Prevalence and Incidence of *Chlamydia Pneumoniae* Antibodies among the Healthy Elderly and Patients with Chronic Obstructive Pulmonary Disease[$]

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Chlamydia pneumoniae is an obligatory intracellular bacteria which can cause both acute and chronic respiratory tract infection. The significance of chronic and recurrent respiratory infection may be of prime importance in chronic obstructive pulmonary diseases(COPD). The purpose of this study was to determine the prevalence and incidence of C. pneumoniae antibodies in elderly COPD patients compared to a healthy elderly control group. C. pneumoniae antibodies were detected by an enzyme-linked immunosorbent assay in serum samples obtained from 127 elderly COPD patients and a 131 healthy elderly control group. The results showed that the seroprevalence of C. pneumoniae infection as determined by the existence of specific IgG or IgA or IgM antibodies was 96.1% in the COPD patients and 75.6% in the control group ($p < 0.01$). The prevalence of individual C. pneumoniae IgG, IgA and IgM in elderly COPD vs healthy control was 85.8% vs 66.4%, 85.0% vs 51.1% and 3.9% vs 0%, respectively. The incidence or seroconversion rate of C. pneumoniae antibodies after one year follow-up was found to be 33 % in the COPD patients and 67.9% in the control group. High prevalence and incidence of C. pneumoniae antibodies indicates that both acute and chronic C. pneumoniae infection play a role in elderly COPD patients. Therefore, antibiotics of choice for C. pneumoniae infection should probably be considered.

Keywords : *Chronic obstructive pulmonary disease, Prevalence of C. pneumoniae antibodies, Incidence of C. pneumoniae antibodies, Healthy elderly, Elderly COPD patients*

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Chronic obstructive pulmonary disease (COPD) is a common cause of disability in older patients in Thailand and worldwide [1, 2]. The high prevalence and incidence of COPD among urban older persons of the Bangkok Metropolis has been reported and they increased with increasing age[3]. Most of the morbidity, mortality and healthcare costs of COPD patients is related to the exacerbation of COPD which is reported to happen at an average of one to four times per year [4-6]. It is estimated that about half of the acute exacerbations are definitely due to bacterial infection[7]. *Chlamydia pneumoniae* is a gram-negative, obligatory intracellular bacteria which can cause a variety of acute and chronic respiratory infections in humans. Seroepidemiological studies have indicated that *C. pneumoniae* infection is common and occurs throughout the world[8-10]. The prevalence of infection due to this organism varies between study regions, age and diseases. In Thailand, the prevalence and incidence of *C. pneumoniae* antibody in elderly COPD patients has never been reported. The aim of the present study was to determine the prevalence and incidence of

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C. pneumoniae antibodies in elderly COPD patients compared to that in a healthy elderly control group.

Material and Method

Subjects

Elderly patients with chronic obstructive pulmonary disease (COPD) who regularly attended the Chest Clinic at Siriraj Hospital from June, 1997 until September, 1998 were enrolled in the present study. Patients were eligible for this trial if they had a clinical diagnosis of COPD together with a forced expiratory volume in one second (FEV_1) of less than 70% of the forced vital capacity (FVC) and had less than a 15% increase of FEV_1 after using an inhaled bronchodilator. The participants were from the Integrated Health Research Program for the Elderly (IHRE), the 12th project of 16 projects that are part of the megaproject on healthcare for the elderly at Siriraj Hospital. One hundred and thirty-one elderly subjects from communities within a radius of 10 km around Siriraj Hospital (they were healthy subjects from the 13th project of the IHRE) participated as the control group in the present study. Determination of the prevalence and incidence of *C. pneumoniae* antibodies in the COPD and control groups was done using a cross sectional study and a cohort study, respectively.

Specimens

To assess the prevalence and incidence of *C. pneumoniae* infection serum samples were collected from the subjects and evaluated for the presence of IgG, IgA and IgM antibodies to *C. pneumoniae* at the time of enrollment in the study (B_0) and after one year later (B_1) from the subjects who tested negative for *C. pneumoniae* antibodies at B_0. Indirect enzyme-linked immunosorbent assay (ELISA, Savyon, Israel) was used to determine the presence of IgG, IgM, and IgA antibodies to *C. pneumoniae* in the sera. Each well of the ELISA-plate was coated with purified *C. pneumoniae* (TW-183) elementary body antigens. Horseradish peroxidase conjugate was used as an enzyme to catalyze the TMB-substrate. The results were read for the absorbency values at 450 nm. Cut-off index (COI) > 1.1 was interpreted as positive for the existence of *C. pneumoniae* antibody. The prevalence of *C. pneumoniae* infection was evaluated by determining the frequency of an existing *C. pneumoniae* IgG or IgM or IgA antibodies in the sera (B_0) obtained at the beginning of the study. The incidence was identified by the frequency of a new case of *C. pneumoniae* infection in COPD patients and in the control group who had seroconversion during the one year period.

Statistical analysis

Chi-square and unpaired student *t*- test were applied for testing the antibody frequencies and the mean COI between COPD patients and the control group, respectively.

Results

The demography of the 127 COPD patients and the 131 control subjects is shown in Table 1. The male to female ratio as well as the percentage of smokers were higher in the COPD patients than in the control group. The pulmonary function was measured in the COPD patients; mean $\pm$ SD of FEV_1 and FVC was 1.2 $\pm$ 0.5 litre and 2.4 $\pm$ 0.6 litre, respectively. Seroprevalence of *C. pneumoniae* IgG or IgA or IgM was detected in 96.1% of the COPD patients which was significantly higher than in the control group (75.6%) (Table2). Prevalence of individual *C. pneumoniae* IgG, IgA and IgM in elderly COPD vs healthy control was 85.5% vs 66.4% ($p < 0.01$), 85.0% vs 51.1% ($p < 0.01$) and 3.9% vs 0%, respectively. Within both groups the frequency of antibodies to *C. pneumoniae* between males and females was not significantly different (Table2). The level of IgG, IgA and IgM antibody to *C. pneumoniae* was demonstrated by mean COI (Table 3). Mean IgA COI in COPD patients (2.4 $\pm$ 1.0) was higher than in the control group (1.8 $\pm$ 0.9) ($p < 0.05$).

There were five COPD patients and thirty-two controls with seronegative sera (B_0). One year following the beginning of the study, sera (B_1) could be collected only from 31 subjects. *C. pneumoniae* infection was found in 1 of 3 COPD patients (33%) and in 19 of 28 subjects in the control group (67.9%);

Table 1. Demographic data, smoking history of COPD elderly patients and control group

	COPD patients	Controls
No. of subjects	127	131
Sex, Male:Female	17:1	0.75:1
Age,mean SD (yr)	69 8	65 5
Smoking (%)		
Non-smokers	2.3	32.8
Ex-smokers	86.6	11.2
Current smokers	11.1	6.2
Unknown	-	49.8

Table 2. Prevalence of *C.pneumoniae* antibody in COPD patients and the control group in the first sera samples(B_0)

Subjects	Sex	No. of subjects with positive *C.pneumoniae* antibody (%)			
		IgG	IgM	IgA	IgG or IgM or IgA
COPD	Male	102(86.4)	5(4.2)	102(86.4)	113(95.8)
	Female	7(77.8)	0	6(66.7)	9(100)
	Total	109(85.8)*	5(3.9)	108(85.0)*	122(96.1)*
Control	Male	42(75)	0	35(62.5)	46(82.1)
	Female	45(60)	0	32(42.7)	53(70.7)
	Total	87(66.4)	0	67(51.1)	99(75.6)

* $p < 0.01$, prevalence of *C. pneumoniae* antibody in COPD patients compared with control

Table 3. Mean cut off index (COI) of positive antibody to *C.pneumoniae* in COPD patients and control group as determined by ELISA tests

Subjects	Sex	Mean COI of *C.pneumoniae* 1.96SE		
		IgG	IgM	IgA
COPD	Male	1.8 0.5	1.5 0.5	2.4 1.0
	Female	1.8 0.4	-	2.4 1.0
	Total	1.8 0.5	1.5 0.5	2.4 1.0*
Control	Male	2.1 0.5	-	1.7 0.8
	Female	1.7 0.5	-	1.9 1.0
	Total	1.9 0.5	-	1.8 0.9

* $p < 0.05$, level of *C. .pneumoniae* IgA antibody in COPD patients compared with control

there was no significant difference in both groups ($p = 0.28$).

Discussion

High seroprevalence of *C. pneumoniae* was demonstrated in healthy and COPD elderly groups. The seroprevalence of *C. pneumoniae* was significantly increased in the COPD group compared to the control group. The prevalence of *C. pneumoniae* infection depends on the subject's age, geographic area of residence and the presence of other chronic diseases. More endemic *C. pneumoniae* infection in Asia has been reported than in Western countries[11]. A high prevalence of *C. pneumoniae* IgG (75.8%) in the elderly control group was reported in the present study which was approximately the same as that found in the elderly population in Seattle, in the United States of America (73%)[12]. The present result was not consistent with that reported by Leowattana et al[13]. They found that the prevalence of *C. pneumoniae* IgG in 11 healthy Thai blood donors aged over 61 was 91%. This may be due to the low number of control subjects enrolled in their study. Moreover, the influence of gender on the seroprevalence of *C. pneumoniae* in adults and elderly has been reported in some countries but it was not observed in the present study or in Japan[10, 14].

The seropevalence of *C. pneumoniae* antibodies, especially IgG (85.8% vs 66.4%) and IgA (85% vs 51.1%), increased in the COPD group compared to the control group. Acute infection of *C. pneumoniae* can be demonstrated by the presence of *C. pneumoniae* IgM and it was found in 3.9% of COPD patients in the present study. The frequency of IgM (4%) in the serum of patients with exacerbation of COPD was also reported by Blasi et al[15]. The high prevalence of *C. pneumoniae* IgG and IgA as well as the presence of IgM antibodies reflects the important role of *C. pneumoniae* infection and that it can probably cause acute or chronic persistent infection in COPD patients. High frequency and high titer of *C. pneumoniae* IgG antibodies were found in European COPD patients[15]. The authors found a high frequency but not a high level of *C. pneumoniae* IgG antibodies in the COPD patients compared to the control, although the existence of IgM antibodies in the COPD patients was approximately the same as that found in the previously referred to study. The association of chronic, persistent infection of *C. pneumoniae* with chronic diseases has been observed and demonstrated by the existence of IgA antibody. A high frequency and high level of *C. pneumoniae* IgA, a marker for active on-going infection or chronic persistent *C. pneumoniae* infection, was found in COPD patients and other chronic disease such as coronary artery heart disease which has also been reported in Thailand and worldwide[13,16]. *C. pneumoniae* IgA disappears within a few months after acute infection but it will continue to exist in the sera of patients who have chronic, persistent infection.

Seroconversion of *C. pneumoniae* antibody was detected in both the elderly COPD and control groups. The age curve of incidence of pneumonia caused by *C. pneumoniae* increased in the elderly.

The incidence of *C. pneumoniae* infection is mostly investigated in children more than in the elderly[9]. The high frequency of seroconversion of *C. pneumoniae* reported in the present study was a preliminary study for the incidence of *C. pneumoniae* infection in the elderly COPD and healthy group. Since the number of negative *C. pneumoniae* antibody sera was limited, the incidence of *C. pneumoniae* in the elderly should be studied further.

Conclusion

A high prevalence of *C. pneumoniae* infection was found in both healthy and COPD elderly groups. No relationship between gender and prevalence of *C. pneumoniae* infection was demonstrated in the present study. A high seroprevalence of *C. pneumoniae* antibodies in COPD elderly patients suggests that it plays a tangible role in causing acute or chronic persistent infections in COPD patients. In clinical practice, the antibiotic of choice for acute exacerbation of COPD should probably be considered.

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References

1. Saenghirunvattana S, Kongngeon V, Aeimrerksiri B, Jerathamopart P, Thamakumpee K, Reechaipichitkul W, et al. Chronic obstructive pulmonary diseases in Thailand: incidence, prevalence, present status and future trends. J Med Assoc Thai 2001;84:1407-11.
2. Fielding JE. Smoking: health effects and control (1). N Engl J Med 1985;313:491-8.
3. Maranetra KN, Chuaychoo B, Dejsomritrutai W, Chierakul N, Nana A, Lertakyamanee J, et al. The prevalence and incidence of COPD among urban older persons of Bangkok Metropolis. J Med Assoc Thai 2002;85:1147-55.
4. Niederman MS. Introduction: COPD-the role of infection. Chest 1997;112:301S-2S.
5. Seneff MG, Wagner DP, Wagner RP, Zimmerman JE, Knaus WA. Hospital and 1-year survival of patients admitted to intensive care units with acute exacerbation of chronic obstructive pulmonary disease. JAMA 1995;274:1852-7.
6. Anthonisen NR, Manfreda J, Warren CP, Hershfield ES, Harding GK, Nelson NA. Antibiotic therapy in exacerbations of chronic obstructive pulmonary disease. Ann Intern Med 1987;106:196-204.
7. Schentag JJ, Pharm D.,Glenn S.,Tillotson, G. S. Antibiotic selection and dosing for the treatment of acute exacerbations of COPD. Chest 1997;112:314S-9S.
8. Laurila A, Bloigu A, Nayha S, Hassi J, Leinonen M, Saikku P. Chlamydia pneumoniae antibodies and serum lipids in Finnish men: cross sectional study. BMJ 1997;314:1456-7.
9. Grayston JT. Infections caused by Chlamydia pneumoniae strain TWAR. Clin Infect Dis 1992;15:757-61.
10. Kanamoto Y, Ouchi K, Mizui M, Ushio M, Usui T. Prevalence of antibody to Chlamydia pneumoniae TWAR in japan. J Clin Microbiol 1991;29:816-8.
11. Grayston JT, Kuo CC, Coulson AS, Campbell LA, Lawrence RD, Lee MJ, et al. Chlamydia pneumoniae (TWAR) in atherosclerosis of the carotid artery. Circulation 1995;92:3397-400.
12. Beaty CD, Grayston JT, Wang SP, Kuo CC, Reto CS, Martin TR. Chlamydia pneumoniae, strain TWAR, infection in patients with chronic obstructive pulmonary disease. Am Rev Respir Dis 1991;144:1408-10.
13. Leowattana W, Mahanonda N, Bhuripunyo K. The prevalence of Chlamydia pneumoniae antibodies in coronary artery diseases. J Inf Dis and Antimicrob agents 2000;17:1-6.
14. Kuo CC, Jackson LA, Campbell LA, JT. G. Chlamydia pneumoniae (TWAR). Clin Microbiol Rev 1995;8:451-61.
15. Blasi F, Legnani D, Lombardo VM, Negretto GG, Magliano E, Pozzoli R, et al. Chlamydia pneumoniae infection in acute exacerbations of COPD. Eur Respir J 1993;6:19-22.
16. von Hertzen L, Isoaho R, Leinonen M, Koskinen R, Laippala P, Toyryla M, et al. Chlamydia pneumoniae antibodies in chronic obstructive pulmonary disease. Int J Epidemiol 1996;25:658-64.

ความชุกและอุบัติการณ์ของคลามัยเดีย นิวโมนิอิ แอนติบอดีในผู้สูงอายุปรกติ และผู้ป่วยที่มีโรคปอดอุดกั้นเรื้อรัง

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เชื้อคลามัยเดีย นิวโมนิอิ (Chlamydia pneumoniae) เป็นแบคทีเรียที่ต้องอาศัยสิ่งมีชีวิตในการเจริญเติบโต และสามารถก่อโรคทางเดินหายใจแบบเฉียบพลันและแบบเรื้อรังได้ การติดเชื้อเรื้อรัง และการติดเชื้อกลับเป็นซ้ำจากคลามัยเดีย นิวโมนิอิ ที่ระบบทางเดินหายใจในวัยหนุ่มสาวอาจทำให้มีความเสี่ยงต่อการเกิดโรคปอดอุดกั้นเรื้อรังเมื่ออยู่ในวัยสูงอายุได้ การศึกษานี้มีวัตถุประสงค์ที่จะหาความชุก และอุบัติการณ์ของแอนติบอดีต่อคลามัยเดีย นิวโมนิอิ ในผู้สูงอายุที่มีโรคปอดอุดกั้นเรื้อรังจำนวน 127 ราย เปรียบเทียบกับกลุ่มควบคุมจำนวน 131 ราย โดยการตรวจหา IgG, IgM และ IgA แอนติบอดีต่อเชื้อคลามัยเดีย นิวโมนิอิ ด้วยวิธี ELISA ผลการศึกษาพบว่าความชุกของแอนติบอดีต่อคลามัยเดีย นิวโมนิอิ ทั้งชนิด IgG หรือ IgA หรือ IgM ในผู้ป่วยสูงอายุที่มีโรคปอดอุดกั้นเรื้อรังเป็นร้อยละ 96.1 มากกว่าในกลุ่มควบคุมซึ่งพบเพียงร้อยละ 75.6 ($p < 0.01$) โดยความชุกของแอนติบอดีแต่ละชนิดคือ IgG, IgA และ IgM ในผู้ป่วยสูงอายุที่มีโรคปอดอุดกั้นเรื้อรังเปรียบเทียบกับกลุ่มควบคุมเป็นร้อยละ 85.8 กับ 66.4, 85.0 กับ 51.1 และ 3.9 กับ 0 ตามลำดับ การศึกษาอุบัติการณ์ หรือ seroconversion ของแอนติบอดีต่อเชื้อคลามัยเดีย นิวโมนิอิ ในผู้ที่ตรวจไม่พบแอนติบอดีในซีรั่มครั้งแรกของผู้ป่วยโรคปอดอุดกั้นเรื้อรัง และผู้สูงอายุปรกติ ตรวจพบแอนติบอดีต่อคลามัยเดีย นิวโมนิอิ ในซีรั่มครั้งที่สองซึ่งเก็บห่างจากซีรั่มครั้งแรกนาน 1 ปี เป็นร้อยละ 33 และ 67.9 ตามลำดับ ผลการศึกษาที่พบว่าความชุกและอุบัติการณ์ของแอนติบอดีต่อคลามัยเดีย นิวโมนิอิ มีความถี่สูงในผู้ป่วยสูงอายุที่มีโรคปอดอุดกั้นเรื้อรังแสดงว่า เชื้อคลามัยเดีย นิวโมนิอิ อาจมีความสำคัญในการก่อโรคติดเชื้อที่ระบบทางเดินหายใจในผู้ป่วย ดังนั้นการใช้ยาปฏิชีวนะต่อการติดเชื้อคลามัยเดีย นิวโมนิอิ จึงอาจมีความสำคัญในผู้ป่วยสูงอายุที่มีโรคปอดอุดกั้นเรื้อรัง