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Changes of Airway Reactivity after *Mycoplasma Pneumoniae* Infection in Children: A Study for Early Precautions against Pediatric Asthma

Han Zhang, Gaomei Lv, Yunxiao Shang, Liyun Liu, Yun Xiang, Jing Feng, and Zhijia Wang

Department of Pediatrics, Shengjing Hospital of China Medical University, Shenyang, Liaoning, PR China

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ABSTRACT

The relationship between *Mycoplasma pneumoniae* (MP) infection and asthma has rarely been explored through examination of airway reactivity. The aim of this study was to determine airway reactivity changes after MP infection in children. First, 106 children were divided into four groups according to the existence of MP infection and/or asthma. Then children with only MP belonged to the MP group; children who had both MP infection and asthma belonged to the MP+A group; children with asthma but not MP infection belonged to the non-MP +A group; normal children were classified as normal control (NC) group.

Each subject underwent a bronchial provocation test (BPT) after effectively controlling the symptoms. Airway hyperresponsiveness (AHR) parameters were compared among the groups. BPT positive rates were also calculated and compared.

All AHR parameters decreased following MP infection, with a more significant decrease of small airway reactivity related indexes. The BPT-positive rate in the MP +A group was significantly higher than that in the MP group. Large airway reactivity showed no significant differences between the MP+A and non-MP+A groups, while the small airway reactivity augmented more significantly in the MP +A group.

MP infection caused increased reactivity of both large and small airways in lungs, and BPT-positive identification in some patients.

Keywords: Allergen bronchial provocation test; Asthma; Bronchial hyperreactivity; Children; *Mycoplasma pneumoniae*

INTRODUCTION

Mycoplasma pneumonia (MP) is the common pathogenic bacterium for community-acquired regional outbreak every 3-8 years.[5] Bronchial asthma, which is one of the most common chronic respiratory diseases, is characterized by chronic airway inflammation and increased airway reactivity. Clinical observations found that many children with MP infections were accompanied with asthma symptoms, even after recovery. Studies have suggested that approximately 30% of the MP infection cases develop wheezing symptoms,[6,7] and it has been reported that pneumonia (CAP) in children aged between five and fifteen.[1] The MP infection rate is 9.6%-66.7%,[2-4] with a

Corresponding Author: Yunxiao Shang, MD;
Department of Pediatrics, Shengjing Hospital of China Medical University, Shenyang 110004, Liaoning, PR China. Tel: (+86 024) 96615 52111, E-mail: yunxiaoshangsci@163.com

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MP infections can induce or exacerbate asthma.[8] Therefore, the relationship between MP infection and bronchial asthma attack has become a research hotspot in medical field in recent years.[9,10]

Possible mechanisms by which MP induces or exacerbates asthma have been proposed recently.[11] MP infection can induce airway epithelial injury, innate immunity activation, or increased Th2-dominant immune responses, which are all involved in the onset or the exacerbations of asthma. In addition, MP can increase airway hyperresponsiveness (AHR) and exacerbate airway remodeling in patients through cysteinyl leukotriene and transforming growth factor-beta.

Bronchial provocation test (BPT) is often performed to determine the changes of pulmonary function and airway reactivity after MP infection. Hypertonic saline BPT is widely used to assess the AHR for its safety and sensitivity.[12] The association between MP infection and asthma has been explored by detecting the MP-IgM in the peripheral blood of the patients with asthma.[13] Although a growing body of basic and clinical evidence implicates MP infection in asthma, the exact contribution of MP to asthma development, exacerbation, and persistence is largely unclear. In the current study, we examined the airway reactivity changes after MP infection and investigated the correlation between airway reactivity changes and asthma after MP infection.

MATERIALS AND METHODS

Subjects

The inpatients and outpatients with MP infection and/or asthma from the Department of Pediatric Respiratory Medicine at Shengjing Hospital of China, Medical University between June 2008 and January 2010 were enrolled in this study.

The diagnostic criteria for MP infection were MP-IgM measurement by particle agglutination (PA). For paired serum samples (with an interval of 1–2 weeks), antibody titer increased by 4-fold or decreased to 1/4 of the original level in the recovery stage. For PA test using only one serum sample, MP-IgM antibody titer continuously increased (MP-IgM > 1:160).

GINA standards for diagnosing pediatric asthma include wheezing, short of breath, chest tightness, cough that vary over time and intensity, and variable exspiratory airflow limitation. The patients with these symptoms caused by other diseases or with atypical clinical manifestations should have at least one positive result in the following tests: 1) BPT or decrease of (Forced expiratory volume in one second) FEV1 test manifestations; 2) bronchial dilation test; 3) variation of PEF (peak expiratory flow) within 1 day (or 2 weeks) ≥20%.

Grouping;

Mycoplasma Pneumonia Infection Group (MP Group)

The MP group included 32 children were diagnosed with MP infection (17 males and 15 females) aged from 4.5 to 14 (mean age: 8.40 ± 2.46 years). All of the patients had respiratory symptoms, such as fever, cough, and nasal discharge. The criteria for MP recovery were as follows: 1) all clinical symptoms disappeared; 2) lung symptoms alleviated or disappeared; 3) normal or improved chest X-ray; and 4) treatment with macrolide antibiotics for two weeks.

MP Infection Plus Asthma Group (MP + A Group)

The MP + A group included 28 patients (16 males and 12 females) with MP infection and asthma aged from 6.5 to 14 (mean age: 8.68 ± 2.18 years). To exclude the possibility of viral infection, viral antibodies in sera of eight pediatric patients were detected using SERION ELISA classic tests. The examined viral antibodies were mumps virus IgM, measles virus IgM, coxsackie virus b1-6 IgM, ECHO virus IgM, respirovirus IgA, adenovirus IgA, influenza virus IgA, and parainfluenza virus IgA. The experiments were carried out following the instructions of the ELISA kit. The exact quantification of pathogen-specific IgM and IgA antibody activities was using the precise 4 parameter logistic function (4 PL).

Non-MP Infection Asthma Group (Non-MP + A Group)

The non-MP + A group included 31 patients (18 males and 13 females) who met the diagnostic criteria for pediatric asthma but did not have MP infection. The age range of the non-MP + A group was 5 to 11 (mean age: 7.83±2.19 years).

Normal Control Group (NC group)

Healthy children who met the following requirements for the control group were selected: no history of asthma, no recurrent infection of the upper respiratory tract; no cardiovascular diseases; no

Table 1. Clinical characteristics of study population

Parameters	MP (n=32)	MP+A (n=28)	Non-MP+A (n=31)	NC (n=15)
Age (yr), mean±SD	8.40±2.46	8.68±2.18	7.83±2.19	9.20±2.90
Boy (%)	17(53%)	16(57%)	18(58%)	8(53%)

congenital diseases that affect lung function; no history of allergic diseases; the first-degree relatives did not have the history of asthma, chronic bronchitis, and respiratory infections in the recent 2 weeks. This group included 15 cases (8 males and 7 females), at the mean age of 9.20±2.90 years (Table 1).

Prerequisite for Bronchial Provocation Test (BPT)

The subjects with ΔFEV1≥75% in routine pulmonary function testing were allowed to undergo BPT. Theophylline, β2 agonists, anticholinergics, and inhaled corticosteroids were discontinued for 12 hours; oral corticosteroids and antihistamines were discontinued for 48 hours. All subjects were examined before BPT to exclude cardiac or pulmonary dysfunction, hypertension, and hyperthyroidism.

BPT was performed 2 weeks after MP infection when the patients were recovered from pneumonia and/or asthma. In that time, thesymptoms were controlled.

Bronchial Provocation Test (BPT)

The children's gender, age, height, and weight were entered into the Power-Cube spirometer (Ganshorn, Niederlauer, Germany) to measure the lung function before and after provocation. During BPT, 4.5% saline (2 ml/min) was inhaled for 30 s, 1, 2, 4 and 8 minutes using an ultrasonic nebulizer. The changes in breath-sounds after each inhalation were recorded. If ΔFEV1<10%, the next step was conducted; if 10%≤ΔFEV1<15%, inhalation was repeated with the same dose of saline. BPT result was negative if ΔFEV1 was always less than 15% and no wheezing was detected during auscultation. BPT was positive if: 1) ΔFEV1>15%, or wheezing was heard during bilateral lung auscultation; 2) ΔFEV1<15%, but wheezing was heard during auscultation. 0.25 ml of ventolin was inhaled to reduce AHR after the test.

Parameters in BPT

The gender, age, height, and weight of the children were entered into the computer of the spirometer, after which the theoretical value for normal lung function was generated. The actual/theoretical value of lung function was compared before and after BPT and the differences were used as research parameters. ΔFVC (forced vital capacity), ΔFEV1 (forced expiratory volume in one second), and ΔPEF (peak expiratory flow) mainly reflect the large airway function, among which the FEV1 is one of the main determinants for AHR. ΔMEF25-75 (mid-expiratory flow) mainly reflects the small airway function, and is an important factor contributing to an increase in airway sensitivity and reactivity.

Ethics Statements

This work was approved by the medical department of Shengjing Hospital of China Medical University which functions as the ethics committee of the hospital. The parents were provided with detailed information about the effect of BPT on human body before written consent was obtained. The children were accompanied by their parents during the test.

Statistical Analysis

Statistical analysis was carried out by SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA). Experimental data were expressed as the mean ± standard deviation (x ± SD). LSD-t test was performed for group comparisons; normally distributed mean values between two groups were compared using, t-tests; and the rates were compared with χ^2 tests. P <0.05 was considered statistically significant.

RESULTS

Airway Reactivity after MP Infection

The linkage between MP infection and asthma has been discovered in recent years that MP infection may precede the onset of asthma or exacerbate asthma symptoms. However, the association between MP infection and asthma is largely unknown. In this study,

we focused on the changes of airway reactivity after MP infection, using BPT. The results indicated that all AHR parameters diminished following MP infection, when compared with the control group. The ΔFVC, ΔFEV1, ΔPEF, and ΔMEF25-75 decreased 6.41, 8.31, 4.34, and 15.34 times in MP group and 6.61, 11.29, 14.00, and 18.32 in MP + A group, respectively. Notably, the reduced small airway reactivity, which was indicated by ΔMEF25-75, was the most significant symptoms of patients in MP group. Nevertheless, AHR parameters showed no statistically significant differences between the MP and MP + A groups (Table 2).

The above results indicated that the airway reactivity did not change in MP infected patients with or without asthma. Then, the airway reactivity was compared in asthma patients with or without MP infection. Detection of BHR parameters in asthma patients with or without MP infection showed that large airway reactivity was not significantly different in two groups. However, the parameter reflecting small airway function significantly decreased in the MP + A group (18.32 vs 12.71), implying that MP infection would cause severe inflammation in the small airway compared with that in the patients with asthma only (Table 2).

BPT-positive Rates in MP, MP + A, non-MP + A, and NC Groups

Next, the BPT-positive rates among the MP, MP + A, non-MP + A, and NC groups were compared. No positive results were found for BPTs in NC group. The BPT-positive rates in MP group, MP + A group, and non-MP + A group were 18.75% (6/32), 46.63% (13/28), and 38.71% (12/31), respectively (Table 3).

Table 2. BHR parameters of the patients in BPT (x ± SD)

Group	n	ΔFVC	ΔFEV1	ΔPEF	ΔMEF25-75
NC	15	2.73±2.69	3.27±2.55	3.47±2.64	4.00±4.29
MP	32	7.13±6.50	8.31±7.65	4.34±8.89	15.34±8.87
MP + A	28	6.61±4.31	11.29±7.81	14.00±10.25	18.32±12.28
Non-MP + A	31	7.45±4.89	12.06±6.57	12.58±7.25	12.71±7.18
t^a		2.50	2.478	3.74	4.68
p^a		0.016*	0.017*	0.001*	<0.001*
t^b		3.16	3.85	3.89	4.35
p^b		0.003*	<0.001*	<0.001*	<0.001*
t^c		0.36	1.48	0.139	1.08
p^c		0.722	0.142	0.89	0.282
t^d		0.700	0.416	0.619	2.180
p^d		0.487	0.679	0.538	0.033*

a. *t*-, *p*-value acquired from comparison between NC and MP groups; b. *t*-, *p*-value acquired from comparison between NC and MP + A groups; c. *t*-, *p*-value acquired from comparison between MP and MP + A groups; d. *t*-, *p*-value acquired from comparison between MP + A and non-MP + A groups. * $p<0.05$, indicating a statistically significant difference

Table 3. BPT-positive rates in NC, MP, MP + A, and non-MP + A groups (x ± SD)

Group	N	BPT		Positive rate (%)	χ^2	*p*
		positive	negative			
NC	15	0	15	0		0.003*[a]
MP	32	6	26	18.75	5.287[b]	0.025*[b]
MP + A	28	13	15	46.63	0.4882[c]	>0.25[c]
Non-MP + A	31	12	19	38.71		

a. Because there were no BPT-positive cases in the normal control group, Fisher's exact probability test of a four-fold table data was used. p refers to the cumulative probability. b. Comparison of the BPT-positive rate between the MP and MP + A groups. All the BPT-positive cases in the MP group showed a ΔFEV>15% 8 min after inhaling saline. One BPT-positive case in the MP + A group showed a ΔFEV>15% 2 min after inhaling saline. One BPT-positive case had wheezing sound in lung auscultation 4 min after inhaling saline. c. Comparison of the BPT-positive rate between the MP + A and non-MP + A groups. Among the BPT-positive cases in the non-MP + A group, one patient had a ΔFEV% >15% 4 min after saline inhalation. * $p<0.05$ was considered statistically significant.

The BPT-positive rate in the MP group was significantly higher than that in the normal control group (p=0.003), implying that MP infection could induce dysfunction of large and small airway ventilation. Not surprisingly, the BPT-positive rate in the MP + A group was significantly higher when compared with the MP group (p=0.025), indicating the typical pathology features of asthma. However, the BPT-positive rates showed no significant difference between the MP + A and non-MP + A groups (p> 0.25).

DISCUSSION

As a common pathogen that causes community-acquired pneumonia, MP has been widely recognized for a long time. During the past decade, due to the in-depth study of the etiology and affecting factors on bronchial asthma, the correlation between MP infection and asthma has attracted widespread attention.[14-16] Clinical observation found that some children with MP infection still had a long time of cough, even after the treatment of infection, which was diagnosed with cough-variant asthma.[17] Nazima et al(Name is different from reference)[18] treated asthma patients with MP infection with macrolides and found that asthma symptoms were significantly relieved and lung function was improved after medication, implying that MP infection can induce the onset, aggravation, or recurrence of asthma.

BPT is widely used for the determination of AHR to assist the diagnosis of asthma. The degree of airway narrowing was judged by a series of pulmonary function indexes after the contraction of bronchial smooth muscle induced by some kind of stimuli. The stimuli include direct inhalation challenges with inhaled methacholine or histamine and indirect challenges such as exercise, dry air hyperpnea, distilled water, hypertonic saline and mannitol, etc.[19] The hypertonic saline test has higher specificity compared with drug trials, due to which it can better reflect the disease progression and drug effect. Besides, BPT with hypertonic saline also has the advantages of low cost of medication, rapid onset of action, and high compliance of patients. No false-positive case has been reported in the literature using hypertonic saline. The ΔFEV1 is still <15% even when a healthy person inhales a large quantity of hypertonic saline. So far, airway reactivity measured with hypertonic saline is consistent with the diagnosis of asthma. In the current study, 4.5% hypertonic saline was used for BPT.[20] Richard et al.[21] performed a BPT to measure airway reactivity in a mouse model 3, 7, 14, and 21 days after MP infection, and found that airway reactivity significantly increased on day 7 and 14, but showed no significant increase on day 21 compared with the control group. Therefore, the BPT was conducted 2 weeks after MP infection, so that the results of the BPT would be more accurate.

Our study showed that parameters reflecting both the large and small airway reactivity decreased in MP group, indicating that children with MP infection had ventilation dysfunction in both large and small airways, even when the disease was well controlled. The airway flow limitation is one of the features of airway mucosal injury, which subsequently leads to AHR in asthma. Further studies should be carried out to explore whether these changes of the airway reactivity in MP infected pediatric patients would contribute to asthma or prolonged cough in the future. Early intervention could be provided if necessary. Besides, the BPT-positive rate was also significantly higher in MP group, which further confirmed that MP infection would induce the onset of asthma.

The reactivity of large and small airway increased in both the MP + A and non-MP + A groups, which was in accordance with the recognition of AHR-positive children with asthma. However, the functional reactivity of small airway in the MP + A group was much higher than that in the non-MP + A group, suggesting that chronic bronchial inflammation occurred after MP colonized on the airway epithelium. Chronic airway (allergic) inflammation is not only one of the basic pathological changes of asthma, but also an important cause of MP induced asthma attacks. It has been known that MP infection could induce asthma through the release of cytokines, immunologic damage of the tissue, and subsequent chronic allergic inflammatory reaction in the respiratory tract.[18,22]

The results also showed that although the asthma symptoms were effectively controlled after treatment, the children in MP + A group still had increased small airway reactivity compared with that in MP group, indicating that MP infection would induce persistent AHR of the airways. The persistent inflammation can be blocked by reducing the expression of neuropeptides, suggesting the involvement of neural mechanisms in MP induced chronic asthma.[23] Besides, MP infection can increase airway reactivity through

directly damaging epithelial cells in the respiratory tract, promoting the release of cytokines, and inducing the generation of IgE or neuropeptides. The children who have AHR or chronic cough after curing pneumonia are likely to develop asthma, so it is necessary to regularly follow up these patients and detect changes in the airway reactivity to prevent MP infection-induced asthma as early as possible.

In summary, our study showed that children with MP infection still had hyper reactivity in both large and small airways even at recovery stage, indicating the potential occurrence of asthma. Examination of the airway reactivity in these children regularly may help to take preventive measures against pediatric asthma.

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