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Molecular Diagnosis of *Chlamydia trachomatis* in Women with Frequent Abortions

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ABSTRACT

Background: *Chlamydia trachomatis* is the commonest cause of bacterial sexually transmitted infections. This research aimed at scrutinizing the *Chlamydia trachomatis* screening tests with vaginal samples and at investigating the correlation between *Chlamydia trachomatis* infection and the abortion incidence.

Methods: The current Cross sectional study was done at gynecology clinic of Razi Hospital in Chalus, Iran between August 2017 and January 2018. Fifty vaginal swabs were collected and detecting C. trachomatis DNA was done. Chi-square test and Independent t-test compared the variables. P <0.001 was significant.

Results: The total *C. trachomatis* infection prevalence was 5(10%) in endocervical swabs. A significant difference was found between duration of sexual activity and *Chlamydia* infection. No significant difference was detected between detection of *Chlamydia* l and abnormal vaginal discharged.

Conclusion: Screening for *Chlamydia trachomatis* infection of the women experiencing a miscarriage should be done and, if positive, they should be treated to inhibit recurrent miscarriages.

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Introduction

Chlamydia trachomatis is the commonest cause of bacterial sexually transmitted infections (STIs). (1). According to the World Health Organization, 92 million new cases are found every year, and 10 million of them live in Europe (2).

C. trachomatis is a non-motile, gram-negative, and obligate intracellular bacterium, which causes *Chlamydia* as a common STIs (3). In patients showing symptoms (e.g. abnormal vaginal discharge, intermenstrual bleeding, dysuria or pyuria), the incubation period lasts from one to three weeks (4). According to reports, more than 80% show no particular symptom. Therefore, high risk of chronic infection, ectopic pregnancy, pelvic inflammatory disease, salpingitis, chronic pelvic pain, or infertility tubular factor (5-6). Colonization of *C. trachomatis* in the reproductive system of pregnant women is associated with early onset delivery (PTB), premature rupture, spontaneous abortion, and perinatal mortality (7). Its effect on miscarriage is not clear, however, women with unrecognized *Chlamydia* l infection, after miscarriage, are more prone to ascending infection (8). This infection in women, hidden causes pelvic inflammatory disease (PID), resulting in tubal infertility. *Chlamydia* l PID can be prevented using on-time antibiotic treatment (9). The treatment costs of infertility and PID caused by *Chlamydia trachomatis* is high thus deveoping screening programs for diagnosing asymptomatic women is of high importance (10).

There are many diagnostic assays to diagnose *Chlamydia trachomatis*, like the gold standard method of the cell culture with a low sensitivity but high specificity provided only in some laboratories (11). Other methods are enzyme-linked immunosorbent assay (ELISA) as well as nucleic acid amplification, like polymerase chain reaction (PCR), which are available in many diagnostic laboratories (12). ELISA kits employ enzyme-labelled antibodies for lipopolysaccharide (13). Such antibodies are able to interact with other species of *Chlamydia*, thus, false positive results are possible. ELISA is less susceptible to cell culture (14). PCR is a highly sensitive and high-tech method and the obtained result is not associated with viability or the target organism intact state. MOMP gene, phospholipase and gene are targeted for diagnosis of *C. trachomatis* (15). The frequency of this infection was 14.99% in female cases in Tehran, with 20.76% in cases who had symptoms, compared to 9.23% in cases in cases with no symptom using PCR method and urine samples (16).

We evaluated the diagnostic utility of a PCR assay for detection of *Chlamydia trachomatis* from endocervical swab specimen, determine the prevalence of *Chlamydia trachomatis* infection in women attending the gynecology clinic in a north Iran (Chalus) tertiary care hospital and abortion for the first time in this area.

Materials and Methods

In the present Cross sectional study study performed from August 2017 until January 2018 on 50 women with frequent abortions and age range between 20-44 visiting the midwifery practices in obstetrics, gynecology wards and prenatal clinic in Razi Hospital, Chalus, Iran. Based on the *C. trachomatis* prevalence in Iranian women (17), The sample size was based on the census completion within the desired time period.

The inclusion criteria was having the experience of three or more abortions.

According to questionnaire, age, history of pregnancy, history of abortion, abnormal vaginal discharge and duration of sexual activity were documented for all women.

Sample collection and processing

The collected vaginal swab using a gynecologist was transferred to the Microbiology Laboratory of Chalous Azad University, followed by centrifugation (12000 g /20 min) and their pellets

underwent suspension in 500 ml phosphate buffered saline, and kept at -20°C for DNA extraction.

DNA Extraction and PCR

The tubes including the samples underwent centrifugation at 30,000 rpm for 30 minutes The supernatant was drained and the precipitates were transferred to a 1.5 ml microtube. DNA extraction kit was used for DNA extraction (preparation of high purity PCR template, Roche, Germany). PCR test: Two specific primers designed for *Chlamydia trachomatis* GeneBank. The following starting sequences were used: Forward: 5'-TGG CGG CGT GGA TGA GGC AT-3 and vice versa: 5'-CTC AGT CCC AGT GTT GGC GG-3 "and the target length of PCR was 300 bp. The total volume for PCR reaction was 25 µl of the main PCR mixture (SinaClon, Iran).

Statistical analysis

SPSS 20 analyzed the results. Chi-square test and Independent t-test compared the variables, and p <0.05 was regarded significant.

Result

The findings of the amplification of *Chlamydia trachomatis* gene by conventional PCR showed that gene was present in 5 (10%) out of 50 vaginal swap and 45(90%) were negative for this bacteria's genomes, and the PCR product of this gene was 243 bp (Figure 1).

The samples' age was 20 to 44 years (29.6±5.9). The mean age of women with *Chlamydia trachomatis* infection was 34.00 ± 2.00 (table 1) it has no significant differences(P<0.001).

Table 1 shows the association of *Chlamydia trachomatis* infection with abortion, the number of pregnancies and duration of sexual activity, abnormal vaginal discharged. 5 women (10 %) had an abortion during the third and fourth pregnancy, this difference of prevalence was significant (P<0.001).The mean length of sexually activity in women with positive *Chlamydia* was 12.40 ± 1.80 and this factor in women with negative *Chlamydia* was 5.7 ± 3.1 which had significant differences (P<0.001). In women with *Chlamydia* infection, 3 women (60%) had abnormal vaginal were discharged and in women without *Chlamydia* infection, 21 women (46.7%) had abnormal vaginal were discharged so there were no significant differences.

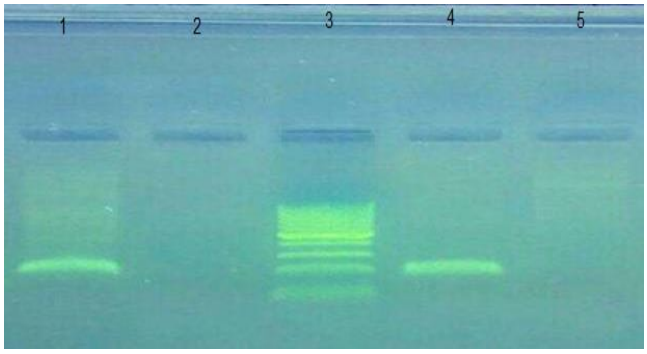

Figure 1. Gel electrophoresis of PCR of *Chlamydia trachomatis* gene. Lanes 1,4: positive results, lanes 2: negative result, lane 5: negative control, lane 3 DNA marker.

Discussion

Chlamydia trachomatis infections is a dormant disease which causes most infected women remain asymptomatic and undiagnosed. Though, screening women infected with *Chlamydia trachomatis* may diminish PID and its complication (18). The PCR assay can detect *Chlamydia trachomatis* infections in females, as it is the more specific, sensitive, inexpensive and rapid tool than the common microbiology tools to investigate infectious agents of the genital tract (19). Also, the diagnosis of bacteria in the medical laboratory is very difficult.

Table 1. Association of *Chlamydia trachomatis* infection with abortion, the number of pregnancies and duration of sexual activity and abnormal vaginal discharge.

		PCR				
		NEGATIVE		POSITIVE		
		Count	Column N %	Count	Column N %	P
Age (Mean±SD(Median))		32.00 ± 6.00(34.2)		34.00 ± 2.00(34.0)		0.296
abortion	3	29	64.4%	1	20.0%	*<0.001*
	4	11	24.4%	4	80.0%	
	5	5	11.1%	0	0.0%	
the number of pregnancies	3	10	22.2%	0	0.0%	*0.001*
	4	18	40.0%	1	20.0%	
	5	14	31.1%	1	20.0%	
	6	2	4.4%	3	60.0%	
	7	1	2.2%	0	0.0%	
duration of sexual activity (Mean±SD(Median))		5.7 ± 3.1(5.0)		12.40 ± 1.80(12.0)		*0.001*
Abnormal discharged activity	NO	24	53.3%	2	40.0%	0.461
	YES	21	46.7%	3	60.0%	

Hence, molecular methods, like PCR offer more positive findings compared to conventional techniques (20). In our study showed the prevalence of 10% (5 cases) for *C. trachomatis* in endocervical specimens of all women. In an urban medical center in USA, the prevalence of *Chlamydia trachomatis* infection was 4.7% in pregnant women (21). Its prevalence was from 4.1% to 25% in young women from various countries in the Europe (22). In Iran, endocervical specimens were applied to detect *Chlamydia trachomatis*.

In Joolayi's study in Ahvaz, the *C. trachomatis* prevalence in infertile women using PCR and IgM was 5% (5 case) and 6% (6 cases)(17). The overall *C. trachomatis* prevalence in women in Tehran, Iran suffering from cervix was 17% (123.21) assessed by PCR-EIA (23). The prevalence of *Chlamydia* infection in 650 women in GBA using PCR in Ahvaz was 18.1% (24). The rate of *C. trachomatis* infection by PCR in 80 asymptomatic and symptomatic women was 27.2% and 18.9%, respectively (25). Intrauterine samples were taken from women with cervical cancer in Tehran. Out of 142 samples, 22 cases (15.5%) were positive for *Chlamydia* PCR (26). In Ahmadi study in Sanandaj result of prevalence was 17/4 % (13). Although the reason for variations in the prevalence rate reported by other studies and our study is unclear, this could be due to demographic and socioeconomic factors (13).

Furthermore, in our study the mean age women with incidence of positive *Chlamydia* was 34.00 ± 2.00 year age, which was in line with other reports. Jenab with co-authors. (27) assessing asymptomatic and symptomatic women in Isfahan, declared an association between 34-45-year age group and *Chlamydia trachomatis* infection . In West Midlands, UK, there was a significant increase in the prevalence of STIs even in older adults (≥45 years old) (28). Parish et al. (29) found that the age range of 25-44 years is more affected by *C. trachomatis* infection in China. In China and other Asian countries, the onset of STIs can be late because of sexual activity starting following reaching adolescence (30). However, this infection is more common in younger ages (31-32). No clear reason is available for the incidence of this infection in older ages.

Its prevalence in woman with abortion was 10%. There was a significant correlation between *Chlamydia* infection and spontaneous abortion

(p<0.001) which has been reported in other studies (33-34-35-13).

C. trachomatis is the commonest curable STI in several developed countries (36). In our research, the prevalence of this infection in women with most duration of sexual activity was statistically significant (p<0.001). *Chlamydia* can cause a purulent vaginal discharge, but it is asymptomatic in 80% of infected women (38-40). In our study, the relationship between *Chlamydia* infection and vaginal discharge was not significant .

Conclusion

An association was found between abortion and molecular evidence of *Chlamydia trachomatis* infection. Some studies suggested that the study of the effectiveness of screening *Chlamydia trachomatis* with sensitive molecular techniques and treatment in pregnant women to inhibit adverse pregnancy outcomes. All females experiencing a miscarriage are suggested to be screened for *C. trachomatis* infection and, if positive, probably treated to inhibit recurrent miscarriages. In addition, pre-natal screening may be suggested to reduce the incidence of unintended pregnancy outcomes.

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Ethics approval and consent to participate

This research was approved by the Mazandaran Azad University of Medical Sciences Research Ethics Committee (IR.IAU.SARI.REC.1397.008). Each participant provided the written informed consent.

Conflict of interest

The authors declare no conflict of interest.

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