Seroprevalence of *Chlamydia pneumoniae* Infection in Thailand

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Abstract

   Chlamydia pneumoniae (*C. pneumoniae*) is an emerging infectious agent, with a spectrum of clinical manifestations, and it has recently been tentatively linked to atherosclerosis. In order to describe the seroprevalence of *C. pneumoniae* in Thailand, we evaluated 1,798 normal healthy subjects (aged 20-93 years) for anti-*C. pneumoniae* IgG and IgA. Specific antibodies were measured by the fully automated ELISA method using elementary bodies of *C. pneumoniae* as an antigen. IgG antibodies against *C. pneumoniae* were presented in 55.16 per cent of men and 41.63 per cent of women. Anti- *C. pneumoniae* IgA were presented in 31.50 per cent of men and 27.49 per cent of women. The prevalence of IgG antibody increased with age and reached 78 per cent in subjects between 51-93 years. The pattern of IgA antibody was a saddle shape, which indicated 2 peaks of chronic infection. Our results suggested that *C. pneumoniae* infection was common in Thailand. The high prevalence of evidence of exposure to *C. pneumoniae* may have implications for the prevention of cardiovascular disease if further study concludes that infection with this organism is a risk for cardiovascular disease.

Key word: Seroprevalence, *C. pneumoniae*, Thailand

   Chlamydia pneumoniae (*C. pneumoniae*) is an obligate intracellular, gram-negative bacterium[1]. It has been recognized as a cause of respiratory tract infections and is considered the most common nonviral intracellular human respiratory pathogen[2,3]. *C. pneumoniae* is involved in 5 – 15

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per cent of community-acquired pneumonia, and recent data indicate its relevance in severe pneumonia and as a respiratory pathogen in immuno-compromised subjects. A causal role for *C. pneumoniae* in the initiation, exacerbation and promotion of asthma has been suggested. Another field of potential great social impact is the possible involvement of *C. pneumoniae* in the pathogenesis of atherosclerosis and related cardiovascular diseases (4-8). This study aimed to evaluate the prevalence of anti-*C. pneumoniae* IgG and IgA in healthy individuals in Thailand.

MATERIAL AND METHOD
Blood samples
A total of 1,798 normal healthy subjects (aged 20-93 years) participated in this study. There were 765 (42.55%) men and 1,033 (57.45%) women. The mean age of men was 34.87 $\pm$ 9.82 years and of women was 37.89 $\pm$ 16.29 years. Fasting blood samples were drawn and placed into 10-ml tubes. These serum sample tubes were allowed to clot and the serum was separated by high-speed centrifugation for 15 minutes. Serums were stored at $-20^{\circ}C$ until determination of anti- *C. pneumoniae* IgG and IgA.

Antibodies to *Chlamydia pneumoniae*
An ELISA method (SeroCP IgG, IgA tests: Savyon Diagnostics, Ashdod, Israel) was used to measure specific anti-*C. pneumoniae* IgG and IgA. Fifty μL of positive control, 50 μL of 1:105 diluted specimens and 3 x 50 μL of negative control were added to the microtiter strips which are coated with intact *C. pneumoniae* elementary bodies. Cover the ELISA plate and incubate at $37^{\circ}C$ for 1 hour in 100 per cent humidity. After washing 3 times with wash buffer, add 50 μL of 1:300 diluted horseradish peroxides (HRP) which conjugated with anti-human immunoglobulin. Cover the plate and incubate for 1 hour at $37^{\circ}C$ in 100 per cent humidity. After washing 3 times with wash buffer, add 100 μL of tetramethylbenzidine (TMB) substrate, cover the plate and incubate at room temperature for 15 minutes. After that, add 100 μL of 1 M H_2SO_4 to stop the reaction. Measure the strip at 450 nm in which positive control absorbance value should be $\geq$ 1.00 and the average absorbance value of negative control should be $>$ 0.10 and $\leq$ 0.40.

Table 1. Seroprevalence of *C. pneumoniae* antibody IgG and IgA distributed by age group.

Age, years	n	IgG (%)	IgA (%)
20 – 30	701	45.79	30.96
31 – 40	727	46.49	24.35
41 – 50	177	50.28	24.86
51 – 60	39	61.54	18.42
61 – 70	39	53.85	48.72
71 – 80	67	46.27	47.76
81 – 93	48	58.33	60.42
Total	1,798	47.39	29.20

RESULTS
The aged-related prevalence of anti-*C. pneumoniae* IgG and IgA were shown in Table 1. It was 45.75 – 50.28 per cent for IgG antibodies and 24.35 – 30.96 per cent for IgA antibodies in subjects aged between 20 – 50 years. In older subjects, the IgG and IgA antibodies response increased with age and reached 61.54 per cent and 60.42 per cent respectively. Overall, *C. pneumoniae* IgG antibody was presented in 55.16 per cent of men and 41.63 per cent of women. In case of *C. pneumoniae* IgA antibody, it was presented in 31.50 per cent of men and 27.49 per cent of women. Fig. 1 shows that the seroprevalence of IgG antibody reaches 55 per cent in men and 41 per cent in women by 20 years of age. After that, it continues to rise until it reaches 77 per cent in men and 51 per cent in women older than 60 years of age. For IgA antibody, the positive rate reaches 36 per cent in men and 27 per cent in women by 20 years of age. Then, it continues to rise with age until it reaches about 48 per cent in men and 49 per cent in women older than 60 years of age (Fig. 2).

DISCUSSION
C. pneumoniae is a relatively new species of Chlamydia, first found in Taiwan in 1965, and later found to be a causative microorganism of respiratory tract infection at Washington University, Seattle, USA. The development of a species-specific microimmunofluorescence (MIF) test made it possible to determine specific antibody prevalence. However, this technique requires highly trained, experienced personnel and depends on individual adjustment for positive or negative results.
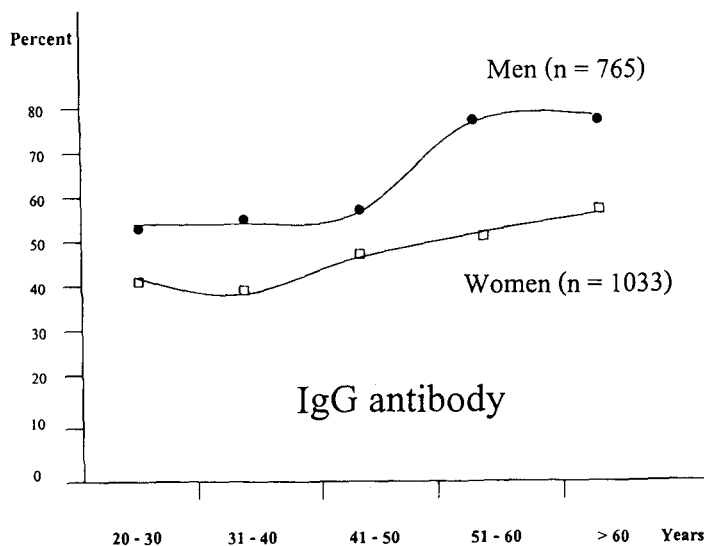
Percent

Men (n = 765)

Women (n = 1033)

IgG antibody

20 - 30   31 - 40   41 - 50   51 - 60   > 60   Years

Fig. 1.   Prevalence of *C. pneumoniae* IgG antibody by age and sex in 1798 normal healthy persons.

Percent

IgA antibody

Men (n = 765)

Women (n = 1033)

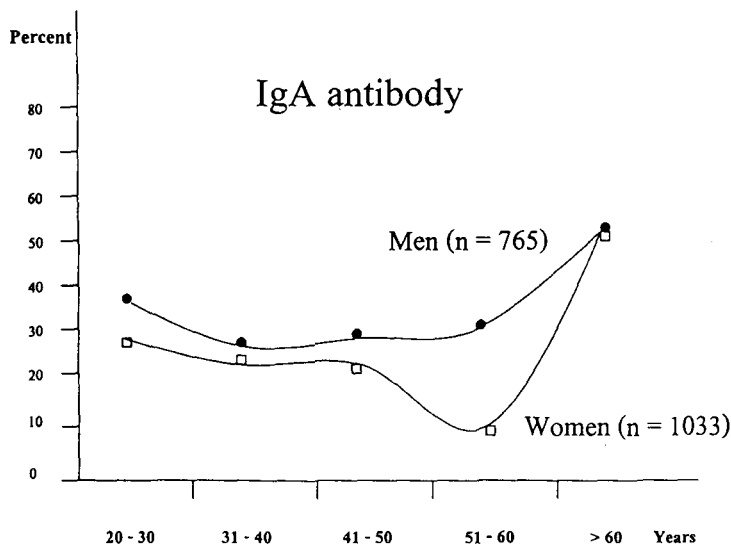20 - 30   31 - 40   41 - 50   51 - 60   > 60   Years

Fig. 2.   Prevalence of *C. pneumoniae* IgA antibody by age and sex in 1798 normal healthy persons.

Recently, purified elementary bodies for *C. pneumoniae* have been applied in the development of a commercially available ELISA for anti- *C. pneumoniae* IgG and IgA. We decided to use this technique for our prevalence study here in Thailand.

Our results show that the prevalence of antibodies to *C. pneumoniae* in a Thai population is common and similar to that found in many countries around the world. Anti- *C. pneumoniae* IgG antibody increased with age and reached 78

per cent in men and 51 per cent in women subjects. The pattern of age related prevalence in Thailand is the same as reports from Seattle, Denmark, Japan, Italy, Korea, Canada and other countries[9-17]. Moreover, we also found that the prevalence of *C. pneumoniae* antibody in men was higher than in women. According to Grayston, *C. pneumoniae* may cause about 10 per cent of all community-acquired pneumonia in adults and 5 per cent of bronchitis and sinusitis[18]. The antibody from first infection is usually lost in 3-5 years. However, we found that IgG antibody did not decrease but increased with age. This prevalence can be best explained by frequent reinfection. Persistent chronic infection may contribute to the high prevalence.

We also evaluated IgA antibody in these subjects and found that the prevalence of IgA antibody in men was higher than in women. However, the pattern of IgA antibody differed slightly from IgG antibody. This pattern was similar to the saddle-shaped epidemiology of *Haemophilus influenzae*, where 2 peaks of chronic infection were found. There was a trend for the IgA antibody to increase rapidly when these subjects reach the age of 60 years or more. This phenomenon confirmed that chronicity is a hallmark of chlamydia infection, since the chlamydia life cycle facilitates establishment of chronic infection[19-21]. The infectious component, the metabolically inactive elementary body, is not affected by antibiotics and may exist in the body for an unknown period before infecting a new cell. For this reason, *C. pneumoniae* infection is difficult to treat.

In conclusion, the infection of *C. pneumoniae* was found to be highly prevalent in Thailand. Young adults were found to have a peak of primary infection, while the older subjects had a peak of reinfection. In this regard, *C. pneumoniae* infection may be involved in the pathogenesis of atherosclerosis in-patients who suffered from cardiovascular disease and antibiotic treatment would be considered in the near future.

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อุบัติการณ์ของการติดเชื้อ *คลามีเดีย นิโมนิเอ* ในประเทศไทย

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การติดเชื้อ *คลามีเดีย นิโมนิเอ* สามารถแสดงอาการได้หลายแบบ เช่น หวัด, หลอดลมอักเสบ, ไซนัสอักเสบ หรือ ปอดบวม และยังมีส่วนเกี่ยวข้องกับการเกิดภาวะหลอดเลือดแข็งตัวด้วย คณะผู้วิจัยต้องการศึกษาถึงอุบัติการณ์ของ การติดเชื้อ *คลามีเดีย นิโมนิเอ* ในประเทศไทยว่ามีความเหมือนหรือแตกต่างจากอุบัติการณ์ที่เกิดขึ้นในต่างประเทศ โดยศึกษาในอาสาสมัครสุขภาพดีอายุตั้งแต่ 20 ปี ถึงอายุ 93 ปีจำนวน 1798 รายเป็นผู้ชาย 765 ราย และผู้หญิง 1033 ราย โดยทำการตรวจหาภูมิต้านทานต่อเชื้อ *คลามีเดีย นิโมนิเอ* ชนิด IgG และ IgA โดยวิธี ELISA ซึ่งใช้ elementary bodies ของเชื้อ *คลามีเดีย นิโมนิเอ* เป็นแอนติเจน พบว่าภูมิต้านทานชนิด IgG ให้ผลบวกในผู้ป่วยชาย 55.16 เปอร์เซ็นต์ ในผู้หญิง 41.63 เปอร์เซ็นต์ ส่วนภูมิต้านทานชนิด IgA ให้ผลบวกในผู้ชาย 31.50 เปอร์เซ็นต์ ในผู้หญิง 27.49 เปอร์เซ็นต์ นอกจากนั้นยังพบว่า ภูมิต้านทานชนิด IgG ค่อยๆเพิ่มอุบัติการณ์ขึ้นตามอายุของอาสาสมัครที่เพิ่มขึ้นและเพิ่มสูงสุดที่ 78 เปอร์เซ็นต์ เมื่ออายุอยู่ระหว่าง 50 – 93 ปี ส่วนภูมิต้านทานชนิด IgA มีอุบัติการณ์ไม่สูงเท่าภูมิต้านทานชนิด IgG และ มีรูปแบบของอุบัติการณ์เมื่อเทียบกับอายุเป็นแบบอานม้าโดยมีช่วงของการติดเชื้อซ้ำ 2 ช่วงอายุคือ 20 – 30 ปี และ 51 – 93 ปี ช่วงอายุระหว่าง 31 – 50 ปี มีอุบัติการณ์ลดลงเล็กน้อยทั้งในเพศหญิงและเพศชาย โดยสรุปพบว่า การติดเชื้อ *คลามีเดีย นิโมนิเอ* พบได้บ่อยในประเทศไทยเช่นเดียวกับที่พบในประเทศต่างๆทั่วโลก หากพิสูจน์ได้อย่างชัดเจนว่า การติดเชื้อ *คลามีเดีย นิโมนิเอ* มีส่วนในการก่อโรคระบบหัวใจและหลอดเลือดจริง การป้องกันการติดเชื้อตลอดจนการรักษา ด้วยยาปฏิชีวนะ ย่อมส่งผลดีต่อผู้ป่วยกลุ่มนี้อย่างแน่นอน

คำสำคัญ : อุบัติการณ์, การติดเชื้อคลามีเดีย นิโมนิเอ, ประเทศไทย

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