Chlamydial conjunctivitis: prevalence and serovar distribution of *Chlamydia trachomatis* in adults

Fruzsina Petrovay,1 István Németh,2 Andrea Balázs1 and Eszter Balla1

1Department of Bacteriology, National Centre for Epidemiology, Budapest, Hungary
2Developmental Drug Metabolism and Pharmacokinetics, Gedeon Richter, Budapest, Hungary

The extragenital manifestation of *Chlamydia trachomatis* infection frequently results in non-specific conjunctivitis among sexually active adults. The aims of the present study were to determine the prevalence of *C. trachomatis*, to describe the distribution of serovars among patients with conjunctivitis and to characterize the relationship between the prevalence and patient demographics such as age and gender. A total of 245 conjunctival specimens were screened for *C. trachomatis* DNA targeting the plasmid gene. Serovar determination of the *C. trachomatis*-positive specimens was carried out by an *omp1* PCR-based RFLP analysis method. Statistical analysis was done using a generalized linear model. *C. trachomatis* was detected in 53 cases (21.6%) of adult conjunctivitis. Molecular genotyping differentiated seven distinct urogenital serovars, the most prevalent being serovar E (16/53), followed by F (15/53), D (6/53), K (6/53), G (4/53), H (4/53) and J (2/53). Statistical analysis showed higher *C. trachomatis* prevalence in the younger age groups, and this peaked at younger age in women than in men. The high prevalence of this pathogen found in ocular samples should alert ophthalmologists to focus on the role of *C. trachomatis* in adult conjunctivitis. The serovar distribution indicated that ocular chlamydial infections usually have a genital source. Nevertheless, conjunctivitis might be the only sign of this sexually transmitted infection. Further comparative genotyping of *C. trachomatis* in ocular and genital specimens might give more detailed epidemiological information about the aetiology of the disease.

INTRODUCTION

Urogenital *Chlamydia trachomatis* infection is the most frequently reported sexually transmitted infection (STI) in Europe (ECDC, 2015). Ocular diseases, which are common extragenital complications of *C. trachomatis* infection, can be divided into three types. Trachoma caused by serovars A–C is the leading infectious cause of blindness in developing countries (Taylor et al., 2014). Neonatal conjunctivitis develops in 20–50 % of babies born to mothers with chlamydial cervical infection (Rours et al., 2008). Adult chlamydial conjunctivitis can develop in sexually active people and up to 80 % of the patients have concurrent genital infection with serovars D–K (Stenberg & Mårdh, 1991; Garland et al., 1995; Postema et al., 1996).

Adult chlamydial eye infections occur through exposure to infectious genital secretions primarily due to autoinoculation or during sexual contact (Haller-Schober & El-Shabrawi, 2002). In typical cases, follicular conjunctivitis develops characterized by mucopurulent discharge, redness and foreign body sensation. If left untreated, this condition may last for several months (Stenberg & Mårdh, 1990; Rao et al., 1996).

The clinical picture of chlamydial conjunctivitis often presents with atypical symptoms and therefore laboratory diagnosis is essential. Moreover, determination of the *C. trachomatis* serovars by molecular typing methods provides epidemiological information about the incidence and prevalence of the actual serovars of the isolated conjunctival *C. trachomatis* strains. However, there are limited data available about the distribution of ocular *C. trachomatis* serovars in adult populations (Garland et al., 1995; Isobe et al., 1996; Kese et al., 2011). The aims of the present study were: (i) to define the prevalence of *C. trachomatis* by PCR in eye samples of individuals with conjunctivitis; (ii) to identify retrospectively the different *C. trachomatis* serovars by genotyping; and (iii) to analyse the relationship between demographics (age and gender) and *C. trachomatis* infection in patients with conjunctivitis.

METHODS

Clinical specimens. Between January 2008 and November 2013, a total of 245 conjunctival eye swabs or conjunctival scrapings from patients with conjunctivitis were sent from the Department of
Ophthalmology (Semmelweis University, Budapest) to the STI laboratory of the National Centre for Epidemiology for C. trachomatis detection. As a routine procedure, ophthalmologists consider C. trachomatis screening when one or more of the following conditions are present: failure of any local treatment; patients have chronic symptoms; clinical signs of follicular conjunctivitis are present; and patients are sexually active young adults. During routine laboratory screening, DNA extraction from the samples was performed as described previously (Petrovay et al., 2009).

**C. trachomatis PCR.** The first 124 DNA samples obtained between January 2008 and May 2011 were tested with an in-house PCR targeting the cryptic plasmid gene of C. trachomatis with primers CTP1 and CTP2, as described previously (Petrovay et al., 2009). The rest of the samples from June 2011 were screened with a PCR targeting the same gene but with different primers, KL1 and KL2 (Mahoney et al., 1993) because the laboratory switched to another PCR method, which also detects the new variant of C. trachomatis. Both PCRs were validated yearly in the international ring trial for external quality control assurance organized by INSTAND e.V., Germany (Reischl et al., 2009).

**PCR-RFLP genotyping.** C. trachomatis-positive samples were analysed retrospectively by an omp1 PCR-based RFLP genotyping method as described previously (Lan et al., 1993; Petrovay et al., 2009). The PCR product was digested with AluI endonuclease (Promega), which can differentiate serovars D, E, F, G and K, as the first step. The H, I and J serovars with similar restriction patterns were further separated with HinfI, EcoRI and Ddel enzymes. Identification of the serovars was performed by 1.5 % agarose gel electrophoresis as described previously using known restriction pattern lengths of the controls (Petrovay et al., 2009).

**Statistical analysis.** The relationship between demographics (age, gender) and C. trachomatis infection was analysed using logistic regression. The model best describing this relationship was selected by means of likelihood ratio tests of nested models (Harrell et al., 2001). The model best describing this relationship was selected by gender, age and age^2. The four models were: gender + age (model 1); gender + age + gender | age (model 2, ‘|’ = interaction); gender + age + age^2 (model 3); gender + age + age^2 + gender | age + gender | age^2 (model 4). For data handling, graphics and statistical analysis, R (version 2.13.0), a free software program for statistical computing, was used (R Core Team, 2011).

**RESULTS**

**Prevalence of C. trachomatis and effect of demographic variables**

Between January 2008 and November 2013, a total of 245 conjunctival specimens were examined. The patients were aged from 2 to 79 years (mean 33.2 years). The patient group comprised 132 females aged 2–70 years (mean 32.5 years) and 113 males aged 3–79 (mean 34.1 years). The duration of symptoms reported by patients varied in a wide range from 1 week to 9 months before chlamydial testing and generally lasted for 1–3 weeks. C. trachomatis was detected as an aetiological agent in 53 cases of adult conjunctivitis (Table 1).

The best model was selected from the four nested models. The log-likelihood values [degrees of freedom (d.f.)] for model 1 to model 4 were −120.6 (3), −117.2 (4), −113.1 (4) and −103.8 (6), respectively. Based on likelihood ratio tests, model 4 was found to be the best model (model 3 vs model 4: \( \chi^2 = 18.532 \), d.f.=2, \( P < 0.0001 \)). The highest probability of C. trachomatis infection was found to be similar between genders (Fig. 1). The probability of having a C. trachomatis infection in a patient with conjunctivitis was highest for individuals at the age of 35 years (28.9 %) and 22 years (49.3 %) for men and women, respectively.

**Genotyping results**

The 53 samples that were positive by C. trachomatis plasmid PCR were successfully amplified by the semi-nested omp1 PCR. Altogether, seven different urogenital serovars of C. trachomatis were identified by RFLP analysis. The distribution of these serovars was as follows: the most prevalent were serovars E (16/53) and F (15/53), followed by D (6/53), K (6/53), G (4/53), H (4/53) and J (2/53).

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
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<tr>
<td>&lt;15</td>
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<td>52.6</td>
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<tr>
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<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Total</td>
<td>16.8</td>
<td>25.8</td>
<td>21.6</td>
</tr>
</tbody>
</table>

**Table 1. Prevalence (%) of C. trachomatis infection by age and gender in patients with conjunctivitis (no. positive/no. screened)**

![Fig. 1. Relationship between demographics (gender and age) and C. trachomatis infection in patients with conjunctivitis. Observed probabilities (\( \chi^2 \), men; \( \phi \), women) were plotted on the mid-point of age class bins except at >54 (\( \chi^2=59 \)). Probability curves (solid line, men; dashed line, women) were predicted by polynomial logistic regression in which age was used as a continuous covariate [logit = \(-7.32 + 1.65 \times \text{women} + (0.37 + 0.14 \times \text{women}) \times \text{age} + (0.005 + 0.006 \times \text{women}) \times \text{age}^2\)].
DISCUSSION

Our results showed that a high proportion of cases with conjunctivitis were caused by *C. trachomatis* infection during the studied period. The overall prevalence of *C. trachomatis* infection was 21.6% in the study population; the affected individuals were between 14 and 45 years of age, which is an alert for the clinicians as a sign of an STI. These results are in accordance with other findings, as *C. trachomatis* is found in chronic conjunctivitis of young sexually active adults in about 5–19% of cases (Haller-Schober & El-Shabrawi, 2002). However the true prevalence ratio of *C. trachomatis* positivity in cases with conjunctivitis might be lower, as the clinical samples analysed in this study were selected for *C. trachomatis* screening by the ophthalmologists.

An age-related prevalence and difference between gender and age was also observed in relation to the occurrence of *C. trachomatis* conjunctivitis (Fig. 1). An association between age and prevalence of infection has also been observed in other studies, indicating that younger age is a proven risk factor for genital *C. trachomatis* infection (HPA, 2008; Simms et al., 2009). The differences in the age-related prevalence of chlamydial conjunctivitis by gender have also been observed in other studies (Stenberg & Mårdh, 1990; Postema et al., 1996; Quirke & Cullinane, 2008). Genital chlamydial infections show the same correlations, as some surveillance reports have found that the proportion of diagnosed chlamydial infections varied by gender across the age groups and *C. trachomatis* was detected more commonly among women than among men in the younger age groups (HPA, 2008; Simms et al., 2009). The disparity in the pattern of positivity between men and women by age may indicate the influence of certain sexual relationships on the transmission of the pathogen, as younger women tend to have older partners (Wadsworth et al., 1996; Johnson et al., 2001).

A heterogeneous serovar distribution was observed among the 53 *C. trachomatis*-positive conjunctival samples, as seven distinct urogenital serovars could be identified. The most prevalent serovars were E (30.2%) and F (28.3%), and this trend is in accordance with the findings of other genotyping studies worldwide. Many studies examining the serovar distribution of *C. trachomatis* in genital samples of different cohorts using genotyping methods have shown that the most frequently observed serovars are E and F (Lysén et al., 2004; Gao et al., 2007; Lima et al., 2007; Kese et al., 2011). The similar distribution of ocular and genital serovars supports the theory that adult chlamydial conjunctivitis infections have a genital source, as has been suggested previously by several studies, and can occur during autoinoculation or transmission from the partner’s infected genital secretions (Stenberg & Mårdh, 1991; Garland et al., 1995; Postema et al., 1996). As genital *C. trachomatis* infections are usually asymptomatic and thus may be left untreated with chronic pathological consequences such as pelvic inflammatory disease, infertility or epididymitis, it is of great value if the ophthalmologists can recognize the aetiological agent of the ocular infection as they may be the first to diagnose this STI. In the case of chlamydial conjunctivitis, a suitable treatment would therefore be systemic because of the associated genital infection rendering local treatment insufficient (Haller-Schober & El-Shabrawi, 2002). Clinicians are also recommended to refer the patients and their sexual partners with genital *C. trachomatis* infection to an STI clinic for further examination (Stenberg & Mårdh, 1990; Postema et al., 1996).

In conclusion, *C. trachomatis* infections can frequently cause conjunctivitis in sexually active young adults, and this may be the only sign of this STI. Ophthalmologists have an important role in recognizing chlamydial conjunctivitis, and the differential diagnosis could be promoted by accurate molecular diagnostic methods. The prevalence and distribution of *C. trachomatis* serovars in the conjunctival samples observed in this study were similar to those of serovars in genital infections. Genotyping methods are useful molecular epidemiological tools for further studies comparing the distribution of *C. trachomatis* serovars in ocular and genital samples of individuals and their sexual partners, which might give a more accurate insight into the aetiology of chlamydial conjunctivitis.

REFERENCES


Kese, D., Potocnik, M., Maticic, M. & Kogoj, R. (2011). Genotyping of *Chlamydia trachomatis* directly from urogenital and conjunctiva


