

## Seroprevalence of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* in Stable Asthma and Chronic Obstructive Pulmonary Disease

*Mycoplasma pneumoniae* and *Chlamydia pneumoniae* have been suggested to take part in the acute exacerbation of bronchial asthma and chronic obstructive pulmonary disease (COPD). Several studies have questioned whether they may play pathogenic roles in connection with bronchial asthma and COPD. This study was designed to evaluate the seroprevalences of *M. pneumoniae* and *C. pneumoniae* in stable asthma and COPD patients, and to compare with control patients. The medical records of one hundred forty patients who underwent *M. pneumoniae* and *C. pneumoniae* serology were retrospectively reviewed. Seroprevalences of *M. pneumoniae* and *C. pneumoniae* in the asthma group (11.1% and 8.3%, respectively) were higher than in the control group (4.4% and 2.2%, respectively) without statistical significance. The seroprevalence of *M. pneumoniae* in the COPD group (16.9%) was significantly higher than in the control group, and the seroprevalence of *C. pneumoniae* in the COPD group (3.4%) was higher than in the control group without statistical significance. This study raises important questions about the relation of *M. pneumoniae* and *C. pneumoniae* infection with stable asthma or COPD.

Key Words : *Mycoplasma pneumoniae*; *Chlamydia pneumoniae*; Asthma; Lung Diseases, Obstructive

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## INTRODUCTION

*Mycoplasma pneumoniae* and *Chlamydia pneumoniae* are common etiologies of atypical respiratory infection. Several recent studies have shown that these organisms play an important role in the acute exacerbation of bronchial asthma and chronic obstructive pulmonary disease (COPD) (1-4). The role of respiratory infection has been recently suggested for the pathogenesis of bronchial asthma, and atypical organisms such as *M. pneumoniae* and *C. pneumoniae* have been recently linked to the onset of asthma (5-7). A few clinical and experimental animal studies have suggested that peripheral airways diseases may be due to the cumulative effects of recurrent respiratory infections over an extended period of time (8). Therefore, the significance of atypical respiratory infections for the development of COPD is now getting a lot of the limelight.

The purpose of this study is to evaluate the seroprevalences of *M. pneumoniae* and *C. pneumoniae* in clinically stable asthma and COPD, and to compare with control subjects.

## MATERIALS AND METHODS

### Study subjects

One hundred forty patients who had been serologically test-

ed for *M. pneumoniae* and *C. pneumoniae* from November 2002 to August 2003 were enrolled as subjects in our study. The clinical charts were reviewed retrospectively, and the subjects were divided into the asthma group, the COPD group, and the control group according to history, physical examination, and the results of spirometry and methacholine challenge test. The diagnosis of asthma was based on demonstration of reversible airway obstruction (post-bronchodilator FEV<sub>1</sub> increase of  $\geq 15\%$ ) and was confirmed by airway hyperreactivity to methacholine (i.e., provocative concentration of methacholine producing a 20% reduction in FEV<sub>1</sub> [PC<sub>20</sub>] of  $< 8$  mg/mL). Entry criteria for the COPD group were the following three conditions: 1) age above 40 yr, 2) smoking history of more than 20 pack-years, 3) maximal FEV<sub>1</sub>/FVC ratio of less than 0.7 and FEV<sub>1</sub> of less than 80% of the predicted value. The patients that were excluded from the asthma group and the COPD group were included to the control group. All enrolled patients did not show any clinical signs of acute exacerbation at that time such as worsening of dyspnea, increased sputum volume, new expectoration of purulent sputum or increased sputum purulence. All the subjects gave their informed consent and the experimental protocol was accepted by the Institutional Review Board of Chonbuk National University Hospital, Jeonju, Korea.

## Serologic test

Anti-*Mycoplasma* antibody titer was detected by the indirect agglutination method (Serodia Myco II, Fujirebio, Japan) that anti-*Mycoplasma* reacted with artificial serum contained membrane of *M. pneumoniae*. According to the manufacturer's instructions, the serologic evidence of infection with *M. pneumoniae* was defined as an antibody titer of higher than 1:64, or a four-fold rise in the convalescent serum compared with base as detected on two tests.

Antibody to *C. pneumoniae* was detected by the microimmunofluorescence (MIF) test using IgG Micro-IF Kit (Fuller Laboratories, California, U.S.A.) and IgM Micro-IF Kit (Viracell, Santa Fe-Granada, Spain). Serologic evidence of an infection with *C. pneumoniae* was defined as an IgG titer of higher than 1:512 or an IgM titer of higher than 1:20.

## Statistical analysis

Data are expressed as mean  $\pm$  standard deviation. Statistical comparisons were performed using the Mann-Whitney U test and chi-square test. For all analyses, all tests were 2-sided, with the level of significance defined as a *p*-value of 0.05 or less.

## RESULTS

### Subject populations and characteristics

A total of 140 patients were enrolled. The average age of

**Table 1.** Comparison of demographic characteristics and spirometry of the asthma group and the control group

Parameters	Asthma group	Control group
Subjects (n)	36	45
Gender (M/F)	20/16	23/22
Age (yr)	60.0 $\pm$ 17.6	57.4 $\pm$ 19.0
FEV <sub>1</sub> (L)	2.0 $\pm$ 0.6	2.3 $\pm$ 0.8
FEV <sub>1</sub> (% pred)	73.0 $\pm$ 20.0	93.1 $\pm$ 17.5
BDR (% changed)	21.0 $\pm$ 7.0	<15%
PC <sub>20</sub> (mg/mL)	7.05 $\pm$ 5.0	>16

Data are presented as mean  $\pm$  SD. n, number; M, male; F, female; FEV<sub>1</sub>, forced expiratory volume in one second; % pred, percent of predicted value; BDR, bronchodilator response; PC<sub>20</sub>, provocative concentration of methacholine causing FEV<sub>1</sub> to fall by 20%.

**Table 3.** *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* seroprevalences in the asthma and control groups

	Asthma group (n=36)	Control group (n=45)	<i>p</i> value
<i>M. pneumoniae</i>	4 (11.1%)	2 (4.4%)	0.255
<i>C. pneumoniae</i>	3 (8.3%)	1 (2.2%)	0.207

Data are presented as the number of seropositive patients (seroprevalence %). n, number of total patients.

the subjects was 62.7  $\pm$  11.0 yr, and there were 85 males and 55 females. There were 36 patients in the asthma group, 59 patients in the COPD group, and 45 patients in the control group. Because the three groups had different characteristics on the demographic parameters and pulmonary function test, statistical comparisons were performed between control and asthmatic groups, and control and COPD groups (Table 1, 2).

### Comparison between asthmatic group and control group

The seroprevalence of *M. pneumoniae* in the stable asthmatic group (11.1%) showed as being higher than the control group (4.4%), and this was without statically significance (*p*=0.255) (Table 3). The seroprevalence of *C. pneumoniae* in the stable asthmatic group (8.3%) showed as being higher than the control group (2.2%), and this was not statically significant (*p*=0.207).

### Comparison between COPD group and control group

The seroprevalence of *M. pneumoniae* in the COPD group (16.9%) was significantly higher than the control group (*p*=0.048), and the seroprevalence of *C. pneumoniae* in the COPD group (3.4%) was higher than the control group without statistical significance (*p*=0.724) (Table 4).

## DISCUSSION

*M. pneumoniae* and *C. pneumoniae* are common etiologic bac-

**Table 2.** Comparison of demographic characteristics, smoking, and spirometry of the COPD group and the control group

	COPD group	Control group
Subjects (n)	59	45
Gender (M/F)	42/17	23/22
Age (yr)	68.5 $\pm$ 10.1	57.4 $\pm$ 19.0
FEV <sub>1</sub> (L)	1.4 $\pm$ 0.6	2.3 $\pm$ 0.8
FEV <sub>1</sub> (% pred)	63.9 $\pm$ 22.0	93.1 $\pm$ 17.5
FEV <sub>1</sub> /FVC (%)	58.0 $\pm$ 10.7	>70%
Smoking (p/yr)	51.3 $\pm$ 31.5	42.9 $\pm$ 30.3

Data are presented as mean  $\pm$  SD. n, number; M, male; F, female; FEV<sub>1</sub>, forced expiratory volume in one second; % pred, percent of predicted value; FVC, forced vital capacity; p/yr, pack years.

**Table 4.** *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* seroprevalences in the COPD and control groups

	COPD group (n=59)	Control group (n=45)	<i>p</i> value
<i>M. pneumoniae</i>	10 (16.9%)	2 (4.4%)	0.048
<i>C. pneumoniae</i>	3 (3.4%)	1 (2.2%)	0.724

Data are presented as the number of seropositive patients (seroprevalence %) n, number of total patients.

teria causing respiratory infection and responsible for community acquired pneumonia. Moreover, respiratory infections have been shown to play an important role in the acute exacerbation of bronchial asthma and COPD. Liberman et al. (3) have reported that the seroprevalence of *M. pneumoniae* in bronchial asthma patients with acute exacerbation was 18%, and the seroprevalence of *C. pneumoniae* in these patients was 8%. They have also reported that the seroprevalence of *M. pneumoniae* in COPD patients with acute exacerbation was 14.2% (4). From the results of several studies, the putative role of atypical respiratory pathogens, such as *M. pneumoniae* and *C. pneumoniae*, in the development of asthma or COPD has been suggested.

Since bacterial infections are known to impair mucociliary clearance and to increase mucus production in the lung, it has been proposed that certain bacterial infections may cause chronic lower airway inflammation. Organisms primarily implicated in this process include *M. pneumoniae* and *C. pneumoniae* (9-11). Hahn (12) has reviewed all the relevant medline articles from January 1984 to March 1999, and he has reported that of 18 controlled epidemiologic studies (with over 4,000 cases/controls), 15 studies have found significant associations between *C. pneumoniae* infection and asthma, and 5 of 6 studies (with over 1,000 cases/controls) have reported significant associations between *C. pneumoniae* infection and COPD.

The most important cause of COPD is well known to be smoking. However, the pathogenesis of asthma is not definitive and it may be multifactorial, and so this ambiguity has gathered interest for the association of atypical respiratory pathogens with the pathogenesis of asthma than COPD (13-18). Martin et al. (11) studied *M. pneumoniae* and *C. pneumoniae* infection rates in chronic bronchial asthma patients and normal control subjects by using polymerase chain reaction (PCR), culture, and serologic test. Their results showed that *M. pneumoniae* and *C. pneumoniae* positive rates in the bronchial asthma group were significantly higher than control group. In contrast to the results of many studies, Foschino Barbaro et al. reported that the seroprevalence of *C. pneumoniae* in stable asthmatics was comparable with the controls (19). Although statistical significance was not noted, our data showed higher rates of *M. pneumoniae* and *C. pneumoniae* infection in the asthma group than for the control group. That this difference was without significance might be resulted from the low number of enrolled patients and/or geographic factors.

In connection with the possible relationship between *M. pneumoniae* infection and the onset of asthma, several studies have shown not only a high level of serum total IgE but also the production of IgE specific to *M. pneumoniae* or common allergens during the course of *Mycoplasma* infection (20, 21). Koh et al. reported that IL-4 levels and IL-4/IFN-gamma ratios in bronchoalveolar lavage fluid were significantly higher in patients with *M. pneumoniae* than in patients with pneumococcal pneumonia or control participants and the bronchoalveolar lavage cytokine data suggested a predominant Th2-like

cytokine response in *M. pneumoniae*, thus representing a favorable condition for IgE production (22). Therefore, further study and evaluation including measurement of serum total IgE, specific IgE to *M. pneumoniae*, and several cytokines are needed to further understanding of the relationship between *M. pneumoniae* or *C. pneumoniae* infection and asthma.

Our results showed that there was a significantly higher positive rate for *M. pneumoniae* in the COPD group than the control group and although statistical significance was not noted, higher rates of *C. pneumoniae* infection in the COPD group than for the control group. What is the reason for the high infection rate of *C. pneumoniae* in patients with bronchial asthma and COPD? In an animal model, non-cultivable *C. pneumoniae* may have been transformed to a cultivable form after immunosuppression by corticosteroids (23). The addition of hydrocortisone succinate enhanced the growth of *C. pneumoniae* in vitro, and steroid medication has been associated with significant elevation of *C. pneumoniae* antibody titers in bronchial asthma patients and COPD patients (24). Taken together, the relatively high positive rate of *M. pneumoniae* and *C. pneumoniae* in patients with bronchial asthma and COPD in our study may be associated with their steroid treatments.

For the clinician, the diagnosis of *C. pneumoniae* infection may be difficult because of the lack of availability of diagnostic facilities for organism identification either serologic testing, and also because of the controversies surrounding the serodiagnostic criteria (12). A serologic method for the diagnosis of *C. pneumoniae* infection that is based on MIF has been introduced for both the laboratory and the clinical setting. A four-fold rise in the IgG titer, an IgM titer higher than 1:16, and an IgG titer higher than 1:512 have been considered to be consistent with acute infection (25). IgG titers between 1:16 and 1:512 are considered to be evidence of prior infection, but not recent infection. Although an IgG titer of higher than 1:64 is occasionally suggestive of recent infection (26), our study defined a serologic positive level for *C. pneumoniae* as an IgG titer of higher than 1:512 to have less false positive results.

In conclusion, *M. pneumoniae* and *C. pneumoniae* infection rates are tended to high in patients with clinically stable bronchial asthma and COPD compared with the control subjects in our study. The results of this study can present the possibility that *M. pneumoniae* and *C. pneumoniae* infections have close associations with stable bronchial asthma or COPD, but it is unclear whether infections of *M. pneumoniae* and *C. pneumoniae* have a direct relation for the pathogenesis of these two diseases. Therefore, greater evaluation and more study are needed to further understanding of the pathogenesis and treatment of asthma and COPD.

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