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ORIGINAL ARTICLE

Prevalence of *Chlamydia trachomatis* infection and evaluation of its genotypes among pregnant women in Tehran, Iran

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ABSTRACT

Background and Objectives: *Chlamydia trachomatis* is an obligate intracellular pathogen. Infection with *C. trachomatis* in pregnant women can result in maternal and fetal death, due to pelvic inflammatory disease. Therefore, we aimed to evaluate this infection in pregnant women and identify circulating genotypes of *C. trachomatis* in Tehran, Iran.

Materials and Methods: Endocervical swabs were obtained from 101 pregnant women and tested by PCR assay to detect *cryptic plasmid* gene. Positive isolates were analyzed for *C. trachomatis* genotypes through amplification and sequencing of the *omp1* gene and alignment with deposited sequences in Gene Bank.

Results: Infection with *C. trachomatis* was observed in 11 cases, yielding an overall prevalence of 10.8% in total. The majority of infected women were asymptomatic and the rate of infection was found more in women at the age of ≥30 years. However, no statistical association was found between *C. trachomatis* infection and risk factors in pregnant women. Analysis of isolated sequences revealed genotypes E (44.4%), D and F (both 22.2%), and K (11.2%) as main genotypes of *C. trachomatis* in this region.

Conclusion: Results of this study showed the prevalence of *C. trachomatis* infections among pregnant women is relatively high. Identifying the precise rate of infection and associated genotypes in other regions is suggested.

Keywords: *Chlamydia trachomatis*; Outer membrane protein 1; Pregnant women; Prevalence; Genotyping

INTRODUCTION

Chlamydia trachomatis (C. trachomatis) is known as the most common bacterial cause of Sexually Transmitted Infections (STIs) among male and female adults. Recent studies reveal that up to 80% of female patients are asymptomatic which not only harbors the risk of long-term complications but also remains the carrier for transmission and dissemination of the infection in the community (1). In pregnant women, as a high-risk group, ectopic pregnancy, preterm delivery, and abortion can occur in case of

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neglecting proper and prompt treatment of infection (2). According to available data, the prevalence of *C. trachomatis* infection among pregnant women has been reported from 2 to 14 percent in different geographical regions of Iran (3, 4).

For many years, diagnosis of *C. trachomatis* infection has been based on common methods including serology, biochemical assessments, direct smears, and especially culture assay as the gold standard method. However, the culture of *C. trachomatis* is a time-consuming procedure and needs to cell culture and invasive sampling from patients (5). Currently, diagnosis of microorganisms through the culture method is being substituted mostly by molecular assays which increased diagnostic sensitivity and decreased the time of infection detection (6, 7).

Furthermore, different serovars of *C. trachomatis* could be identified by employing these molecular techniques. Based on 4 variable segments of the *omp1* gene, *C. trachomatis* has been divided into 19 genotypes, of which eight genovars D, E, F, G, H, I, J, and K are mainly isolated from adult patients with genito-urinary diseases and neonates with conjunctivitis (8). Understanding the epidemiological pattern of *C. trachomatis* genotypes in the population of different regions could provide valuable data on establishing a proper strategy for the prevention and control of STIs (9).

Unfortunately limited data are available from Iran concerning the distribution of *C. trachomatis* genotypes among different groups of population. Therefore, we aimed this study to evaluate *C. trachomatis* infection and its genotypes among pregnant women at term in Tehran, Iran.

MATERIALS AND METHODS

From 1st August to last January (2019), pregnant women attending a university hospital in Tehran (Iran University of Medical Sciences) were recruited for the study. Participation was voluntary and the only exclusion criterion was antibiotic usage within the past three weeks of sampling. Informed consent was obtained from each subject and a questionnaire containing demographic and medical data was fulfilled by interviewing. The ethical committee of the Iran University of Medical Sciences approved this project (IR.IUMS.REC1396.30909).

Endocervical specimens were collected with sterile Dacron swabs based on the relevant protocols (rotating swabs in the endocervical canal). Swabs were placed separately into the collection tubes containing 2ml sterile phosphate buffer saline (PBS) and transported to the research laboratory at the Institute of Immunology and Infectious Disease (Iran University of Medical Sciences). Upon arrival, samples were prepared (vortexing and centrifugation at 4000 × g for 20 minutes) and DNA extraction was performed using the phenol/ chloroform method (10). Then samples were assessed through an in-house PCR assay using kl1/kl2 primers to amplify a 241 bp fragment of *cryptic plasmid* gene from *C. trachomatis.* Samples with positive results of *cryptic plasmid* gene were prepared for nested PCR assay and amplification of the *omp1* gene with previously developed primers by Gao et al. (11). In brief, using 1 µl of each forward and reverse primers of (NLO&NRO) and in a 1.5ml reaction tube containing 12.5 µl of PCR Mastermix (Cinnagen, Iran), 7 µl of extracted DNA and up to 25 µl distilled water, a 1125 bp fragment of *omp1* gene was amplified under PCR condition of primary denaturation at 95ºC for 5 min, followed by 40 cycles of denaturation at 95ºC for 60 sec, annealing at 45ºC for 60 sec, and extension at 72ºC for 60 sec and a final elongation step at 72ºC for 8 min. Then 2 µl of the first PCR product were used as a template in the second PCR to amplify a 1050 bp fragment in the same reaction mixtures and conditions as the first PCR, except for the primers (NLI&NRI) and annealing temperature of 49ºC. Final reaction products were analyzed through electrophoresis in 1% agarose gel and visualized by UV.

Positive samples with 1050 bp fragment of *omp1* gene were purified and sequenced in both directions by a Big Dye Terminator cycle sequencing kit (ABI PRISM, Applied Biosystems, USA). The obtained sequences were aligned with deposited sequences of *C. trachomatis* genotypes in Gene Bank (National Center for Biotechnology Information) and a phylogenetic tree with 1000 bootstrap replicates was constructed based on likelihood method (Mega software, ver.6.0) to identify the relations between sequences (12). Table 1 shows the primers used for amplification of the *omp1* gene.

Chi-square test and/or Fisher's exact test were used to compare data and determine risk factors of infection. The statistical analysis was performed using MedCalc software (Ver. 20) and the P-value was set at <0.05 (13).

Table 1. Oligonucleotides primers used for nested-PCR assay

Nested-PCR		Sequences	Size
Primary	NLO	ATGAAAAAACTCTTGAAATCG	1125 bp
	NRO	CTCAACTGTAACTGCGTATTT	
Secondary	NLI	TTTGCCGCTTTGAGTTCTGCT	1050 bp
	NRI	CCGCAAGATTTTCTAGATTTC	

RESULTS

A total of 103 pregnant women enrolled for the study, of which 2 were excluded due to missing samples. Subjects were in the age range from 21 to 43 years (mean: 27.0 years) and a high proportion of them was multigravida (69.7%). Preterm delivery was recorded in 18 women (17.8%) and having a history of STIs and abortion was documented in 21.7% and 16.8% of pregnant women, respectively.

Based on the results of the first PCR assay (*Cryptic plasmid* gene), infection with *C. trachomatis* was found in 11 cases, yielding an overall prevalence of 10.8% (95% Confidence Interval: 5.4 - 19.4) among pregnant women. The majority of infected women were asymptomatic and a higher rate of infection was observed in women at the age of ≥30 years old. However, no statistical association was found between *C. trachomatis* infection and risk factors in pregnant women.

The *omp1* gene (1050 bp fragment) was successfully amplified in 9 of 11 samples. Sequence alignment between clinical isolates and deposited genotypes in the Gene Bank resulted in the identification of 4 different genotypes, in which E was found as the most frequent genotype (44.4%), followed by D and F (both 22.2%), and K (11.2%). No significant association was found between specific genotypes and risk factors in pregnant women. Fig. 1 reveals the phylogenetic tree of identified genotypes in this study.

The number at the nodes indicates bootstrap values. The accession numbers and genotypes of *C. trachomatis* are given with capital letters plus numbers. The genotypes detected in this study are represented as Sample1-9. The sequence of *Chlamydophila psittaci* (JN411078) *omp1* gene was used to root the tree.

DISCUSSION

The epidemiology of prenatal *C. trachomatis* infection in Iran is almost unknown and most studies have only investigated the rate of infection during pregnancy. In the current study, we investigated chlamydial infection and its genotypes in pregnant women at term. To our knowledge, this is the first data from Iran on circulating genovars of *C. trachomatis* among pregnant women.

In particular, our finding on the rate of *C. trachomatis* infection among pregnant women at term (10.8%) is within the ranges reported in the previous surveys from Tehran. In the last published study from Tehran, for example, the prevalence of *C. trachomatis* infection among pregnant women at term was reported as 8.8% which is similar to the results of this study (14). Also, based on another report from this region the *C. trachomatis* DNA was detected in urine samples of 11.2% of pregnant women at different trimesters (15). However, some other studies reported a low rate of infection (between 2 and 4%) in this group (3). Consistently, a similar condition is noticeable in reports from other countries. In Europe, for example, the prevalence of *C. trachomatis* infection is reported from 1 to 37 percent, depending on geographical regions (16, 17). Differences in the sensitivities of diagnostic tests and sampling methods as well as environmental and economic conditions of studied populations may justify some of these variations (18). Moreover, it is mentioned that living in large cities with a high crowded population like Tehran, could facilitate the conditions needed for transmission of *C. trachomatis* and increase the rate of infection (19, 20).

In the current study, pregnant women with ages ≥30 years old were more likely to have *C. trachomatis* infection. This finding is advocated by reports of some studies from Iran. Conversely in developed countries, females at the ages of <25 years are considered a high-risk group for chlamydial infection (21, 22). Social and cultural variations among populations and some religious beliefs regarding the limitation of sexual activities before marriage could probably explain this finding.

Analysis of the *omp1* gene sequences of clinical isolates in this study revealed 4 distinct genovares of E

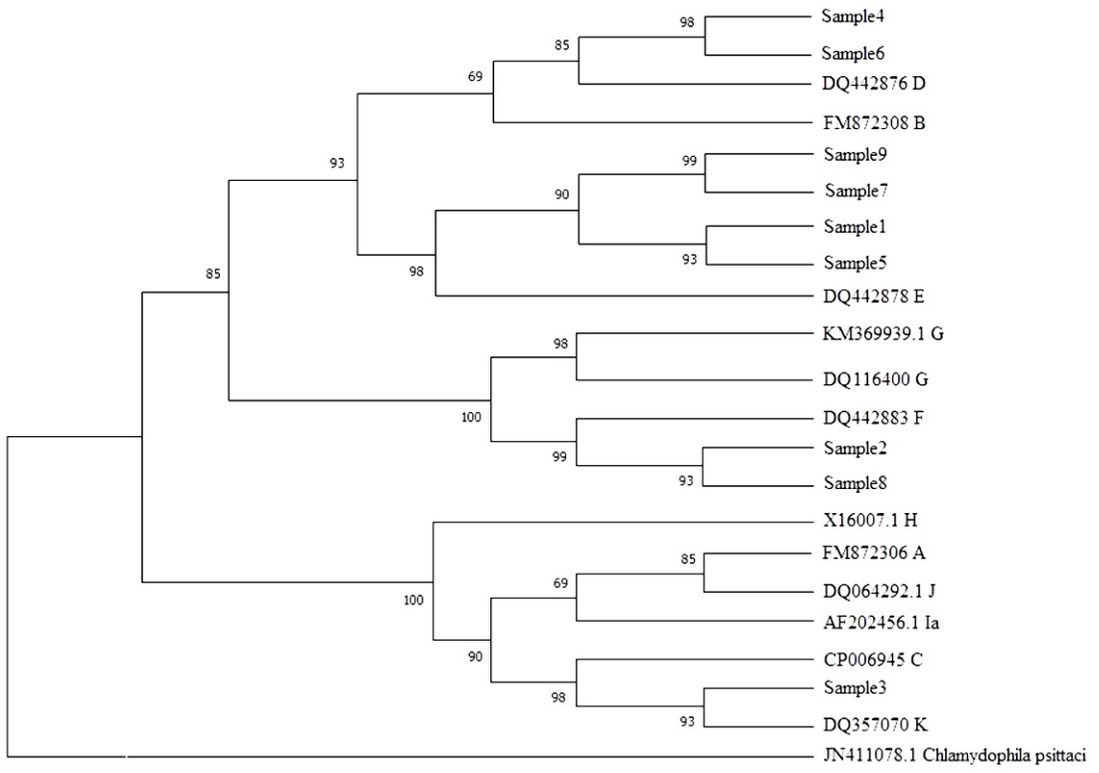

Fig. 1. The phylogenetic analysis of *omp1* gene based on maximum likelihood methods

(44.4%), D and F (both 22.2%), and K (11.2%), as circulating genovars among pregnant women. Similar findings have been reported by 2 published studies from Iran in which genovars E, D, and F were mainly identified in clinical isolates of infected non-pregnant women. In Ahvaz, for example, among 108 *C. trachomatis* positive specimens obtained from symptomatic women attending gynecological clinics, genovars E (31.5%), F (23.1%), D (13%), and K (9.2%) were identified as the most common genovars (23). Moreover, E and D (both 50%) were the only identified genotypes of *C. trachomatis* in pap smear swab samples of infected women in Birjand (24).

Finally, it should be noted that we did not find a significant association between *C. trachomatis* infection with preterm delivery, abortion, history of STIs, and clinical symptoms in pregnant women. This may be due to some limitations in our study such as the small sample size and consecutively the few numbers of related cases in analyzed variables.

CONCLUSION

This study revealed that the rate of *C. trachomatis* infection among pregnant women in Tehran, Iran is relatively high. Also, genotypes E, D, F, and K were identified as circulating genovars of *C. trachomatis* among patients. Since the cases were mostly asymptomatic, it seems that a prenatal screening test for *C. trachomatis* is necessary. At last, more epidemiological studies in different regions of Iran as well as in other groups are suggested.

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