the only controller. Furthermore, 78% ($n = 216$) of the children fulfilled the serum atopy criterion (total serum IgE level >77 kU/L), and the average total serum IgE level was 478.92 kU/L (± 691.8 kU/L).

The prevalence of methacholine-induced AHR was 70.7% ($n = 195$) in our study population. Compared with the children without AHR, the children who responded to methacholine were slightly younger (7.23 years in AHR+vs. 7.96 years in AHR−, $p = 0.019$) and had a higher IgE level (559.62 kU/L in AHR+vs. 282.02 kU/L in AHR−, $p < 0.005$). Additionally, a negative correlation was found between the IgE level and PC20 in the patients with AHR ($R = 0.02$, $p = 0.045$). Sex, current medications, and the duration

since asthma diagnosis did not differ between the patients with and without AHR ($p = 0.420$, 0.369, respectively).

The patient characteristics were similar in the four season groups (Table 2). The seasonal distribution of MCCTs is shown in Table 3, and the fewest children underwent MCCT during winter ($n = 30$, 10.9%). Methacholine-induced AHR prevalence was the highest (80.0%) during winter, but the difference among seasons was not significant. Furthermore, when assessing these children who had positive MCCT results, no significant seasonal change in mean PC20 was observed. The severity of AHR was almost equally distributed among the borderline, mild, and moderate levels, even between different seasons (Fig. 1).

In the subgroup analysis (Table 4), we analyzed whether sex, age, serum atopy, and the total serum IgE level were related to methacholine hyperreactivity among seasons. The results were mostly nonsignificant. The older children (≥ 6.7 years) and the children with a total serum IgE level above the 75th percentile (612.25 kU/L) had a higher proportion of positive MCCTs in fall and summer, respectively.

Table 2 Patient characteristics in four season groups.

	Spring (%) Mar–May	Summer (%) Jun–Aug	Autumn (%) Sep–Nov	Winter (%) Dec–Feb	p
Total	34 (12.3%)	146 (52.9%)	66 (23.1%)	30 (10.9%)	
Male	23 (67.6%)	90 (61.6%)	41 (62.1%)	19 (63.3%)	0.932
Age	7.79 ± 2.4	7.44 ± 2.4	7.37 ± 2.0	7.23 ± 2.7	0.792
Serum Atopy ^a	28 (82.4%)	115 (78.8%)	51 (77.3%)	22 (73.3%)	0.844
Total IgE KU/L	446.4	526.3	437.0	431.0	0.843
Duration of asthma diagnosis (year)	1.85	2.11	2.18	1.8	0.470
Medication					
LTA	21 (61.8%)	106 (72.6%)	47 (71.2%)	21 (70%)	0.185
ICS	3 (8.8%)	7 (4.8%)	2 (3%)	0	
LTA + ICS/LABA	4 (11.8%)	5 (3.4%)	4 (6.1%)	5 (16.7%)	
ICS + LABA	6 (17.6%)	28 (19.2%)	13 (19.7%)	4 (13.3%)	

LTA: leukotriene antagonist.

ICS: inhaled corticosteroid.

LABA: long-acting beta agonist.

^a IgE level > 77 kU/L as serum atopy.

Table 3 Prevalence of methacholine-induced AHR obtained in different seasons.

Season	No. of AHR N (%) MCCT	P	PC20 in P AHR (+)
Spring Mar–May	34 22 (64.71%)	0.480	3.53 0.720
Summer Jun–Aug	146 105 (71.92%)		3.00
Autumn Sep–Nov	66 44 (66.67%)		3.66
Winter Dec–Feb	30 24 (80.0%)		3.20
Total	276 195		3.24

4. Discussion

Although AHR is a key feature of bronchial asthma and assists in diagnosis,⁵ the prevalence varies from 52% to 90% among studies.^{3,4,14} This is because AHR in children with asthma is unstable. Its presence can be affected by the chosen modality, such as age group, country, medication, or acute infection.^{5,15} For those sensitive to methacholine provocation, their response to indirect stimuli, such as mannitol or exercise, can be negative instead. In our study, 70.7% of the children had AHR to methacholine, and they were younger than the patients without AHR. Many studies have suggested that younger children are more sensitive to methacholine because their lung functions continue to increase until adolescence.¹⁶ Younger age of asthma onset, parenteral asthma, and second-hand smoke exposure are reported variables that affect the presence of AHR in children with asthma.¹¹

The frequency and degree of AHR are associated with atopy severity in the general population, but the correlation in the asthmatic group remains controversial.^{17,18} We found that higher total serum IgE levels were associated with higher methacholine hyperreactivity prevalence but failed to determine a relationship between serum atopy (total IgE > 77 kU/L)¹² and AHR. In the literature, a higher prevalence of methacholine-induced AHR (PC20 < 4 mg/mL) was found in patients with atopic asthma compared with their nonatopic partners in 214 ten-year-old current wheezers.¹⁹ Another study on 136 school-aged children with asthma also reported a higher prevalence of methacholine-induced AHR in patients with atopy than those without.² A retrospective study comparing the relationship between the degree of atopy and response to methacholine found increased

methacholine reactivity in patients with a higher number of positive skin-prick responses.²⁰ However, "atopy" was defined as a positive skin-prick test in all the aforementioned studies. The total serum IgE level was positively associated with skin-prick test reactivity.²¹ For conflicting reports stating no association existed between atopy and methacholine-induced AHR, several postulated explanations have been offered. First, variables other than atopy may influence the presence of AHR, such as younger age, earlier symptom presentation, and parenteral allergy.¹¹ Second, patients might be specifically hyperresponsive to a particular allergen rather than have atopy itself.²² For example, sensitization to indoor allergens was related to increased AHR more than sensitization itself.^{23,24} Third, the stimulus types used to provoke AHR have led to varying results in the literature.^{25–27} The AHR frequency in response to methacholine was similar in asthmatics with and without atopy in some studies, whereas AHR in response to indirect stimuli (i.e., mannitol and adenosine 5'-monophosphate) was correlated significantly with atopy in children. Methacholine complications directly cause airway narrowing by action on airway smooth muscles receptors, and because younger children have smaller airways, they may have a higher prevalence of AHR to methacholine even if they are nonatopic.²⁷ AHR in response to indirect stimulus is believed to produce a more positive correlation with the underlying airway inflammation because it causes bronchoconstriction through the degranulation of mast cells and inflammatory mediator release.

Our study has highlighted that the prevalence and severity of methacholine-induced AHR in children with asthma may not be influenced by seasons in Taiwan, a subtropical country. This result conflicts with those of studies conducted in temperate regions.^{7–9} Joseph et al. reported a 22.4% reduction in positive MCCTs in summers compared with those performed in the rest of the year in children suspected of having asthma but not in adults. Other studies have reported a higher proportion of positive MCCTs in winter and spring.⁷ Factors contributing to the seasonal variability of MCCTs are similar to the triggers of asthma, including temperature, humidity, pollen exposure, mite sensitization, and viral infection. Hay fever worsens in spring in North America, Europe, and Japan, whereas pollen allergies are less common in Taiwan.²⁸ Most children with asthma in Taiwan are sensitive to mites,²⁹ which usually survive in humid environments. The weather is humid all

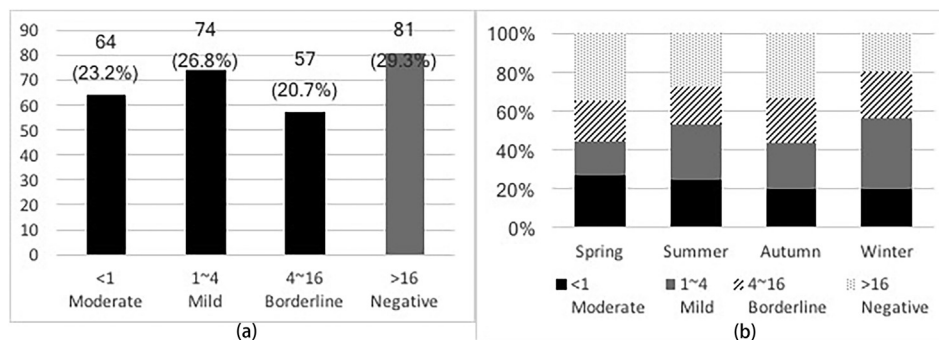


Figure 1 a. Number of patients (%) with negative, borderline, mild, or moderate AHR. b. Prevalence of methacholine-induced AHR and severity in different seasons; no significant difference in severity distribution among seasons.

Table 4 Prevalence of methacholine-induced AHR in four seasons with patients of different sex, age, and atopy status.

	Spring N (%)	Summer N (%)	Autumn N (%)	Winter N (%)
Male	16 (69.9%)	61 (67.8%)	26 (63.4%)	16 (84.2%)
Female	6 (54.5%)	44 (78.6%)	18 (72.0%)	8 (76.7%)
<i>P</i>	0.459	0.178	0.593	0.641
Age ≤ 6.7 year ^a	10 (71.4%)	58 (72.5%)	29 (80.6%)	16 (88.9%)
Age > 6.7 year ^a	12 (60.0%)	47 (71.2%)	15 (50.0%)	8 (66.7%)
<i>P</i>	0.380	0.500	0.009	0.150
No serum atopy ^b	3 (50.0%)	22 (64.7%)	10 (66.7%)	5 (62.5%)
Serum atopy ^b	19 (67.9%)	23 (74.2%)	34 (66.7%)	19 (86.4%)
<i>P</i>	0.640	0.830	1.000	0.300
• IgE 25th percentile ^c	3 (50.0%)	23 (69.7%)	10 (66.7%)	7 (70.0%)
<i>P</i>	0.641	0.826	1.000	0.372
• IgE 75th percentile ^c	6 (75.0%)	31 (88.6%)	11 (68.8%)	5 (100%)
<i>P</i>	0.681	0.016	1.000	0.553

^a 6.7 years is the median age of the total population.

^b IgE level > 77 kU/L as serum atopy.

^c IgE 25th percentile of the total population: 83.35 kU/L compared with the rest of the group; 75th percentile: 612.25 kU/L compared with the rest of the group.

year round in Northern Taiwan, and the average humidity over the past 10 years was 76.6% (73.0%–80.6%, Taipei weather station) according to published data provided by the Central Weather Bureau, Taiwan (<http://www.cwb.gov.tw>). In winter, the incidence of respiratory tract infections gradually increases, but unlike in temperate climates, the clear-cut seasonality of respiratory viral infections in Taiwan, such as influenza virus, adenovirus, and respiratory syncytial virus, is less.^{30,31} Although the rate of positive MCCTs is the highest in winter (80%) in our study, the result did not reach statistical significance ($p = 0.480$). Corresponding to previous studies, we speculate that our result contradicts others because Taiwan is located in a subtropical region, where seasonal differences in temperature, humidity, and respiratory viral infection are less obvious compared with those in countries in temperate regions. In addition, instead of seasonal allergies, sensitization to perennial allergens is more common in Taiwan.

In the subgroup analysis for seasonal variability, a higher proportion of positive MCCTs was found in the older children (aged > 6.7 years, the median age) in fall and in the children with the highest quartile of the total serum IgE level in summer. We reviewed the literature but failed to uncover any satisfactory explanation for the higher prevalence of hyperresponsiveness. Although the MCCTs of children are reported to be more seasonally dependent than in adults, a previous study did not compare the seasonal variability for different age groups of children.⁹ Studies have shown that AHR increases during pollen seasons in subjects with pollen allergies.³² The increase in house dust mites in fall also contributed to more severe AHR in van der Heide's study.³³ Therefore, we postulated that children with the highest IgE level exhibited more AHR in summer because these children may be sensitive to certain allergens abundant in summer. One in five children in Taiwan are sensitive to cockroaches, and a study reported that cockroach presence was related to asthma attacks in summer.^{29,34} However, we could not demonstrate the correlation because a specific IgE level in relation to cockroaches was not obtained.

The major limitation of this study is that the prevalence of positive MCCTs was obtained from a cross-sectional survey; thus, the seasonal variability of individuals must be identified in a longitudinal follow-up study. Additionally, we did not obtain the results of specific serum IgE to mites, cockroaches, or any aeroallergen, which may correlate more with different seasons. The difference in the sample size among the four seasons may result in bias in our study. The incidence of acute exacerbation and asthma related-hospitalization increases after August and peaks in the winter season in Taiwanese children. March–April is another period associated with increased asthma attacks.^{35,36} By contrast, asthma control is more stable during summer. Thus, clinicians tend to perform more MCCT follow-ups during this period. This explains the smallest sample size in the winter group. Last, the timing of the lung function test is also a study limitation. Among those diagnosed with asthma at a younger age, although they constitute a small proportion, their first lung function test may be delayed until the age of 6 years, leading to a varying duration in asthma diagnosis in our study population.

In conclusion, we reported that 70.7% of all the children with asthma in Taiwan responded to methacholine, whereas younger age and a higher IgE level were associated with positive MCCTs. However, no seasonal variability in either the prevalence or severity of methacholine-induced AHR was found in our study. The probability of detecting AHR is equal in all four seasons.

Conflicts of interest

None.

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