Original Article

Chlamydia trachomatis Serovar Distribution in Patients with Follicular Conjunctivitis in Iran

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Abstract

Objectives: *Chlamydia trachomatis* infects the urogenital tract and eyes. Anatomical tropism is correlated with serovars which are characterized according to the variation in the major outer membrane proteins encoded by the *ompA* gene. The aim of the present study was to determine the distribution of *C. trachomatis* serovars among patients with follicular conjunctivitis in Iran.

Materials and Methods: A total of 68 conjunctival specimens from symptomatic adults were studied for the presence of *C. trachomatis* using polymerase chain reaction (PCR) analysis. Serovars were determined by Omp1 PCR-RFLP analysis.

Results: *C. trachomatis* was detected in 38 (55.9%) of patients with follicular conjunctivitis, with higher *C. trachomatis* prevalence in the younger age groups. Twenty-six (38.2%) of these patients had a history of urinary tract infection. Four distinct serovars were identified in the conjunctiva samples using molecular genotyping. The most prevalent was serovar E, followed by G, I, and F.

Conclusion: Our serovar distribution indicated that chlamydial follicular conjunctivitis usually has a genital source. Genital serovars may cause eye diseases, especially in sexually active adults. On the other hand, conjunctivitis might be the only sign of sexually transmitted infection. Therefore, genotyping *C. trachomatis* in ocular and genital specimens could be beneficial for acquiring more detailed epidemiological information about the etiology of the disease and monitoring treatment success.

Keywords: *Chlamydia trachomatis*, follicular conjunctivitis, serotype, PCR, RFLP

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Introduction

Besides genital infections, *Chlamydia trachomatis* can cause eye infections.[1] Serotypes D-K of *C. trachomatis* cause neonatal or adult inclusion conjunctivitis.[2] Symptoms of this disease include redness of the eye along with mucopurulent discharge, conjunctival hyperemia, papillary lymphoid hyperplasia, and follicle formation.[3] Global increases in urogenital infections by *C. trachomatis* have caused a concurrent increase in *C. trachomatis* eye infections.[4] *C. trachomatis* serotypes D-K are among the most important causes of urogenital and eye infections in developing countries.[3] Additionally, *C. trachomatis*-induced inclusion conjunctivitis has a high prevalence in Iran and strong correlation with genital tract infection.[5]

Usually, nucleotide sequence variations of the *ompA* gene are used to identify the different serovars of *C. trachomatis.*[6] The *ompA* gene encodes the major outer membrane protein (MOMP) of *C. trachomatis* and contains four symmetrically spaced variable domains (VDs; VDI to VDIV) which encode major antigenic determinants. These regions are interspaced with five conserved domains.[7] Compared to other methods of *C. trachomatis* serotyping in clinical specimens, PCR-restriction fragment length polymorphism (RFLP) analysis of the amplified *ompA* gene encoding MOMP is one of the simplest methods for genotyping *C. trachomatis.*[8]

Despite the high prevalence of disease caused by *C. trachomatis* in Iran, little information is available regarding the prevalent serovars of *C. trachomatis* causing infection. Determination of circulating *C. trachomatis* serovars within our population can provide important information regarding the epidemiology and pathogenesis of infections caused by this bacterium. This information allows for monitoring treatment success and may play a role in developing better strategies for disease control.[6]

Materials and Methods

Sample Collection: A total of 68 conjunctival swabs were taken using a Dacron swab from the upper subtarsal conjunctiva of patients referred by optometrists to Farabi hospital between September 2016 and September 2018. The following signs were taken into consideration: secretions, hyperemia, small follicles in low quantities and large follicles in quantities over five, tarsal scarring, papillae, scarring of limbic follicles, entropion, pannus, leucoma, xerosis, and trichiasis.[9] A *Chlamydia* strain isolated from a genital infection and identified as genotype E by sequencing was used as the positive control.

DNA Extraction: Swabs were placed into 1.5 mL of sterile water and vortexed. After spinning, the DNA was isolated using the Accuprep Genomic DNA Extraction Kit (Bioneer Co., South Korea) as specified by the manufacturer.

PCR amplification of chlamydial DNA extracted from the conjunctival swabs was performed using CT1 (5'-GCCGCTTTGAGTTCTGCTTCCTC-3') and CT5 (5'-ATTTACGTGAGCAGCTCTCTCAT-3') primers, designed from the first and fifth conserved domain of the *Omp1* gene, respectively. Five microliters of each extracted sample from either the chlamydial reference or clinical swabs were used for amplification in a 50 µL solution containing 200 µM (each) dATP, dCTP, dGTP, and dTTP; 50 mM KCl; 10 mM TrisHCl (pH 8.4); 1.5 mM $MgCl_2$; 0.5 µM of each oligonucleotide primer (CT1 and CT5) and 1.25 U of Taq DNA polymerase.[10]

Thirty cycles of amplification were performed in an automated thermocycler. Each cycle consisted of 1 min of denaturation at 95 °C, 1 min of annealing at 55 °C, and 2 min of extension at 72 °C.[9] The PCR products were analyzed by electrophoresis of 10 µL of the amplification mixture through a 1% agarose gel.

Nested PCR: One microliter of the primary *Omp1* PCR product (CT1-CT5) was pipetted with an aeroseal-tipped pipette into a prepared PCR mixture containing nested primers including MF21 (5'-CCGACCGCGTCTTGAAAACAGATGT-3') and MB22 (5'-CACCCACATTCCCAGAGAGCT-3'), designed from *Omp1* constant regions flanking variable segments VS1 and VS2 (416-base pair DNA fragment).[11] The amplification conditions of the nested PCR were the same as those of the primary *Omp1* PCR. Amplified products were analyzed by electrophoresis on a 2% (w/v) agarose gel.

RFLP Assay: RFLP analysis of PCR and nested PCR products was carried out as described previously.[8] Briefly, amplified DNA products were digested separately overnight, in one reaction with 10 U *AluI* and in a second reaction with 10 U of each of the three enzymes: *HpaII*, *EcoRI*, and *HinfI.* Analysis of digested DNA was performed by electrophoresis of the total reaction on an 8% polyacrylamide gel. Patterns were compared visually.

Results

Sixty-eight clinical samples from patients with chronic conjunctivitis were collected for this study. The patients included 31 women (45.6%) and 37 men (54.4%). The patients ranged in age from 17 to 76 years but were mostly in the 21-30 age range. Of these patients, 26 (38.2%) had a history of urinary tract infection.

Direct PCR amplification with CT1 and CT5 primers yielded a band of approximately 1200 base pairs from 30 of the ocular specimens tested and the reference strain. Thus, 30 (44.1%) of the 68 isolates were positive for *C. trachomatis* using the CT1 and CT5 primers. In eight samples, *Omp1* was amplified after nested PCR with the MB22 and MF21 primers. Therefore, *C. trachomatis* was detected in a total of 38 patients (55.9%).

Thirty-five of the 38 specimens that showed positive result in the *Omp1* PCR were clearly typed in RFLP analysis, while 3 samples could not be typed.

Figure 1 shows the results of *AluI* and *HpaII, EcoRI,* and *HinfI* RFLP analysis of MB22-MF21 amplified *Omp1* gene from three specimens. The reference *C. trachomatis* specimen was genotyped to serve as a reference for the genotyping of clinical specimens. Sequence analysis confirmed it to be a genotype E strain.

RFLP profiles of the 35 ocular specimens showed 20 samples (57.1%) were genotype E, 8 (22.9%) were genotype G, 5 (14.3%) were genotype I, and 2 samples (5.7%) were genotype F.

Discussion

Genotyping of *C. trachomatis* plays a critical role in global epidemiological studies and provides necessary information regarding infection transmission and recurrence.[12] Previous studies have shown an association between *ompA* type and host phenotype and it is believed that MOMP is an important target of immunity; therefore, *ompA* variants could elicit different host immune responses and different clinical symptoms.[13,14]

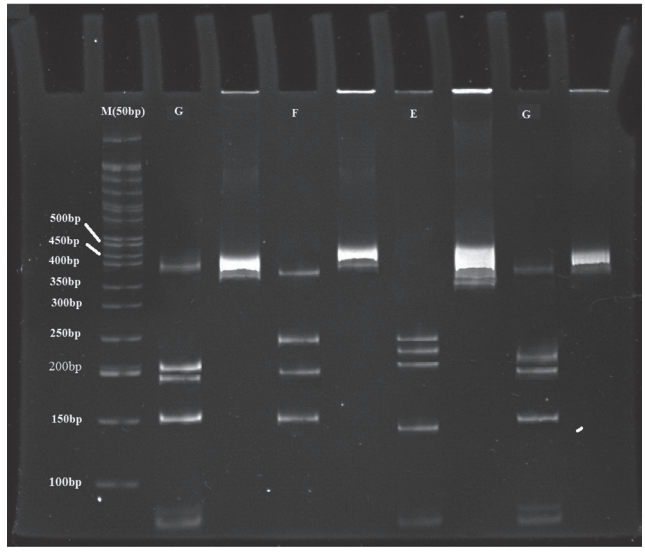

Figure 1. Polymerase chain reaction-restriction fragment length polymorphism genotyping of three *Chlamydia trachomatis* strains cleaved and uncleaved with the endonucleases. Lane M contains the DNA molecular weight marker (bp: base pairs). The letters above the lanes indicate serovars E, F, and G of *C. trachomatis*

Many studies have used RFLP pattern analysis of amplified MOMP DNA for the differentiation of *C. trachomatis* serovars in ocular and genital samples.[11] In the present study we investigated the distribution of serovars of *C. trachomatis* isolates which were obtained from patients with follicular conjunctivitis using RFLP patterns of the *Omp1* gene. We determined that 38 out of 68 samples were positive in *Omp1* PCR, which demonstrates a high prevalence of *C. trachomatis* infection (55.9%) among these samples.

An association between age and infection has also been observed in our study, as most patients were in the age range of 21-30 years, indicating that younger age is a risk factor for follicular conjunctivitis caused by *C. trachomatis*. Age-related differences in the prevalence of chlamydial conjunctivitis have also been observed in other studies.[15]

Four distinct serovars of E, G, I, and F could be identified in 35 isolates of our ocular samples. The most prevalent serovar was E (57.1%). The high percentage of serovar E among our ocular samples is in agreement with previous research that identified this serovar as the most prevalent serovar from *C. trachomatis*-positive conjunctival samples.[6] It has also been stated that *C. trachomatis* serovar E could have an enhanced capability of infecting the conjunctival mucosa compared to other serovars.[16]

Many studies have shown serotype E as the most common serotype in genital samples.[12,17] It has also been shown that there is a difference in the distribution of genital serotypes in correlation with patient age, with a predominance of genotype E in adolescents.[16]

However, the genotype distribution that we observed in patients with follicular conjunctivitis differs from those reported by other researchers who found that genotype E was the most prevalent, followed by F and D.[6,17] In the present study, genotypes G (22.9%), I (14.3%) and F (5.7%) were the most prevalent serovars after E, while serovar D was not detected. This difference may be explained by the fact that most of the cited studies examined conjunctival or urogenital samples and there are few studies examining follicular conjunctivitis. On the other hand, previous studies have shown a negative association between genotype D and ocular *C. trachomatis* infections compared to genital infections.[16]

An investigation of *C. trachomatis* genotyping from genital samples conducted in Ahvaz, Iran showed that the most prevalent genotype was E, followed by F, D, K, I, G, H and J.[18] These findings may indicate that there is a correlation in the distribution of *C. trachomatis* genotypes between urogenital and follicular conjunctivitis samples in Iran. The similar distribution of ocular and genital serovars supports the theory that adult chlamydial conjunctivitis infections have a genital source and can occur due to autoinoculation or transmission from a partner's infected genital secretions.[15]

It was of interest that in the present study, 26 (38.2%) of patients with follicular conjunctivitis had a history of urinary tract infection. Previous studies have indicated that *C. trachomatis* infections can frequently cause conjunctivitis in sexually active young adults, and this may be the only sign of sexually transmitted infection.[15] Mohamed-Noriega et al.[19] in their study of patients with adult inclusion conjunctivitis found that the majority of patients had concurrent asymptomatic genital infection. On the other hand, 8.3% of women with confirmed chlamydial genitourinary disease had a positive direct fluorescent antibody test for *C. trachomatis* from conjunctival scrapings but none had ocular signs or symptoms.

C. trachomatis infections are usually asymptomatic and thus may remain untreated, possibly leading to chronic pathological consequences such as pelvic inflammatory disease, epididymitis, and infertility. Therefore, it is valuable for ophthalmologists to be able to recognize the etiological agent of ocular infection, as they may be the first to diagnose sexually transmitted diseases. In the case of chlamydial conjunctivitis, systemic treatment would be appropriate because associated genital infection may cause local treatment to be insufficient.[15]

Study Limitations

The small number of patients with follicular conjunctivitis and the retrospective, non-randomized, and descriptive design are limitations of our study. In addition, the limited resolution of the PCR-RFLP used can be considered another limitation of this study.

Conclusion

Genotyping methods are useful molecular epidemiological tools for studying the distribution of *C. trachomatis* serovars in the community and investigating the source of *C. trachomatis* ocular and genital infections in patients and their sexual partners, and may give a more accurate insight into the etiology of chlamydial conjunctivitis.

Ethics

Ethics Committee Approval: Shahid Beheshti University of Medical Sciences Ethics Committee (no: 113/3).

Informed Consent: Obtained.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: Z.A., F.F., F.A., B.B., F.D., Design: Z.A., F.F., F.A., B.B., F.D., Data Collection or Processing: Z.A., F.F., F.A., B.B., F.D., Analysis or Interpretation: Z.A., F.F., F.A., B.B., F.D., Literature Search: Z.A., F.F., F.A., B.B., F.D., Writing: Z.A., F.F., F.A., B.B., F.D.

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