Serological Study of *Mycoplasma pneumoniae* Infections

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Mycoplasma pneumoniae antibody was determined in 811 sera of different patients admitted to Siriraj Hospital with respiratory tract infection from July 1, 2000 to August 31, 2003 by agglutination with gelatin particle agglutination test kit (SERODIA-MYCO II, Fujirebio Inc. Japan) in microtiter plates. Three hundred and three sera were positive (37.36%). The five most positive titer were found in patients 5-9 yr (40.26%), followed by patients 1-4 yr (24.75%), 10-14 yr (19.80%), 30-39 yr (5.28%) and 20-29 yr (3.96%). The positive titers ranged from 40 to > 20480. Female: male ratio in positive patients was approximately the same (1.19:1). High titers (≥ 320) were found in 146 out of 303 patients (48.18%). The infection was mostly found in children aged 5-9 yr. Detection of antibody to M. pneumoniae infection showed that 37.36 % of patients who were suspected of having atypical bacterial pneumonia were positive.

Keywords : *Serological study, Mycoplasma pneumoniae, Atypical bacterial pneumonia*

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Mycoplasma pneumoniae infection is a disease of the upper and lower respiratory tracts. Cough, fever and headache may persist for several weeks. Convalescence is slow. Mycoplasmas are spherical to filamentous cells with no cell wall. There is an attachment organelle at the tip of the filamentous *M. pneumoniae, M. genitalium* and several other pathogenic mycoplasmas. Fried-egg-shaped colonies are seen on agar. Mycoplasmas are fastidious bacteria. Many require cholesterol, a unique property among prokaryotes[1,2].

Mycoplasma pneumoniae attaches to sialoglycoprotein or sialoglycolipid receptors of the tracheal epithelium via protein adhesion on the attachment organelle. The major adhesin is a 170-kilodalton protein, named P1. Hydrogen peroxide and superoxide radicals (O_2-) excreted by the attached *M. pneumoniae* cause oxidative tissue damage. Pneumonia is induced largely by local immunologic and phagocytic responses to this organism. Sequelae of *M. pneumoniae* infection is mainly hematologic and neurologic. Macrophage activation, cytokine induction and superantigen properties of some mycoplasmal cell components can be considered as pathogenicity factors[3-5].

Mycoplasma pneumoniae infection occurs worldwide and is more prevalent in the colder months. It affects mainly children aged 5 to 9 years. It is spread by close personal contact and has a long incubation period[3,6-10].

Culture of *M. pneumoniae* from sputum or a throat swab is possible, but very slow; therefore diagnosis is usually based on serologic tests. Tests using diagnostic DNA probes and amplification of specific genomic mycoplasma sequences by the polynmerase chain reaction (PCR) are being developed[11-13].

There is no certified vaccine for *M. pneumoniae*. Treatment with erythromycin or tetracycline is effective in reducing symptoms in *M. pneumoniae* infection[3].

Material and Method

Eight hundred and eleven sera from different patients admitted to Siriraj Hospital with respiratory tract infection suspicious of having atypical bacterial pneumonia by physicians during a 3-year period i.e., July 1, 2000 to August 31, 2003 were studied for *M. pneumoniae* antibody. The test was performed by the Serodia Myco II gelatin particle agglutination test

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(Fujirebio, Japan). It is based on the principle that gelatin particles sensitized with *M. pneumoniae* cell membrane components are agglutinated in the presence of *M. pneumoniae* antibody. Serum samples were inactivated at 56 ^{0}C for 30 minutes. Twenty-five microliter serum samples were two-fold diluted to give dilutions of 1 in 10 to 1 in 10240. Sensitized and unsensitized lyophilized gelatin particles were suspended in diluent. Twenty-five microliter drops of the unsensitized particles were added to the 1 in 10-serum dilution to give a final dilution of 1 in 20. Twenty-five ml drops of the sensitized particle suspension were then added to the remaining wells giving final dilutions of 1 in 40 to 1 in 20480. The plates were shaken for 30 seconds and then covered and left undisturbed on a level surface at room temperature for three hours or overnight[14].

The test was initially calibrated using the control sera dilution series. Each batch of tests included control wells containing 25 ml of diluent and 25 ml of the particles suspensions and dilutions of a reactive control serum of known titer, supplied with the kit.

Buttons or compact, smooth rings of particles in the bottom of the wells were read as negative agglutination patterns and a more extensive ring as positive. Titers of $\geq$ 40 were regarded as positive for *M. pneumoniae* antibody.

Results

Table 1 shows 811 serum samples were tested with Serodia Myco II particle agglutination. Three hundred and three of 811 (37.36%) serum samples were positive for *M. pneumoniae* antibody. The five most positive titers were found in patients 5-9 yr (40.26%) with median age was 8.1 years, followed by patients 1-4 yr (24.75%), 10-14 yr (19.80%), 30-39 yr (5.28%) and 20-29 yr (3.96%). Table 2 shows that the positive titers ranged from 40 to> 20480. Female:male ratio in positive patients was approximately the same (1.19:1). High titers (> 320) were found in 146 out of 303 patients (48.18%). The highest titer (> 20480) was found in 7 patients i.e., 4.29% of all sera tested.

Table 1. Positive serum from patients of different age-groups

Age (yr)	Number of positive serum			Percent of positive serum
	Male	Female	Total	
0-1	1	0	1	0.33
1-4	43	32	75	24.75
5-9	60	62	122	40.26
10-14	20	40	60	19.80
15-19	2	3	5	1.65
20-29	5	7	12	3.96
30-39	3	13	16	5.28
40-49	0	6	6	1.98
50-60	1	1	2	0.66
> 60	3	1	4	1.32
Total	138	165	303	100.00
Median age (yr)	7.1	9.1	8.1	

Table 2. Positive serum from patients with different titers

Titer	Number of positive serum			Percent of positive serum
	Male	Female	Total	
40	13	21	34	11.22
80	28	36	64	21.12
160	24	35	59	19.47
320	21	20	41	13.53
640	12	13	25	8.25
1280	6	5	11	3.63
2560	10	5	15	4.95
5120	4	12	16	5.28
10240	9	8	17	5.61
20480	4	4	8	2.64
> 20480	7	6	13	4.29
Total	138	165	303	100.00

Discussion

Isolation of *M. pneumoniae* from atypical bacterial pneumonia is considered to be the gold standard for diagnosis. However, isolation requires 2-4 weeks, which limits its clinical usefulness. Serology is probably the most frequently used method to diagnose *M. pneumoniae* infections. The cold agglutination test remains a fairly sensitive and specific test for the diagnosis of *M. pneumoniae* infection[5]. This type of pneumonia usually occurs in young people[7]. Community-acquired pneumonia has a polymicrobial etiology, of which the prevalence of *Mycoplasma pneumoniae* was 35 %[6]. IgM serology diagnosed a *current M. pneumoniae* pneumonia in many children. The age of 21% of the patients diagnosed as having *M. pneumoniae* pneumonia was < 5 years[10]. This observation is in contrast to the recent Canadian guidelines for the diagnosis and managenent of community-acquired pneumonia, which recommended detection of *M. pneumoniae* IgM only in $\geq$ 5 years of age[15]. Gendrel et al[16] diagnosed *M. pneumoniae* infection in 42% of the

patients. The present study was focused on hospitalized patients, but interestingly, the authors had a rate of 37.36% *M. pneumoniae*. The incidence of overt disease caused by *M. pneumoniae* was dependent on the prevalence of the infection in the population and appears to be related to age[5]. Longitudinal studies found that the rate of endemic *M. pneumoniae* pneumonia was highest in children 5-9 years of age, the next highest rate was in children 10-14 years of age[17]. A smaller study from Baltimore, identified *M. pneumoniae* infection in only 1.9% of the patients[18]. Evidence of infection was a fourfold increase in titer, or a titer of 1:160 or greater. It was not specified if this assay (passive agglutination test, Serodia-Myco II; Fujirebio, Japan) detected IgG or IgM antibodies[5].

Conclusion

Detection of antibodies to *Mycoplasma pneumoniae* infection showed that 37.36% of patients who were suspected of having atypical bacterial pneumonia were positive. The infection was mostly found in children aged 5-9 yr (40%). Seven out of 55 (12.72%) patients demonstrated a fourfold titer increase between acute and convalescent phase sera taken at onset and several weeks later when sera were tested by using twofold serial dilutions.

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การศึกษาการติดเชื้อ Mycoplasma pneumoniae ด้วยวิธีซีโรโลยี

สมพร ศรีเฟื่องฟุ้ง, วนิดา เตชะไชยวิวัฒน์, เชิดศักดิ์ ธีระบุตร

การตรวจหาแอนติบอดีต่อเชื้อ Mycoplasma pneumoniae ในซีรั่มของผู้ป่วยระบบทางเดินหายใจในโรงพยาบาลศิริราชตั้งแต่ 1 กรกฎาคม 2543 ถึง 31 สิงหาคม 2546 จำนวน 811 ราย โดยใช้ชุดทดสอบ SERODIA-MYCO II (วิธี agglutination ด้วย gelatin particle) พบว่ามีตัวอย่างซีรั่ม 303 รายที่ให้ผลบวก (37.36%) ซึ่งเรียงตามลำดับช่วงอายุดังนี้คือ 5-9 ปี (40.26%), 1-4 ปี (24.75%), 10-14 ปี (19.80%), 30-39 ปี (5.28%) และ 20-29 ปี (3.96%) ผลทดสอบบวกจะอยู่ระหว่าง 1:40 ถึง 1: >20480 ผู้ป่วยหญิงจะให้ผลบวกใกล้เคียงกับผู้ป่วยชายในอัตราส่วน 1.19:1 ในจำนวนผู้ป่วยที่ให้ผลบวก 303 ราย มี 146 ราย (48.18%) ที่ให้ผลบวกไตเตอร์สูง (1: >320) ดังนั้นการติดเชื้อ M. pneumoniae จะพบมากที่สุดในเด็กอายุระหว่าง 5-9 ปี และการตรวจหาแอนติบอดีต่อเชื้อ M. pneumoniae ในผู้ป่วยที่สงสัยว่าเป็น atypical bacterial pneumonia จะให้ผลบวก 37.36%