



Detection and Isolation of *Chlamydia trachomatis* from Urine and Its Association with Preterm Labor

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ABSTRACT

Aims Preterm labors and abortions are among dangers that threaten mothers' and infants' lives. Several bacterial, viral, and parasitic agents can cause preterm labor and abortion. Among these bacterial agents, *Chlamydia trachomatis* plays a very key role. The aim of this study was to detect and isolate *Chlamydia trachomatis* from urine and its role in preterm labor.

Materials & Methods This cross sectional study was carried out from 2016 to 2017 in Al-Mahdi Clinic under the supervision of Tehran University of Medical Sciences. 119 pregnant women who were in the first trimester of pregnancy (between 12 and 14 weeks) referred to a health center for screening tests were selected using available sampling method and were collected their urine samples. The obtained samples were centrifuged and the DNA was extracted from urine sediment. Using specific PCR kits for detecting *Chlamydia trachomatis*, the presence or absence of this bacteria was investigated in the urine sediment. The data were analyzed using Chi square test.

Findings The rate of isolating *Chlamydia trachomatis* from the urine of these pregnant women was 6.72%. There was no significant relationship between chlamydia infection and history of abortion ($p=0.93$).

Conclusion The level of the isolation of *Chlamydia trachomatis* from the urine of pregnant women is twice more than the level of its isolation from samples taken from vaginal and cervical discharge in pregnant women. Therefore, urine is an appropriate sample for the diagnosis of *Chlamydia trachomatis* since the sampling method is non-invasive and has more positive cases.

Keywords Preterm Labor; Chlamydia trachomatis; Pregnancy; Abortion

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[1] Term and preterm labour are associated with distinct microbial community structures in placental membranes which are independent of mode of delivery [2] The prevalence and clinical significance of urethritis and cervicitis in asymptomatic people by use of multiplex polymerase chain reaction [3] Performance evaluation of a DNA chip assay in the identification of major genitourinary pathogens [4] Evaluation of Seeplex® STD6 ACE Detection kit for the diagnosis of six bacterial sexually transmitted infections [5] Asymptomatic bacteriuria and pyuria in pregnancy [6] Prevalence of *Chlamydia trachomatis* infection in Samoan women aged 18 to 29 and assessment of possible risk factors: a community-based study [7] Human papillomavirus positivity in women undergoing intrauterine insemination has a negative effect on pregnancy rates [8] Roles of bovine Waddlia chondrophila and *Chlamydia trachomatis* in human preterm birth [9] Cervicovaginal coinfections with human papillomavirus and *Chlamydia trachomatis* [10] *Chlamydia* related bacteria (*Chlamydiales*) in early pregnancy: community-based cohort study [11] Comparison of first void urine and urogenital swab specimens for detection of *Mycoplasma genitalium* and *Chlamydia trachomatis* by polymerase chain reaction in patients attending a sexually transmitted disease clinic [12] Assessing the presence of *Chlamydia trachomatis* genome in pregnant women with spontaneous abortion using polymerase chain reaction method in Yasuj: First report from Southwest of Iran

Introduction

Preterm labors and abortions are among dangers that threaten mothers' and infants' lives. Several bacterial, viral, and parasitic agents can lead to preterm labors and abortions. Statistics show that more than 90% of preterm labors are caused by an infectious agent and an inflammation in the placenta or amniotic fluid [1]. Among the bacterial agents, *Chlamydia trachomatis* plays a very key role. *Chlamydia trachomatis* is a gram-negative and obligate intracellular bacterium that belongs to *Chlamydiaceae* and is pleomorphic and immobile. As an obligate intracellular bacterium, it cannot grow on bacteriological culture environments; therefore, a cell culture is used for isolating and reproducing it. *Chlamydia trachomatis* is the main cause of trachoma which may result in abortion, birth defects in infants, endometritis, preterm labor, and stillbirth. Since bacterial culture is not easily feasible and serological methods are not very accurate in its detection, molecular methods, such as polymerase chain reaction (PCR), are used to detect *Chlamydia trachomatis*.

In the vast majority of studies and reviews, samples taken from cervical and vaginal discharge by a speculum were used for detecting *Chlamydia trachomatis*. A limited number of studies have been conducted on urine samples.

Since, in the current study, pregnant women who were in the first trimester of pregnancy were studied, among whom the risk of abortion is high and the use of speculum may also add to this risk, a urine retention sampling method was used to avoid maternal and fetal harms.

The aim of this study was to detect and isolate *Chlamydia trachomatis* from urine and its role in preterm labor.

Materials and Methods

This cross sectional study was carried out from 2016 to 2017 in Al-Mahdi Clinic under the supervision of Tehran University of Medical Sciences. 119 pregnant women who were in the first trimester of pregnancy (between 12 and 14 weeks) referred to a health center for screening tests were selected using available sampling method and were collected their urine samples.

Considering the accuracy of 7% for the diagnosis of the considered difference and given the prevalence rate of *Chlamydia trachomatis* in women, the required number of samples was obtained 119.

Initially, personal information questionnaires were answered by these women and sampling was performed after obtaining their full written consent. These women were admitted to the clinic for routine screening tests so there was no need for ethical considerations. The best urine sampling method for performing the PCR test aimed at detecting *Chlamydia trachomatis* was in a way that these

women should hold their urine for 4 hours prior to the test. This increased the chance of isolation. After 4 hours of urinary retention, the patients were trained to collect their urine in urine culture containers. Urine samples were taken from the patients in sterile urine culture containers. In sterile conditions, 10ml of urine was centrifuged at 3,000rpm for 10 minutes. Then, about 1 to 2ml of urine sediment was transferred to sterile microfuges and stored at -70°C until the PCR tests were performed.

DNA extraction from the samples was conducted using Tissue DNA Extraction Kit with A101211 code (Pars Toos; Iran). After the extraction, the DNA samples were stored at -70°C until the PCR tests were performed.

To conduct the PCR tests, ready-to-use commercial kits named *Chlamydia trachomatis* PCR Kit with A101122 code (Pars Toos; Iran) were used. All the employed kits had both positive and negative controls.

The data were analyzed using Chi square test.

Findings

The mean age of the studied pregnant women was 28.94±5.50 years old (Table 1).

The rate of isolating *Chlamydia trachomatis* from the urine of these pregnant women was 6.72%. There was no significant relationship between chlamydia infection and history of abortion (Table 2; Figure 1).

Table 1) Frequency distribution of education level, history of variables including abortion, preterm labor, and multiple pregnancies and the number of pregnancies and methods of contraception in the studied pregnant women (n=119)

Variables	Number	Percentage
Education level		
Primary school	10	8.40
High school	18	15.13
Diploma	69	57.98
Associate degree	8	6.72
Bachelor	14	11.76
Abortion history		
Non	88	73.95
One	25	21.01
More	6	5.04
Preterm labor		
No	116	97.48
Yes	3	2.52
Multiple pregnancies		
No	116	97.48
Yes	3	2.52
Pregnancy time		
1	35	29.41
2	47	39.50
3	28	23.53
4	7	5.88
5	2	1.68
Pregnancy prevention method		
Natural	85	71.43
Condom	12	10.08
LD	12	10.08
IUD	8	6.72
Injection	2	1.68

Table 2) Relationship between cases of positive *chlamydia* in urine samples of the pregnant women and history of abortion (n=119; the numbers in parentheses are percentages)

Abortion	Positive	Negative	P.Value
No	6 (75.0)	82 (73.87)	0.93
Yes	2 (25.0)	29 (26.13)	

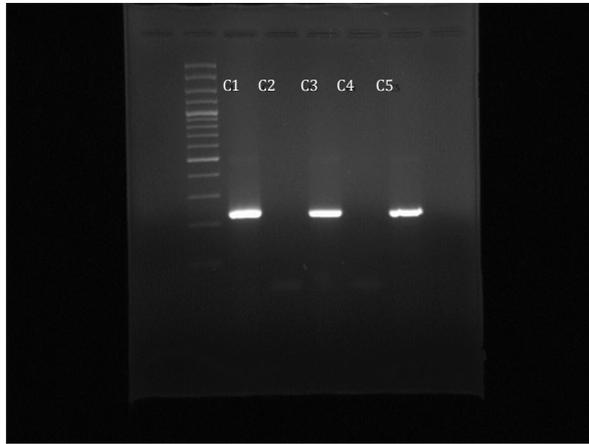


Figure 1) Gel electrophoresis of PCR products of *Chlamydia trachomatis* (C1: control positive, C2: control negative, C3, C4 & C5: urine samples)

Discussion

Among genitourinary tract infections, sexually transmitted infections are different from other infections because they are mostly asymptomatic and should be treated concurrently in couples. In such infections, treating the women alone is inadequate and, sometimes, patients' responses to a treatment are not effective. Moreover, many cases of recurrence of these infections were reported [2]. Sexually transmitted infections are often caused by bacterial agents, such as *mycoplasma* and *chlamydia*, or viral agents, all of which are hard to grow and routine culture methods used for these agents usually have false negative results and take a long time to reach the final result. The contamination of clinical samples is another problem with routine culture methods. Serological methods are also not valid enough due to generating false positive results. Thus, in order to detect these infectious agents, molecular detection PCR methods are more appropriate [3, 4].

Reviewing previous carried out studies have indicated that, in most cases, samples taken from cervical and vaginal discharge using invasive sampling methods were studied for molecular detection methods. These invasive methods are often not accepted by women, and in addition to being unfavorable, in the case of pregnant women, the risk of abortion also threatens the mother. Therefore, the use of alternative samples that do not include invasive sampling methods and produce valid results is desirable. Collecting urine samples is one of these alternatives [5].

A study conducted by Samoa examined the existence of *Chlamydia trachomatis* in 239 women aged 18 to 29 years using PCR. The results showed that 36% of these women (mostly aged 18 to 24) were infected. For the purpose of this study, urine sampling was used [6]. In addition, a retrospective study carried out on 590 women, from 2010 to 2014, demonstrated that 11% and 6.9% of women were infected with human papillomavirus (HPV) and *Chlamydia trachomatis*, respectively [7].

According to a study conducted in Switzerland, 146 pregnant women who had preterm labors were compared with 261 women who had normal deliveries. The results demonstrated that the former group dealt with a higher proportion (with a significant difference) of *Chlamydia trachomatis* infection than the latter group. The results were obtained by using the PCR test [8].

In USA, on average, 3 to 6% of apparently healthy women below 25 years of age are infected with *Chlamydia trachomatis*. In general, the incidence of *Chlamydia trachomatis* infection varies in different countries so that, according to the conducted studies, the prevalence rates of this infection are 3.9% in the United Kingdom, 6% in Finland, 17% in France, 9% in Poland, and, according to various studies, between 6.5 and 22% in Iran. In these studies, endocervical samples obtained from women who had a history of abortion and were not pregnant during sampling were used [9].

Based on another study carried out on urine samples of 847 pregnant women who were in the first trimester of their pregnancy in France, the rate of positive *Chlamydia trachomatis* detected by the PCR method was about 1.7% [10].

In another study conducted by Jensen *et al.*, the researchers concluded that the use of urine samples for detecting *Chlamydia trachomatis* by the PCR method was better than the use of urogenital swab samples. They figured out that urine samples were better than cervical samples for detecting these agents [11].

In the same line, a study carried out in Iran evaluated the prevalence rate of colonization with *Chlamydia trachomatis* in pregnant women and reported that this rate was 2.8%. The studied samples in this study were taken from the cervical discharge of women who were in the third trimester of their pregnancy when the risk of abortion is less than the first trimester. Based on another similar study conducted in Iran, the number of positive cases of *Chlamydia trachomatis* detected by PCR in pregnant women who experienced abortions or preterm labors was 14%. Cervical, urine, and placenta samples taken from pregnant women were used in the mentioned study [12].

The design constraints included some women who were reluctant to complete the questionnaire so they excluded of study. Other limitations were that

women who had not their gestational age between 12 and 14 weeks were also excluded.

It is recommended that the number of more samples be used for future studies and in addition to the urine sample, vaginal discharge is also used for diagnosis *Chlamydia trachomatis*.

The results indicated that considering these 119 pregnant women under study and using the PCR method, the rate of the isolation of *Chlamydia* from the urine samples of these pregnant women who referred to the healthcare center to conduct the first-trimester screening tests was 6.72%. According to the results of previous studies, the level of the isolation of *Chlamydia* from urine samples taken from women in society was reported to be 3% on average. Based on the one sample t-test, no significant difference was found between these two groups ($p=0.10$). Considering the absence of a significant difference between the two groups, as the reported percentages showed, the level of the isolation of *Chlamydia trachomatis* from the urine of pregnant women was twice more than the level of its isolation from samples taken from vaginal and cervical discharge in pregnant women, because, according to the results of previous studies, the level of its isolation from samples taken from vaginal and cervical discharge was reported to be 3%. Therefore, urine is an appropriate sample for the diagnosis of *Chlamydia trachomatis* since the sampling method is non-invasive and has more positive cases.

Conclusion

The level of the isolation of *Chlamydia trachomatis* from the urine of pregnant women is twice more than the level of its isolation from samples taken from vaginal and cervical discharge in pregnant women. Therefore, urine is an appropriate sample for the diagnosis of *Chlamydia trachomatis* since the sampling method is non-invasive and has more positive cases.

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Statistical analyst/ Discussion author (10%)

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