Seroprevalence of *Chlamydia pneumoniae* in Male Adults in Jordan

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**ABSTRACT**

No data are available on the prevalence of antibodies to *Chlamydia pneumoniae* infection in adult subjects in Jordan. In this study, we investigated the seropositivity for *C. pneumoniae* in a Jordanian sample of blood donating male adults by microimmunofluorescence antibody assay. Adults involved in this survey aged 18 to 50. The mean prevalence of IgG antibodies against *C. pneumoniae* at titer $\geq 1:16$ was 61.1%. The antibody prevalence was notably low in adults under the age of 20 years (16.6%), and the highest antibody prevalence reached up to 69.2% at fifth decade of life. Most individuals acquired infection during the third decade of life. In conclusion, a high seroprevalence of infection with *C. pneumoniae* was detected in adults, suggesting that *C. pneumoniae* infection is highly endemic in Jordan. This survey should serve as a basis for further analysis of the incidence of symptomatic and asymptomatic chlamydial infections in general population, and to unveil the correlation between *C. pneumoniae* and related diseases in Jordan.

*Keywords: Chlamydia pneumoniae, Seroprevalence, Microimmunofluorescence, Jordan*

1. **INTRODUCTION**

*Chlamydia pneumoniae* is an obligate intracellular bacterial pathogen and is an important cause of acute respiratory illnesses, including pneumonia, bronchitis, pharyngitis, and sinusitis. Approximately, 10% of cases of community-acquired pneumonia are associated with *C. pneumoniae* (Grayston et al., 1990; Kuo et al., 1995). In addition, *C. pneumoniae* has a tendency to cause persistent and chronic infection, which has been associated with conditions such as coronary heart disease (Kuo et al., 1993; Saikku, 2000), chronic obstructive pulmonary disease (COPD) (von Hertzen et al., 1997), chronic asthma (Hahn et al., 1991), and arthritis (Braun et al., 1994).

Since seroepidemiologic studies have demonstrated that 50 to 70% of adults have antibody to *C. pneumoniae*, it is estimated that nearly everyone acquires at least one *C. pneumoniae* infection during lifetime (Kuo et al., 1995). According to some studies conducted mainly in industrialized Western countries, asymptomatic infections may be common (Gnarpe et al., 1991; Emre et al., 1994; Gaydos et al., 1994; Hyman et al., 1995; Block et al., 1997). High titers of anti-*C. pneumoniae* antibodies detected in serum samples, bacterial isolation and positive polymerase chain reaction (PCR) findings without any respiratory symptoms may be signs of an asymptomatic infection with *C. pneumoniae*. In Jordan, prevalence of genital infections caused by Chlamydia trachomatis in symptomatic and asymptomatic Jordanian patients has been investigated (Awwad et al., 2003). In most developing countries, including Jordan, there have been no or limited data on the incidence of *C. pneumoniae* among the populations and on the association of this pathogen with several serious diseases, thought to be chronic like persistent cough and coronary heart diseases. In this study, the species-specific microimmunofluorescence (MIF) technique was used to determine the seroprevalence of *C. pneumoniae* in male individuals in Jordan.

2. **MATERIALS AND METHODS**

2.1. Patients

Serum samples were obtained during the period from October to December, 2006 from 126 male individuals (aged 18 to 50 years), who visited Al-Hussein Center for Cancer, Amman, Jordan, for blood donation. Sera were stored at $-70^\circ$C until testing.
Table 1. Prevalence of anti-\textit{C. pneumoniae} IgG antibody by age in sera of Jordanian male blood donors

<table>
<thead>
<tr>
<th>Age group (Years)</th>
<th>No. of samples tested</th>
<th>No. of +ve samples (^a)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>6</td>
<td>1</td>
<td>16.6</td>
</tr>
<tr>
<td>20-29</td>
<td>71</td>
<td>43</td>
<td>60.6(^*)</td>
</tr>
<tr>
<td>30-39</td>
<td>36</td>
<td>24</td>
<td>66.7(^*)</td>
</tr>
<tr>
<td>40-50</td>
<td>13</td>
<td>9</td>
<td>69.2(^*)</td>
</tr>
<tr>
<td>Total</td>
<td>126</td>
<td>77</td>
<td>61.1(^*)</td>
</tr>
</tbody>
</table>

\(^{a}\) IgG titer $\geq$ 1/16
\(^{*}\) \(p < 0.05\) compared with the group under the age of 20 years.

Table 2. Prevalence of recent (acute) infection with \textit{C. pneumoniae} in infected Jordanian blood donating males according to age.\(^a\)

<table>
<thead>
<tr>
<th>Age group (Years)</th>
<th>Total no. of +ve samples</th>
<th>No. of +ve samples with high titer (^a)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>1</td>
<td>1</td>
<td>NA(^b)</td>
</tr>
<tr>
<td>20-29</td>
<td>43</td>
<td>9</td>
<td>20.9</td>
</tr>
<tr>
<td>30-39</td>
<td>24</td>
<td>11</td>
<td>45.8(^*)</td>
</tr>
<tr>
<td>40-50</td>
<td>9</td>
<td>4</td>
<td>44.4(^*)</td>
</tr>
<tr>
<td>Total</td>
<td>77</td>
<td>25</td>
<td>32.5(^*)</td>
</tr>
</tbody>
</table>

\(^{a}\) IgG titer $\geq$ 1/256.
\(^{b}\) not applicable, as specimens from ages under 20 years were underrepresented.
\(^{*}\) \(p < 0.05\) compared with the age group 20-29 years.

2.2. Serology

Diagnosis of \textit{C. pneumoniae} infection was conducted using the IgG indirect microimmunofluorescence (MIF) test kit according to manufacturer’s instructions (Ani Labsystems Ltd., Vantaa, Finland). The MIF test is based on indirect detection of IgG antibodies against \textit{C. pneumoniae} using FITC-conjugated anti-human IgG antibodies. Serum was diluted from 1:8 to 1:256. Ten microliters of serum dilutions as well as a drop of positive and negative control sera provided with the kit was pipetted to MIF slide wells dotted with inactivated \textit{C. pneumoniae} elementary bodies. Slides were then incubated for 30 min in a moist chamber. After being washed with PBS (phosphate buffered saline; pH 7.2) for 10 min, slides were rinsed with distilled water and dried. Next, 10 µl of FITC-labeled anti-human IgG were added to the wells, and the slides were incubated again in a moist chamber for 30 min. Finally, specimens were washed, dried, mounted with a mounting medium, and then covered by the proper coverslips. The slides were examined under fluorescence microscope (Olympus, Hamburg, Germany) at 40X magnification or by Leica TCS-SP laser scanning confocal microscope with a krypton/argon laser. Photomicrographs were processed using Adobe Photoshop 7.0 (Adobe Systems) and Microsoft Power-Point. Specimens displaying a bright uniform fluorescence associated with bacterial particles were considered positive. A titer of $< 1:16$ was
considered negative, whereas a titer of \( \geq 1:16 \) was considered positive. A titer of \( \geq 1:256 \) was considered acute (recent or current) infection, as recommended by others (Miyashita et al., 2001; Lin et al., 2004).

Figure 1. Seroprevalence of IgG antibodies to \( C. \) pneumoniae in Jordanian male individuals according to age.

2.3. Statistical Analysis

The statistical analysis of the results was carried out using the Student’s \( t \) test. \( P \) values less than or equal to 0.05 were considered to be statistically significant.

3. RESULTS

The serum samples of adults were divided into four age groups, each containing from 6 to 71 samples (mean \( \pm \)SD: 31.5\pm29). These age groups are shown in Fig. (1) and Table (1). When microscopically examined, sera were considered positive when spotted \( C. \) pneumoniae elementary bodies displayed a bright strong uniform fluorescence (Fig. 2A), compared with bacterial spots coated with negative (Fig. 2B) and positive (Fig. 2C) control sera included in the kit. Of 126 specimens tested, 77 specimens were seropositive at titer \( \geq 1:16 \), when tested by MIF assay (Table 1). The overall prevalence of anti-\( C. \) pneumoniae IgG antibodies was 61.1%.

There were differences in the prevalence rates of \( C. \)
pneumoniae infection (IgG antibody titer ≥ 1:16) in specimens taken by age groups. The age-related seroprevalence of IgG antibodies to C. pneumoniae in the four age groups is shown in Fig. (1) and Table (1). The antibody prevalence in each group was as follows: <20 years old, 16.6%; 20–29 years old, 60.6%; 30–39 years old, 66.7%; and 40–50 years old, 69.2%. A rapid increase in the rate of seropositivity was observed at age 20–29 years. The seroprevalence in persons under age 20 years was notably lower than those 20 years of age and older.

Recent C. pneumoniae infection with IgG antibody titer ≥ 1:256 was found in 25 (32.5%) cases out of 77 persons which showed seropositivity at titers ≥ 1:16 (Table 2). When the persons group with ages under 20 years is excluded, as relatively small number of individuals have been included, compared to other age groups, considerable high percentages of subjects with acute C. pneumoniae infection were observed in the age groups 30-39 (11/24, 45.8%) and 40-50 (4/9, 44.4%) years old (Table 2).

4. DISCUSSION

The overall prevalence of antibody was 61.1%, as 77 male adults out of 126 were positive by having an anti-C. pneumoniae IgG titer of ≥1:16. The Prevalence rate of IgG antibody to C. pneumoniae in adults younger than 20 years was low (16.6%). Thereafter, the antibody prevalence increased rapidly with age. The most prominent increase was noted in the next age group (20–29 years) and remained high afterwards. Thus, it is likely that most individuals acquired infection during the second and third decades of life. The progressively increasing antibody prevalence with age is similar to that described by others. (Kanamoto et al., 1991; Aldous et al., 1992; Kern et al., 1993; Einarsson et al., 1994; Choi et al., 1998). In consideration of the short duration of C. pneumoniae-specific antibody (Aldous et al., 1992; Kuo et al., 1995), the high seroprevalence in the older populations indicates that persistent infection or reinfection with C. pneumoniae during adulthood is common.

Seroepidemiologic studies worldwide have shown that seroprevalence of C. pneumoniae was 40-70% in adults and 10–20% in children (Kanamoto et al., 1991; Aldous et al., 1992; Karvonen et al., 1992; Montes et al., 1992 and 1993; Kern et al., 1993; Einarsson et al., 1994; Kese et al., 1994; Choi et al., 1998). In the present study, a high prevalence of antibody to C. pneumoniae was observed in adults 20 years and older (63.3%), whereas it was 16.6% in young adults. The general seroprevalence, observed here, among adults (61.1%) was one of the highest reported worldwide. In general, studies from different underdeveloped countries have also shown general higher antibody prevalence in adults than in developed industrialized Northern countries (Kuo et al., 1995). C. pneumoniae antibody prevalence in adult populations was shown to be 43.4% in Japan (Kanamoto et al., 1991), 40–52% in USA (Aldous et al., 1992), 53% in Iceland (Einarsson et al., 1994), 56% in Finland (Karvonen et al., 1992), 67.8% in Sweden (Gnarpe et al., 1993), 61.6% in Korea (Choi et al., 1998), 50.6% in Slovenia (Kese et al., 1994), 61.5% in mainland China (Ni et al., 1996), and 64% in Sudan (Mahmoud et al., 1994). A major factor responsible for such differences in the antibody prevalence to C. pneumoniae could be the population density (Karvonen et al., 1992; Saikku, 1992). Consistently, high seroprevalence of C. pneumoniae infection in Jordan could be attributable to the high population density in the main cities. Importantly, we believe that the relatively high rate of acute infection can strongly contribute to the high overall seroprevalence observed in this study.

Of the 77 seropositive cases, 25 individuals (19.8%) had acute (recent) infections with an IgG titer of ≥1:256. In many studies, the percentages of acute infections in adult persons may not exceed 10% (Lin et al., 2004; Miyashita et al., 2006). However, other studies reported a surprisingly high proportion of individuals, who met the serologic criterion for acute C. pneumoniae infection. For instance, Gaydos et al. (1994) and Hyman et al. (1995) detected acute infections in 18.8% and 18.4% of adults, respectively. Moreover, Kern et al. (1993) indicated that 12.9% (19 of 147) of adult subjects were found to have serologic evidence of acute C. pneumoniae infection. In our study, 19.8% of Jordanian adults had titers suggestive of acute infection, consistent to former reports (Kern et al., 1993; Gaydos et al., 1994; Hyman et al., 1995). The high rate of acute infection observed here is most likely due to the performance of our study during a C. pneumoniae epidemic. This view is supported by previous studies which indicated a high incidence of this pathogen during winter, especially from October to December (Hsu et al., 2007), the time period during which sera of this study were collected.

In conclusion, we demonstrate for the first time the high prevalence of antibody to C. pneumoniae in Jordan,
indicating that *C. pneumoniae* infection is endemic in this country. The primary infections are most probably acquired before the age of 30, and persistent infection or reinfection with *C. pneumoniae* is common during adulthood as evidenced by a persistent high prevalence of *C. pneumoniae* antibody through old age. This study represents a good basis for future surveys that should (1) include a large size of population representing both sexes and all ages, and (2) be expanded over the whole year.

**REFERENCES**


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