Prevalence of Specific Antibodies to *Chlamydia pneumoniae* in Korea

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To clarify the endemic status of *Chlamydia pneumoniae* in Korea, the incidence of antibodies in 564 serum samples from healthy individuals, patients with respiratory infection, and cord blood specimens was evaluated. We conclude that *C. pneumoniae* infection is highly endemic in Korea and that this infection is associated with acute respiratory diseases.

*Chlamydia pneumoniae* is one of the new, emerging infectious agents, with a spectrum of clinical manifestations, including upper and lower respiratory tract infections, and it has recently been tentatively linked to atherosclerosis (2, 4, 15, 21). *C. pneumoniae* is spread through person-to-person transmission by droplet, and outbreaks of infection have been reported in families, schools, military barracks, and nursing homes (1, 8, 13). Infection with *C. pneumoniae* is usually mild or asymptomatic, but it can be severe, especially in the elderly, probably as a result of underlying illness (11).

*C. pneumoniae* is notoriously difficult to cultivate in a cell culture system. PCR assay, antigen enzyme immunoassay, and direct immunofluorescence tests have been described for *C. pneumoniae*; however, their efficiencies have not been properly validated (6, 11). The microimmunofluorescence (MIF) test is specific for *C. pneumoniae* and is the standard method for *Chlamydia* serology today. Little is known about the prevalence of *C. pneumoniae* antibodies in healthy individuals and the association between *C. pneumoniae* and respiratory infection in Korea. This study aimed to evaluate the prevalence of *C. pneumoniae* antibodies in healthy individuals and patients with acute respiratory disease in Seoul, Korea.

**Specimens.** We collected a total of 564 serum samples for *C. pneumoniae* antibody testing. Three hundred forty-nine serum samples were obtained from healthy individuals who had no acute respiratory diseases. Cord blood samples were collected from 70 healthy babies whose mothers did not have pelvic inflammatory disease or acute respiratory disease. One hundred forty-five patients with acute respiratory disease (98 with acute pneumonia, 24 with acute bronchitis, and 23 with acute pharyngitis) who visited Hanyang University Hospital in Seoul, Korea, from January 1996 to November 1997 were enrolled in this study. A diagnosis of acute pneumonia was made if there was a compatible clinical illness and if a pulmonary opacity was present on radiographs. All patients with acute bronchitis and acute pharyngitis were examined clinically.

**Antigen preparation.** *C. pneumoniae* (TW-183) was provided by the Centers for Disease Control and Prevention and propagated in HeLa cells. The elementary bodies (EBs) of *C. pneumoniae* were partially purified by differential centrifugation followed by gradient centrifugation in Percoll (17). The EBs were resuspended in a solution of 2% yolk sac in phosphate-buffered saline containing 0.02% formalin. The EBs and sonicated HeLa cells (negative control) were dotted on clean slides. The slides were dried at room temperature for 2 h and fixed in acetone for 15 min (3).

**Serologic testing.** An MIF test was used to measure chlamydial antibodies (3). The presence of chlamydial antibodies in the immunoglobulin M (IgM) or IgG serum fractions was detected with fluorescein isothiocyanate conjugates of antihuman IgM or IgG (Dako, Copenhagen, Denmark). Dots were evaluated for homogeneity and intensity of the fluorescence with a fluorescence microscope. The endpoint was the highest serum dilution with positive fluorescence. Fluorescence in the negative control (HeLa cells) negated other reactions at that dilution. Serological diagnosis of a previous infection was made when IgG antibody titers were 1:32 or higher. A single titer of anti-*C. pneumoniae* antibody of $\geq 1:512$ for IgG or $\geq 1:16$ for IgM was considered to indicate a recent infection. When high titers of antibody (IgG $\geq 1:512$) or positive IgM results were observed in healthy individuals, their records were examined to determine whether respiratory infection had occurred within 3 months of serum collection. The significance of the data was determined by the chi-square test. A probability value ($P$) of $<0.05$ was considered significant.

**Rheumatoid factor assay.** A false-positive MIF IgM antibody test may occur if the patient has circulating rheumatoid factor, the prevalence of which increases with age (24). Rheumatoid factor was detected with the Array 360 system (Beckman, Fullerton, Calif.). The sera positive for rheumatoid factor were absorbed with a goat anti-human IgG antibody reagent (Gaulloisorb; Gull Laboratories, Inc., Salt Lake City, Utah) as prescribed by the manufacturer and retested for the presence of *C. pneumoniae*-specific IgM antibody in the MIF test.

**Results.** The prevalence of antibodies to *C. pneumoniae* in the sera of healthy individuals was evaluated by the MIF test (Table 1). For cord blood, the antibody was detected in 50% of the samples. The antibody detection rate in healthy individuals was 52%, broken down by age as follows: $\leq 1$ year old, 39%; 2 to 5 years old, 11%; 6 to 10 years old, 22%; 11 to 20 years old, 44%; 21 to 40 years old, 53%; 41 to 50 years old, 64%; 51 to 60 years old, 71%; and $\geq 61$ years old, 80%. Overall, *C. pneumoniae* antibody was present in 53% of males and 51% of females. However, in subjects over 21 years of age, *C. pneumoniae* antibody was present in 66 of 94 males (70%) and 82 of 146 females (56%). There were 11 cases (3%) of recent infection with *C. pneumoniae* in healthy individuals, as determined by IgG, and 2 cases (0.6%) for IgM. Antibody titers of 1:256 or higher were observed in 18% of the healthy individuals who tested positive for the IgG antibody. On reviewing the past

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TABLE 1. C. pneumoniae antibodies according to age in healthy individuals and cord blood

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>No. of samples</th>
<th>No. (%) positive for IgG (≥1:32)</th>
<th>No. (%) of recent infections</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>IgG (≥1:512)</td>
</tr>
<tr>
<td>Cord blood</td>
<td>70</td>
<td>35 (50)</td>
<td></td>
</tr>
<tr>
<td>Individuals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–1</td>
<td>31</td>
<td>12 (39)</td>
<td></td>
</tr>
<tr>
<td>2–5</td>
<td>28</td>
<td>3 (11)</td>
<td></td>
</tr>
<tr>
<td>6–10</td>
<td>18</td>
<td>4 (22)</td>
<td></td>
</tr>
<tr>
<td>11–20</td>
<td>32</td>
<td>4 (14)</td>
<td></td>
</tr>
<tr>
<td>21–30</td>
<td>106</td>
<td>56 (53)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>31–40</td>
<td>32</td>
<td>17 (53)</td>
<td>2 (6)</td>
</tr>
<tr>
<td>41–50</td>
<td>22</td>
<td>14 (64)</td>
<td></td>
</tr>
<tr>
<td>51–60</td>
<td>31</td>
<td>22 (71)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>61–</td>
<td>49</td>
<td>39 (80)</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Total</td>
<td>349</td>
<td>181 (52)</td>
<td>11 (3)</td>
</tr>
</tbody>
</table>

The presence of C. pneumoniae antibodies in patients with acute respiratory infection was detected by the MIF test (Table 2). The incidence of C. pneumoniae antibody in the group of patients with acute respiratory diseases was 67%. They included patients with acute pneumonia (69%), acute bronchitis (54%), and acute pharyngitis (70%). Antibody titers of 1:256 or higher were observed in 26% of the patients with acute respiratory infection who tested positive for the IgG antibody. Recent infection (IgG ≥ 1:512) with C. pneumoniae in patients with acute respiratory disease totaled 14 cases (10%): 12 cases of acute pneumonia (12%), 1 case of acute bronchitis (4%), and 1 case of acute pharyngitis (4%). IgM antibodies were detected in eight patients (6%), of which six had acute pneumonia. Two of the six patients with acute pneumonia had high titers of IgG (≥512) and IgM (≥1:16) antibodies. Two of eight patients who had IgM antibodies showed detectable rheumatoid factor. After absorption with anti-human IgG in these two samples, the C. pneumoniae IgM-positive sera became C. pneumoniae-negative in the MIF assay.

**Discussion.** Grayston et al. (9) suggested that antibody titers of IgG (≥1:16) indicate past infection, and this antibody (IgG) is used in population antibody prevalence surveys. However, IgG titers of 1:32 or higher have been reported in several populations around the world (12, 19, 22). Saikku et al. (23) suggested that the prevalence of C. pneumoniae is associated with population density, which may explain the higher rate of positivity in Seoul, a city with a high population density.

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A recent infection has been defined as one in which there is a fourfold rise in titer of the antibody, a high titer of IgG antibody (≥1:512), or a positive IgM antibody (9). We found 11 cases (3%) of high IgG antibody titers (≥1:512) and 2 cases (0.6%) of high IgM antibody titers in healthy individuals. However, review of the medical histories of these individuals (with IgG titers of ≥1:512) for the 3 months preceding serum collection revealed diseases such as pneumonia in only a portion of them. Of these 11 individuals, 2 had developed acute pneumonia, 2 had suffered from acute pharyngitis, 3 had asthma, and 4 had no illness. The medical records of the two healthy individuals with IgM antibodies (≥1:16) were also reviewed. One was a 26-year-old male with a history of a previous bout of acute pharyngitis, and the other was a 24-year-old female with no illness in her past history. Both had high titers of IgG antibodies (1:256). We considered that they might have a primary infection of C. pneumoniae. This shows the need to be cautious in interpretation of a high titer of IgG antibody to C. pneumoniae in healthy individuals, because the antibody might be due to a previous illness, such as pneumonia, bronchitis, or pharyngitis, and mild cold-like syndromes. Careful review of the patient’s medical history should aid in differential diagnosis (8). The presence of a high titer of antibody alone provides a much less precise serological diagnosis than a fourfold rise in titer from paired sera (14). This is especially true for elderly patients, who may have had multiple C. pneumoniae infections in the past and may have persistently high IgG titers. Kuo et al. (14) reported that in a study of persons over 65 years of age, 8% had persistent IgG antibody titers of ≥1:512. Our results were similar in that 6% of those over 61 years of age had a high titer of IgG antibody (≥1:512).
In our study, the prevalence of *C. pneumoniae* antibodies (IgG) in patients with acute pneumonia was higher than that of healthy individuals (*P* < 0.05), but there was no evidence of an increase of *C. pneumoniae* antibody in patients with bronchitis or acute pharyngitis (*P* > 0.05). The percentage of recent infection with *C. pneumoniae* in patients with acute pneumonia was 12 cases (12%) for those with IgG antibodies and 6 cases (6%) for those with IgM antibodies. The recent infection rates of *C. pneumoniae* in patients with acute pneumonia were higher than those of healthy individuals (*P* < 0.05). Ten percent of the patients with pneumonia and 4% of those with bronchitis have been reported to have TWAR infection (9). *C. pneumoniae* infection may also be associated with acute exacerbation of chronic obstructive pulmonary disease (16), but it is detected in 1% of patients with pharyngitis (7). *C. pneumoniae* may also have a causal association with wheezing, asthmatic bronchitis, and adult-onset asthma (10).

We conclude that *C. pneumoniae* infection is highly endemic in Seoul, Korea, as it is in Western countries, and that this infection is associated with acute respiratory diseases.

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**REFERENCES**