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Chlamydia pneumoniae and exacerbations of asthma in adults

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Background: *Chlamydia pneumoniae* is a frequent causative agent of acute respiratory disease and has been recently reported as a possible cause of asthma.

Objective: We assessed the prevalence of *C. pneumoniae* infections in adult patients with acute exacerbations of asthma.

Methods: One hundred sixty-eight adult patients with acute exacerbations of asthma and 108 control subjects matched for age, sex, and smoking status were studied. Nasopharyngeal swab specimens were obtained from all subjects and analyzed by isolation in cell culture and polymerase chain reaction (PCR) test for *C. pneumoniae*. Serum samples were also obtained and tested for *C. pneumoniae*-specific antibodies by the microimmunofluorescence test.

Results: *C. pneumoniae* was isolated from two (1.2%) asthma patients and none from controls and detected by PCR from nine (5.4%) cases and one (0.9%) control. Both culture positive specimens were also positive in PCR. Further, serologic evidence of acute *C. pneumoniae* infection was present in 15 (8.9%) of asthma patients and in three (2.8%) of controls ($P = .048$). The prevalence of *C. pneumoniae*-specific IgG and IgA was significantly higher in asthma cases than in controls (IgG $\geq$ 1:16: 85.1% versus 67.6%, $P = .001$; IgA $\geq$ 1:16: 47.6% versus 16.7%, $P < .001$). Mean titer of IgG and IgA was also significantly greater in asthma cases than in controls (IgG: 38.8 versus 18.1, $P = .0001$; IgA: 17.2 versus 6.1, $P = .0001$).

Conclusions: Our data suggest that *C. pneumoniae* infection may trigger acute exacerbations of adult asthma.

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INTRODUCTION

Asthma is a chronic lung disease characterized by airway obstruction, inflammation, and bronchial hyperresponsiveness to a variety of stimuli, including infections. Infections with viruses such as respiratory syncytial virus, parainfluenza virus, influenza A and B virus, coronavirus, adenovirus, and rhinovirus have been identified in 10% to 60% of children and adults with acute exacerbations of asthma.[1–5] Infection with other organisms, such as *Mycoplasma pneumoniae*, have also been associated with acute exacerbations of asthma.[6,7]

Another atypical respiratory pathogen, *Chlamydia pneumoniae*, has been established as an important cause of acute respiratory illness, including pharyngitis, bronchitis, and pneumonia.[8,9] In a previous prospective study designed to assess the role of acute *C. pneumoniae* infection in bronchitis and atypical pneumonia, Hahn et al[10,11] reported an association between serologic evidence of acute *C. pneumoniae* infection and adult-onset asthma and asthmatic bronchitis. Emre et al[12] also examined the prevalence of *C. pneumoniae* infection in children with acute exacerbations of reactive airway disease. *C. pneumoniae* was isolated from 11% of children in their study.

To study the role of *C. pneumoniae* in acute exacerbation of adult asthma, we assessed the frequency of *C. pneumoniae* infection in adult patients with exacerbations of asthma by isolation and polymerase chain reaction (PCR) from nasopharyngeal swabs and by serology.

SUBJECTS AND METHODS

Study Population

Patients studied were 168 adults (16 to 80 years of age, mean 49.2 years; 91 males and 77 females) with asthma having recurrent episodes of reversible wheezing and breathlessness[13] who were seen at the Kawasaki Medical School Hospital between January 1995 and December 1996 for acute wheezing attacks, often with status asthmaticus. The initial mean percent of predicted FEV_1 was 70.3 (range 38 to 124 and SD 16.2). The mean duration of wheezing was 18.2 years (range 1 to 52 years); 124 (74%) patients were on regular treatment with bronchodilators. Twenty eight percent were current smokers, 42% were ex-smokers and 30% had never smoked. Non-asthmatic controls matched for age, sex, and smoking status were selected from patients attending the same hospital. The criteria for inclusion were no signs and symptoms of acute respiratory illness and normal pulmonary function test with a percent of predicted FEV_1 of 90 or greater (mean 101.6, range 90–134, and SD 9.2). Informed consent was obtained from all subjects. The mean duration of follow up was 3.2 months (range 2 to 12 months).

Culture and PCR

Nasopharyngeal swab specimens were obtained from all patients and controls for isolation in cell culture and PCR. The swab specimens were placed in sucrose-phosphate-glutamate (SPG) transport medium. Culture for *C. pneu-*

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moniae was performed in cycloheximide-treated HEp-2 cells grown in a 24-well cell culture plate as reported previously.[14] All specimens were passed once. Culture confirmation was done by fluorescent-antibody staining with *C. pneumoniae* species-specific and genus-specific monoclonal antibodies.[14,15]

The *C. pneumoniae*-specific primers used for PCR were from the DNA base sequence within the 53-kD protein gene as reported previously.[16] Cell culture grown *C. pneumoniae* strain KKp-15[17] was used as positive controls, and chlamydia transport medium was used as negative controls in every run. After electrophoresis of amplification products on a 1.5% agarose gel at 100 V, the band was visualized by staining with ethidium bromide. The appearance of a 499 base pair amplification product was taken as positive.

Serology

Paired serum samples were collected at intervals of 4 weeks for controls. Also serum samples of asthma patients were collected several times for at least 4 weeks after onset. All sera were stored at −70 °C until testing. The microimmunofluorescence (MIF) test was used for titration of IgG, IgA, and IgM antibodies against chlamydial species,[18] using formalinized elementary bodies of *C. pneumoniae* KK-pn15, *C. trachomatis* L2/434/Bu and *C. psittaci* 6BC strains as antigens. Rheumatoid factors were absorbed with Gullsorb (Gull Laboratories, Salt Lake City, USA) before IgA and IgM titrations.[19] Acute or current infection was defined by IgM ≥ 1:16, IgG ≥ 1:512 or a fourfold rise in IgG. Chronic or pre-existing antibody was defined by IgM < 16 and IgG 1:16 to 1:256. Criteria for definition of IgA levels as indicative of acute versus pre-existing infection have not been established. In this study the positive IgA antibody was therefore, defined as titers ≥ 1:16 by conventional criteria.

Complement fixation (CF) tests were done in all patients for antibodies to adenovirus, influenza A and B virus, respiratory syncytial virus, cytomegalovirus, parainfluenza virus types 1, 2 and 3, and *M. pneumoniae*. A seroconversion or fourfold rise in antibody was taken as indicating infection.

Table 1. Demographic Characteristics of Study Population

Characteristics	Asthma Cases n = 168	Controls n = 108	*P* Value*
Age, yr			
mean ± SD	49.2 ± 10.2	48.6 ± 9.6	0.626
range	16–80	18–78	—
Gender (male)	91 (54.2)†	60 (55.6)	0.901
Smoking status			
Current	47 (28)	28 (26)	—
Ex	71 (42)	42 (39)	—
Never	50 (30)	38 (35)	0.357‡
C. pneumoniae infection			
Culture	2 (1.2)	0	0.522
PCR	9 (5.4)	1 (0.9)	0.094

* The Student's *t* test was used for mean age comparison and the Fisher's Exact test for other statistical analyses.
† Number (%).
‡ Ever-smokers versus never-smokers.

Statistical Analysis

Statistical analysis was done by two tailed Fisher's Exact test. Mean age comparison was done by the Student's *t* test and geometric mean titer (GMT) comparison by the Mann-Whitney U test. A P value < .05 was considered to be significant.

RESULTS

Demographic characteristics of study populations are shown in Table 1. There were no significant differences between cases and controls in age, sex, or smoking status.

C. pneumoniae was isolated from the nasopharynx from 2 (1.2%) asthma patients and none from controls. *C. pneumoniae* was detected by PCR from 9 (5.4%) cases and one (0.9%) control. Culture positive specimens were all positive by PCR.

Antibodies against *C. pneumoniae* are shown in Table 2. IgG and IgA antibodies were present more often in asthma patients than controls (IgG ≥ 1:16: 85.1% versus 67.6%, $P = .001$; IgA ≥ 1:16: 47.6% versus 16.7%, $P < .001$). The GMTs of IgG and IgA were significantly higher in cases than in controls (for IgG 38.8 versus 18.1, $P = .0001$; for IgA 17.2 versus 6.1, $P = .0001$). Serologic evidence of acute *C. pneumoniae* infection was found in 15 (8.9%) cases (only 3 cases had IgM) and in 3 (2.7%) controls ($P = .048$). No acute *C. trachomatis* or *C. psittaci* infection was found. No differences in frequencies of *C. trachomatis* and *C. psittaci* antibodies were observed between asthma patients and controls.

Other respiratory tract pathogen including viruses and *M. pneumoniae* were identified in 21 (12.5%) patients by serologic test. Fourteen (8.3%) were found to have infections with viruses (seven influenza A, three parainfluenza 3, three respiratory syncytial virus, one dual infection with influenza A, and adenovirus) and seven (4.2%) with *M. pneumoniae*. Dual infections of *C. pneumoniae* and viruses or *M. pneumoniae* were not observed.

The results of laboratory tests and treatment of nine culture or PCR-positive patients are summarized in Table 3. Five of nine patients who were culture or PCR positive had serologic evidence of acute infection. Three patients had stable IgG antibody titer, all of them had IgA. One had no IgG and IgA antibody. These four patients, however, had clinical and laboratory evidence of infection. All had symptoms of acute respiratory tract infection and elevation of inflammatory signs, CRP, and ESR. *C. pneumoniae* was successfully eradicated from the

Table 2. *Chlamydia pneumoniae* Serology

Antibodies	Asthma Cases n = 168	Controls n = 108	*P* Value*
IgG			
≥1:16	143 (85.1)†	73 (67.6)	0.001
GMT	38.8	18.1	0.0001
IgA			
≥1:16	80 (47.6)	18 (16.7)	<0.001
GMT	17.2	6.1	0.0001
Acute‡	15 (8.9)	3 (2.8)	0.048

* Statistical analysis was done by Fisher's Exact test. Geometric mean titer comparisons were done by the Mann-Whitney U test.
† Number (%).
‡ IgM ≥ 1:16, IgG ≥ 1:512, or a fourfold rise in IgG.

nasopharynx of all infected patients following antibiotic therapy. Five patients were treated with new macrolide antibiotics (clarithromycin in four and roxithromycin in one) and four patients with levofloxacin for 14 to 21 days (Table 3). All nine patients were treated with combined therapy with intravenous theophylline (in all nine patients) and 5 to 7-day courses of oral prednisone (in six patients) and antibiotics because of severe asthma attacks and achieved remission.

DISCUSSION

Respiratory tract infections with viruses and *M. pneumoniae* have been considered as the most common triggers of asthma in all age groups.[1–7] Hahn et al[10] reported a strong relationship between acute *C. pneumoniae* infection and the diagnosis of asthmatic bronchitis in adults who had wheezing at the time of enrollment after an acute respiratory tract illness. Subsequently, Emre et al[12] identified *C. pneumoniae* infection by culture in 13 (11%) of 118 children and by serology in 16 (22.8%) of 70 children with asthma exacerbations and reported improvement in asthma after the eradication of *C. pneumoniae* by antibiotics. In this study, we were able to detect *C. pneumoniae* infection by culture or PCR in nine (5.4%) of 168 adult patients with exacerbations of asthma. Furthermore, we found a significantly higher frequency (85.1%, $P = .001$) and GMT ($P = .0001$) of anti-*C. pneumoniae* IgG antibody in patients with exacerbations of asthma. Serologic data also suggest at least 15 (8.9%) exacerbations were associated with *C. pneumoniae*. Our results are consistent with those reported by Hahn et al,[10] on association between acute *C. pneu-*

Table 3. Laboratory Findings in 9 Patients with Acute Exacerbations of Asthma Attributed to *Chlamydia pneumoniae* Infection

Patient No.	Sex	Age, yr	Follow-up (days after onset)	Culture	PCR	Antibodies			Antibiotics/Duration, days
						IgM	IgG	IgA	
1.	F	20	10	+	+	32	256	8	clarithromycin/21
			20	+	+	32	256	16	
			46	−	−	0	256	8	
			116	−	−	0	128	0	
2.	M	48	5	−	+	0	32	32	levofloxacin/14
			24	−	−	0	512	128	
			68	−	−	0	512	64	
3.	F	56	1	−	+	0	64	32	clarithromycin/21
			32	−	+	0	1024	128	clarithromycin/14
			96	−	−	0	1024	32	
4.	F	19	8	−	+	16	0	0	levofloxacin/14
			22	−	−	16	128	8	
			63	−	−	0	256	8	
5.	M	54	3	+	+	0	64	32	roxithromycin/14
			41	−	−	0	256	64	
6.	M	46	8	−	+	0	32	16	levofloxacin/14
			28	−	−	0	32	16	
7.	F	21	3	−	+	0	16	8	clarithromycin/14
			35	−	−	0	16	0	
8.	M	26	7	−	+	0	16	8	clarithromycin/14
			43	−	−	0	16	0	
9.	F	18	5	−	+	0	0	0	levofloxacin/14
			14	−	+	0	0	0	
			51	−	−	0	0	0	
			98	−	−	0	0	0	

Note. + = positive; − = negative.

moniae respiratory infection and acute exacerbation of asthma, and antibody titers and wheeze.

In our study, exacerbations of asthma associated with *C. pneumoniae* were seen more often in older age groups, while exacerbations of asthma not associated with *C. pneumoniae* antibody were seen more often in young adults. Seroepidemiologic studies showed older patients were more likely to be having reinfections with *C. pneumoniae*, while younger patients were more likely to be having a primary infection.[20] In our study, most patients with acute *C. pneumoniae* infection had no IgM antibody titers suggesting a reinfection in these patients. The sample size in our study is too small to confirm the epidemiologic observations.

It has been suggested that presence of serum IgA antibodies is a useful marker for diagnosis of some chronic bacterial infections because the half life of serum IgA is less than 1 week[21]; therefore, its continuous presence may indicate persistent antigenic stimulation to the immune system. In support of IgA antibodies as a marker for chronic infection, microbe-specific serum IgA has been shown to associate with a variety of chronic illnesses by bacterial pathogens, including *C. trachomatis*.[22] In particular, *C. pneumoniae*-specific IgA has been associated with *C. pneumoniae* reinfection,[23] chronic obstructive pulmonary disease,[24] and symptomatic asthma.[25] Our data also showed a significant association of *C. pneumoniae*-specific serum IgA antibodies and symptomatic reversible airway obstruction in adults. These findings indicate that chronic infection or reinfection with *C. pneumoniae* is common in asthma patients. Asthma exacerbations in patients with evidence of *C. pneumoniae* infection occurred in older ages when infection with *C. pneumoniae* is often a reinfection. It has been proposed that chronic *C. trachomatis* infection induces immunopathologic disease.[9] Reinfection with *C. pneumoniae* may therefore trigger an immunopathologic process in the lungs, perhaps involving epithelial damage and mediator release,[26,27] or induce delayed hypersensitivity to chlamydial protein antigens[28] resulting in chronic airway inflammation characteristic of asthma.

The rates of infections with viruses and *M. pneumoniae* in our study are similar to some reports,[3,4] but is remarkably lower in comparison to some other reports.[5,6] The low prevalence of viral and mycoplasmal infection found in our study is probably due to the use of the CF test which has a low sensitivity and specificity.

In conclusion, we have shown an association of *C. pneumoniae* with acute exacerbations of asthma in some asthmatic adults. We were not able to assess whether eradication of *C. pneumoniae* after antichlamydial therapy produced improvement of asthma symptoms because bronchodilators or corticosteroids were also used.

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