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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

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Abstract:

CONTEXT: *Chlamydia trachomatis* is one of the most prevalent factors of sexually transmitted diseases worldwide, which causes abortion, premature rupture of membrane, uterine cervicitis, pelvic inflammatory diseases, and ectopic pregnancy.

AIMS: The aim of this study was to investigate the presence of *C. trachomatis* genome in pregnant women with abortion in Yasuj, Iran.

SETTINGS AND DESIGN: This is a descriptive–analytical study in Shahid Mofateh Gyneco-obstetrics clinic and Emam sajjad Hospital.

SUBJECTS AND METHODS: Specimens of cervix, urine, placenta, and blood of 107 pregnant women were simultaneously collected at maternity ward and operation room and were stored for molecular tests. The molecular tests were performed, and the results were analyzed.

STATISTICAL ANALYSIS USED: Analysis was performed using SPSS software 9.1 (IBM, Illinois, Chicago, USA) with descriptive–analytical statistics.

RESULTS: Of 107 women, 15 had polymerase chain reaction-positive tests for *C. trachomatis*. The age of the studied individuals was 15–38 years with mean age of 26.6 years. The highest infection rates were in the age group of 21–25 years (7.47%), and the lowest frequency was in the age group of 36–40 years. A significant difference was noted between the number of first abortions in the case and control groups.

CONCLUSIONS: Our results showed that *C. trachomatis* as a sexually transmitted agent causes severe complications such as abortion; thus, it is important to screen pregnant and sexually active women who are craving to carry a baby.

Keywords:

Abortion, *Chlamydia trachomatis*, pregnant women

Introduction

Chlamydia trachomatis is an intracellular, small, coccoid bacteria and is of the most prevalent factors of sexually transmitted disease worldwide. It has increased approximately 20% annually

since 1996 according to the World Health Organization (WHO) statistics. The annual global occurrence of chlamydia infection is 90 million.^[1-3]

In general, all sexually active women are at risk of such infection. Women in the age

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group of 15–25 years have highest risk of chlamydia infection.^[4] Approximately 60%–80% of chlamydial infection in women is asymptomatic, and due to complications during pregnancy, it is important.^[5] The recommendation of WHO is controlling and preventing the infection with emphasis on quick diagnosis and treatment.^[6] When the organism attacks the covering tissue of cervix, it can affect the immune system of the host and become chronic. In case of no treatment, the infection remains for months and causes intensive problems in the genital system with numerous short and long runs. In addition, the infection could move to upper genital system and cause infertility and ectopic pregnancy (EP) without any evident symptom.^[4,7,8]

The importance of the bacterium in midwifery is preterm delivery, abortion, rupture of aquatic sac, and neonatal infection. More than 60% of infants born to mothers infected with untreated chlamydia are infected with the bacterium during birth and suffer conjunctivitis and pneumonitis. Furthermore, the bacterium is one of the prevalent factors, which causes tubal infertility, cervicitis, pelvic inflammatory diseases, epididymitis, proctitis, EP, and arthritis.^[9,10] With respect to frequency of the infection and its complications, huge expenses (cost) are imposed to the community because the prevalence of the infection and its quick treatment is quite necessary with the purpose of controlling the complications. It is obvious that for designing proper policies toward controlling and preventing the infection in the community, the first step is to specify the frequency and prevalence of the infection in different populations, and meanwhile, the identification of population and groups at risk is necessary to design and provide proper guidelines for timely prevention and treatment of the infection in any population.^[11,12]

Among the population in whom the bacterial infection is of particular importance are the sexually active women, pregnant women, women with genital infection, and also women with the history of abortion. In a study by Fallah *et al.* from a urine specimen of women suffering from cervicitis, 14.9% of the studied specimen was infected with *C. trachomatis*.^[13] Furthermore, in a similar study, 15.5% of the women suffering from cervicitis were infected.^[14]

Sexually active women are another group in whom *C. trachomatis* is very important, so that in a study by Bakken *et al.* on women in fertility age, the prevalence of infection was reported to be 4.1%.^[15] In addition, in a study conducted in Australia on sexually active women in the age group of 15–35 years, the prevalence of chlamydia infection was reported to be 13%.^[16]

Pregnant women are also another group who are very sensitive in view of the bacterial infection, so that in case of the existence of *C. trachomatis* infection, it is

possible that the infant will be infected with the bacteria during passing through delivery channel and may cause premature rupture of aquatic-sac, preterm delivery, and even sometimes abortion.^[17] The prevalence of *C. trachomatis* infection in pregnant women was reported to be 18.2%.^[18] In a study on 340 pregnant women referring to Gyneco-obstetrics clinics of Tehran, the prevalence of chlamydia infection was reported to be 11.2%.^[19] Another important factor of *C. trachomatis* in pregnant women is the bacterium's effect on abortion, so that in 2002, Salari *et al.* studied pregnant women with habitual abortion for the existence of chlamydia, and 7.2% of them tested positive for the direct immune florescent test.^[20]

Although the population of pregnant women with a history of abortion is very important in view of contamination to *C. trachomatis*, no remarkable study has been carried out in our country so far. The aim of this study was to determine the presence of *C. trachomatis* infection in women with history of abortion in Yasuj, Iran, as being aware of the rate of prevalence in the sensitive population, would enable medical personnel to emphasize the necessity of screening of the infection during pregnancy and even before pregnancy, and provide preventive and sanitary plans in this field.

Subjects and Methods

In this descriptive–analytical cross-sectional study performed from September 2010 to June 2014, 107 pregnant women referring to gyneco-obstetrics clinic and maternity center of Yasuj, Southwestern Iran, with current abortion, history of abortion, or with normal delivery were included in the study. Of these women, 30 were in the control group and 77 were in the patients group. In this study, women with definite diagnosis of abortion or fetal death were in the patients group and women in delivery process or with the history of successful delivery were in the control group. The taken specimens included 2 ml of urine sediment, 10 ml of blood, and 25 g of placental tissue and cervix swab. Mentioned specimens were homogenized in brain–heart infusion broth (BHIB). Sampling was done in Yasuj city simultaneously at Shahid Mofateh Gyneco-obstetrics clinic, maternity center and operation room of Emam Sajjad Hospital. The specimens used were urine, cervix, blood, and placenta. The research tools in this study were questionnaires that gathered personal information, including age, job, level of education, place of residence, number of pregnancies, number of abortions, timing of abortion in weeks, and history of fertility.

After identification of the patients and explaining the objectives of the study, sampling, and procedure, informed consent was obtained from the participants. Then, sampling was done and transferred to the laboratory in BHIB. Urine specimens were transferred after enrichment by centrifuge.

The samples were stored at -20°C temperature until the time of DNA extraction. After collecting all of the samples, extraction of DNA was performed simultaneously. DNA extraction was performed using Cinagen extraction kit (DNP kit and dn8115c code) after taking the samples out of -20°C storage. After adding proteinase K, the samples were kept at 55°C for 1.5 h. Then, after adding 200 μl lyse solution and 300 μl sediment solutions, the specimens were stored at -20°C for 24 h. Consequently, the samples were centrifuged at 13,000 rpm, and the surface solution was discarded. By adding 500 microliters of washing solution to the obtained sediment and centrifuging several times, the solution containing pure DNA was obtained for polymerase chain reaction (PCR) and was kept in microvial at -20°C temperature until centrifuging was performed. Consequence of primers used and size of the obtained product in the study and Polymerase chain reaction (regime) are shown in Tables 1 and 2 respectively.

Results

The age range of the participants was 15–38 years, with the mean age of 26.6 years. Of 107 women who participated in the study, 15 had a positive test for PCR of *C. trachomatis*. The highest infection rate was in the age group of 21–25 years (7.47%), and the lowest frequency was observed in the age group of 36–40 years [Table 3]. The number of abortion cases due to chlamydial infection

Table 1: Consequence of primers used and size of the obtained product in the study on *Chlamydia trachomatis*

Target	Primer sequence	Product size (bp)
Omp2	Ch.t.F: CCT GGA GAT CTT GTG TTG GGA GAT Ch.t.R: ACC ACA GTC AGA GCA GCT CTT	225

Table 2: Polymerase chain reaction (regime) to study *Chlamydia trachomatis*

Stage	Temperature ($^{\circ}\text{C}$)	Time
Preincubation	95	5'
Denaturation	94	1'
Annealing	60	40''
Polymerization	72	40''
Stabilization	72	5'

Table 3: Frequency and percentage of *Chlamydia trachomatis* with respect to number of abortions in the two groups

Abortion	Control		Test		Total
	Positive	Negative	Positive	Negative	
Without abortion	4.67	18.69	0	0	23.36
First abortion	0	3.74	7.47	48.59	59.82
Second abortion	0	0.93	0.93	10.28	12.15
Third and more abortion	0	0	0.93	3.73	4.67
Total	4.67	23.36	9.34	62.61	100

was also studied in this research, and the highest number of isolated cases of *C. trachomatis* was revealed in the first abortion (7.47%) and the lowest one in the second and third abortion. Although no significant difference was observed between the number of abortion and chlamydial infection, a statistical difference was observed among the first abortion in the groups ($P = 0.02$). In this study, the education status of participants varied in the range of illiterate to university education. The highest frequencies of chlamydia infection were in individuals with high school education (5 cases [4.67%]) and the lowest was in illiterate individuals (1 case [0.93%]). The electrophoresis image shows the products of polymerase sequential reaction of different samples [Figure 1].

Discussion

C. trachomatis is one of the most prevalent factors of sexually transmitted disease worldwide. The importance of the bacterium in midwifery is abortion, premature rupture of membrane, preterm birth, and neonatal infection during delivery. The objective of this research is to determine the frequency of *C. trachomatis* infection in women with the history of abortion in Yasuj city, Southwestern Iran.

According to the results obtained from the present study, 15 cases (14.02%) of *C. trachomatis* were isolated from the collected samples. The frequency was higher in the test group (10 cases, 9.34%), and there was a lower rate in the control group (5 cases, 4.67%) and no significant difference was observed between the studied groups in view of suffering from the bacterium. Different research cases have been carried out by other researchers, with different methods and different results.

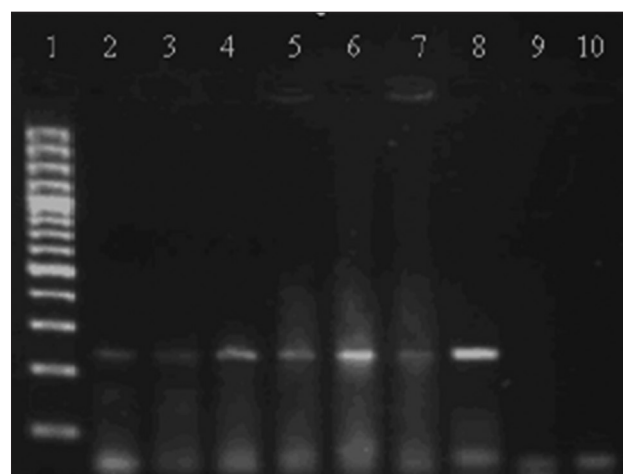


Figure 1: Polymerase chain reaction product gel electrophoresis: 1-100 bp marker; 2–7 - Clinically positive samples having omp2 gene of *Chlamydia trachomatis* (225 bp product); 8 - Positive control; 9 - Negative control; 10 - Clinically negative sample

In a study by Chamani *et al.* in Tehran, women with and without a history of abortion were studied for frequency of chlamydia infection. In this research, urine specimen was also studied, the participants were in reproductive ages, and they were also studied for history of abortion, education, job, and age of fertility. Nearly 11.7% of women with history of abortion had chlamydia infection.^[19]

In another study done by Willems *et al.* in Zaire (Congo, 1990), the percentage of chlamydial infection was studied in pregnant women and 9% of the participants were infected.^[21]

A study conducted by Somji *et al.* in Pakistan reported that 18.2% of pregnant women had positive results of chlamydia test.^[18]

Magon *et al.* showed a significant correlation between *C. trachomatis* and abortion in pregnant women, which is in contradiction to the results of present research.^[22]

In the present research, the highest isolation level of *C. trachomatis* is in the age group of 21–25 years (4, 3.73%) that is two times more than the frequency of test and control groups, and the lowest frequency was in the age group of 36–40 years, the results are similar in both groups. The correlation between age and *C. trachomatis* infection was not significant.

Chen *et al.* in China showed a significant correlation between infection rate and age, so that the highest positive cases were in the age group of <25 years. As it was observed in the first abortion among groups, it was not seen not in other abortions, which could be due to producing antibody and protection property of antibody in the subsequent abortions. It is recommended that pregnant women should be screened in view of the existence of the bacterium.^[23]

Conclusions

Results of current study showed that *C. trachomatis* as a sexually transmitted agent causes severe complications such as abortion; thus, it is important to screen pregnant and women in child bearing age craving to carry a baby.

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Conflicts of interest

There are no conflicts of interest.

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