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Full Length Research Paper

Prevalence of *Chlamydia trachomatis* infections in symptomatic women by polymerase chain reaction (PCR) immunofluorescence and Giemsa stain

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Chlamydia trachomatis **is a ubiquitous human pathogen that is responsible for the most prevalent bacterial sexually transmitted disease worldwide. Studies show that polymerase chain reaction (PCR) is more sensitive than cellular culture for detection of C.** ***trachomatis*** **infections. The aim of this study is to compare different laboratory methods, including Giemsa staining, direct immunofluorescence assay (DFA) and PCR for detection of** ***C. trachomatis*** **in women with urethral symptoms. In this study, 130 women with urethral symptoms admitted in the gynecology clinic, were used and specimens were obtained with endocervical swab for Giemsa staining, DFA and PCR. All the cases underwent these three techniques. Demographic data and the medical history of patients were obtained by direct interview; however, the mean age of cases was 33.8±9.06. Clinical symptoms included abnormal vaginal discharge in 101 cases (77.7%), spotting in 14 cases (10.8%), dysmenorrheal in 7 cases (5.4%), irritation in 6 cases (4.6%) and dysuria in 2 cases (1.5%). In DFA technique, 5 cases (3.8%) were positive and 3 (2.3%) were suspicious, while in the PCR technique, 6 cases (4.6%) were positive for** ***C. trachomatis.*** **However, 3 suspicious cases with DFA were negative in PCR. There was no positive case for** ***C. trachomatis*** **in Giemsa staining. In conclusion,** ***C. trachomatis*** **was not frequent in this study and it can be concluded that this infection was not a major hygienic problem in the same populations that were previously studied. Consequently, the causes that necessitate monogamy could be related to religious causes. Frequency of Chlamydia detection of DFA and PCR was same in the two groups. Nonetheless, Giemsa staining is not a reliable method for evaluating** ***C. trachomatis*****.**

Key words: *Chlamydia trachomatis*, polymerase chain reaction (PCR), direct immunofluorescence assay (DFA).

INTRODUCTION

Chlamydia trachomatis is an obligatory intracellular organism which causes different clinical symptoms like cervicitis, vaginitis, endometritis, pelvic inflammatory disease (PID), ectopic pregnancy, infertility and urethritis (Balabanov et al., 2006). About 70% of chlamydial infections in women are asymptomatic and only 30% are symptomatic (Patel et al., 2010). Annual incidence of chlamydial infection is around 92 million in the world (Mahon et al., 2007). Urogenital chlamydia infections are one of the most prevalent sexually transmitted diseases and it is a major cause of concern in public health (Tellis

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et al., 2009). Studies show that each year, around 3 to 4 million cases of new chlamydial infections are detected in the United States with an estimated annual expense of about 1.4 billion dollar (Stamm, 1999). According to its complications, as well as its social and economical problems, early diagnosis and treatment of *C. trachomatis* infection can be of great importance (Gerris, 2005).

There are many laboratory methods used to detect infections caused by *C. trachomatis*. Studies show that polymerase chain reaction (PCR) is more sensitive than the cellular culture for detection of *C. trachomatis* infection. Comparison of PCR with other Chlamydia diagnostic tests, such as direct immunofluorescence (DFA) and enzyme-linked immunosorbent assay (ELISA) shows that PCR is more sensitive and specific and has lower rates of false positive results (Santos et al., 2003, Schechter et al., 1997).

The aim of this study was to compare three different laboratory methods for diagnosis of symptomatic vaginal infection in women. These methods included Giemsa staining, polymerase chain reaction and direct immunofluorescence.

MATERIALS AND METHODS

This study is a perspective cross-sectional study which was carried out in the microbiology laboratory of Rasoul-e-Akram Hospital. In this study, 136 endocervical specimens were taken from women suffering from vaginal symptoms including abnormal vaginal discharge, spotting, postcoital bleeding, dysuria, lower abdominal pain, dyspareunia and dysmenorrheal.

For specimen collection, cervix was inspected and any mucous or vaginal fluid found was removed. A sterile Dacron swab was inserted into the endocervix for 1 to 2 cm, turned around for 10 to 30 s and taken out afterwards. A total of 3 swabs were prepared from each patient. One swab was used for phosphate buffer saline (PBS) containing microtube for PCR, while the second was prepared for DFA and the third was for Giemsa staining.

Direct immunofluorescence assay (DFA)

In this method, the specimens were preserved in microtubes containing PBS at 2 to 8°C, and then centrifuged for 15 min with a microcentrifuge apparatus. The supernatant was thrown away and the slide was prepared from the precipitate. The precipitate in the microtubes was transferred to the slides surface by a 50 γ sampler. The slides were air-dried for 5 to 10 min, followed by fixation in methanol (0.5 ml), while the conjugated antibody and mounting media were taken out from the refrigerator and held at room temperature for up to 10 min. Subsequently, 1 drop each of the conjugated antibody was put on the control slide and on each test slide. The antibody covered the specimen surface thoroughly. Then the control slide and test slides were put within a wet jar and held at room temperature and darkness for 15 min. After 15 min, the slides were taken out from the wet jar and washed in Becker with distilled water for 15 to 20 s, and were allowed to air-dry. Afterwards, one drop of the mounting media was then put on each test slide. The control and test slides were covered by the slide cover, while the prepared slides were observed under immunofluorescence microscope. If it were not possible to observe the slides right after preparation, the slides were preserved at a dark place for 2 to 8°C and maximally for 24 h. First, positive and negative control slides were observed, while the test slides were observed afterwards (Förstl et al., 2005).

PCR

Preparation of the samples for PCR and DNA extraction was performed by the method described by Santos et al. (2003). Briefly, endocervical smears were collected in 400 µl of TE (10mM Tris-HCl pH8.0 and 1mM EDTA). Each sample was supplemented with 4 µl triton 10% (v/v) and 4 µl proteinase K (10 mg/ml), followed by incubation at 55°C for 90 min and then at 95°C for 30 min. The samples were maintained at -20°C, until they were used (Santos et al., 2003). Primers KL1-5´ TCCGGAGCGAGTTACGAAGA 3´ and KL2-5´AATCAATGCCCGGGATTGGT 3´ were used for insertion of plasmid segment 24 bp of Chlamydia (KL1 and KL2).

A reactive system was used with the ultimate volume of 50 microlitres containing DNA, 5 microlitre Mgcl2 25 mmol, dNTP 25 mmol, KL1 and KL2 primers 1 mmol and Taq polymerase 1.5 unit. Amplification was performed at termocycler (MINICICLRE TM, MJ), with RESEARCH MODEL -150 for 35 cycles, as follows: 1 min of denaturation at 93°C, 1 min of anilation at 64°C, 1 min of polymerase at 72°C and a final phase of PCR at 72°C for 5 min. The products were analyzed at gel-agar by electrophoresis. The demographic data and past medical histories were collected by direct interview. However, P-value of less than 0.05 was taken as significant (Santos et al., 2003).

RESULTS AND DISCUSSION

This study was performed to evaluate the different laboratory methods of *Chlamydia* infection in symptomatic women. A total of 136 cases were included within the sample size, from which 3 cases were excluded due to poor sample quality and lack of cellularity, and 3 others were excluded due to unknown medical history. Consequently, the analysis was performed on 130 cases.

The range of patients' age was between 20 and 66 years old with the mean age of 33.8 ± 9.06, while the number of gestations was between 0 and 6 times for each patient, with the mean of 1.63 ± 1.57 times. Number of patients' children was 0 to 5, with the mean of 1.45 ± 1.46. About 110 patients had no previous history of abortion, while 16 patients (12.3%) had 1 abortion, 3 patients (2.3%) had 2 abortions and 1 patient (0.8%) had 3 abortions. A total of 69 patients (53.1%) had no previous history of antibiotic therapy, but 61 patients (46.9%) had the history of taking 1 to 4 different antibiotics, mostly doxycycline and metronidazole. In addition, 74 patients (56.9%) had no history of cervicitis, while 56 patients (43.1%) reported previous history of cervicitis. The most common clinical symptoms in this study were abnormal vaginal discharge in 101 cases (77.7%), spotting in 14 cases (10.8%), dysmenorrhea in 7 cases (5.4%), irritation in 6 cases (4.06%), and dysuria in 2 cases (1.5%).

Most of the patients had more than 1 symptom. Menstrual irregularities and vaginal discharge were the 2 most common symptoms, although 2 patients suffered

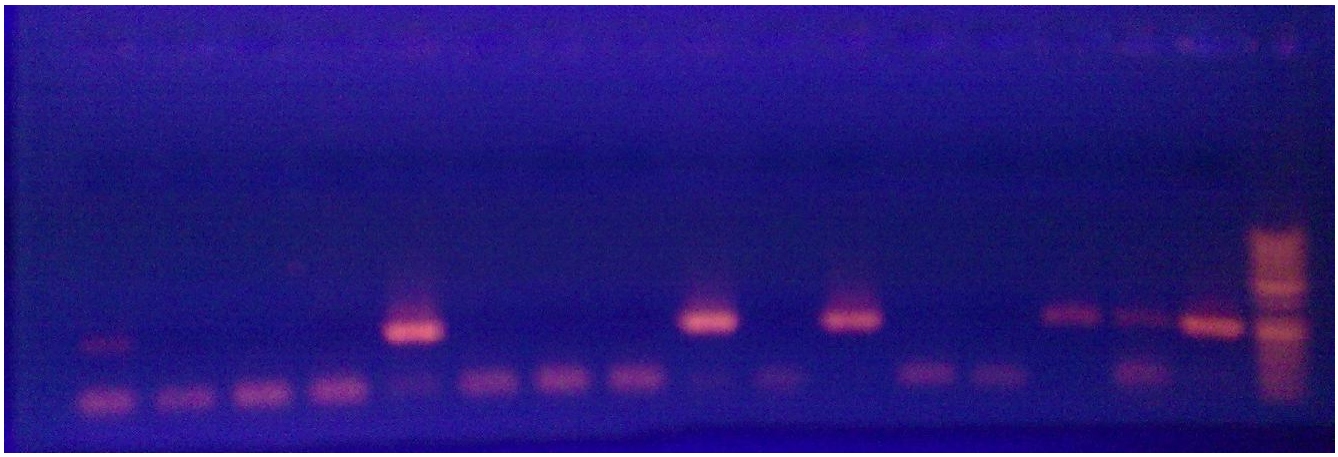

Figure 1. Results of gel electrophoresis of the PCR product of *C. trachomatis* KL1 and KL2.

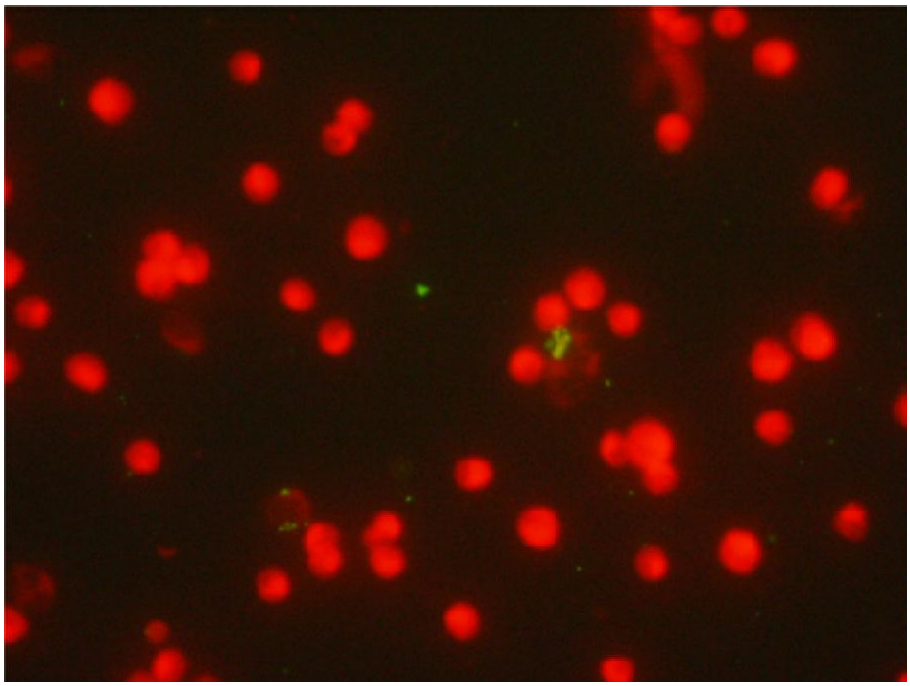

Figure 2. Specimen slide of positive *C. trachomatis* by DFA method.

from primary infertility. Five patients (3.8%) were positive for *C. trachomatis* infection by DFA method, while 3 other patients (2.3%) had suspicious results. In the PCR method for detection of *Chlamydia* DNA, 6 cases (4.6%) were positive. The 3 suspicious cases by the DFA method were proved to be negative at the PCR, which means the patients with the suspicious cases were not infected by *Chlamydia* (Figures 1 and 2). However, no positive results were achieved in Giemsa staining method (Table 1).

In the present study, *Chlamydia* infection was evaluated in women with vaginal symptoms and was performed on women from the general population. The sampling technique was cervical swab which is similar to that of most mentioned studies. The mean age of the study group was 33 years and this indicated that the majority were young and in the reproductive age. About 70% of the cases had previous history of pregnancy, while the average of gravidity was about 1.5 times. The other considerable point in this study was the previous history of abortion. In total, 21 patients were reported to have history of abortion; however, none of these had *Chlamydia* infection.

In this study, there was no relationship between *Chlamydia* infection and abortion. About half of the cases reported previous antibiotic therapy, but there was no

Table 1. Comparison of different laboratory methods for detection of *C. trachomatis*.

Result	DIF		PCR		Giemsa	
	Frequency	Percent	Frequency	Percent	Frequency	Percent
Positive	5	3.8	6	4.6	0	0
Suspicious	3	2.3	0	0	0	0
Negative	122	93.9	124	95.4	130	100
Total	130	100	130	100	130	100

significant relationship between antibiotic usage and *Chlamydia* infection in this study (3 patients in the antibiotic group and 3 patients in those with no previous history of antibiotic therapy). The other important point in this study was the absence of any relationship between cervicitis and *Chlamydia* infection. Five cases of the *Chlamydia* infected patients in this study had no previous history of cervicitis. In this study, the 2 patients with primary infertility were positive for *Chlamydia* in PCR method.

The diagnostic techniques of *Chlamydia* detection in this study were Giemsa, DFA and PCR, and there were no positive results in Giemsa staining method. The number of positive cases in DFA method was less in comparison with that of the PCR method, which is compatible with this study's expectation and that of previous studies which have been carried out by other investigators such as Santos et al. (2003); Krepelet et al., (2005); Ostergaard et al. (1991).

This study is among the limited studies which have been done in Iran for comparison of different laboratory diagnostic methods for detection of chlamydia infections. In the study of Sohrabi et al. (2007), 79 healthy pregnant women attending Imam Khomeini Hospital in Ahwaz during a period of one month were analyzed for *Chlamydia* immunoglobulin (IgG) with ELISA method. There were 8 samples (10%) positive against *C. trachomatis* (Sohrabi et al., 2007). In a study by Zaeimi et al. (2006), 142 endocervical samples were taken from women suffering from cervicitis, attending Mirzakoochakkhan women Hospital in Tehran, and DFA and PCR techniques were used to detect *C. trachomatis* in the endocervical samples. Twenty two (15.5%) of the 142 samples were diagnosed as *Chlamydia* positive according to PCR results, while DFA diagnosed 20 (14.1%) positive cases. No statistically significant difference was found between the two diagnosis methods applied in this study. In a study, Fatholahzadeh et al. (2004) first voided and then examined the urine of sixty-seven (62 males and 5 females) patients suffering from urethritis. In addition, discharges from the beginning of the urethral duct were collected for bacteriologic exanonation. The results of this study showed that fifteen (22%) of the specimens with *C. trachomatis* were positive with PCR method. In Hashemi's et al. study (2007), a sensitive diagnostic polymerase chain reaction-based enzyme immunoassay (PCR-EIA) method was developed which detects *Chlamydia* in women with cervicitis. Endocervical swabs collected from 123 women (20 to 55 years) with cervicitis were tested by both conventional PCR and PCR-EIA assays. The frequency of positive *Chlamydia* infection by conventional PCR and PCR-EIA assay was 7 and 17%, respectively. In a study by Nazer et al. (2008), one hundred and forty asymptomatic women were randomly selected from those who attended gynecology out patient department of Hazraat e Rasool Hospital in Tehran. First catch urine specimen were collected from all the participants and a total of 140 urine specimen were tested for the determination of *C. trachomatis* infection. *C. trachomatis* omp-1 was detected in 22.1% of the cases (Nazer et al., 2008).

In a study by Santos et al. (2003), the incidence of *Chlamydia* infection in sexually active young women diagnosed by PCR, was reported as 20.7% (Santos et al., 2003). In Fedorora's et al. (2007) study on 225 women, suspected to have Chlamydiasis, it was proved that PCR has the highest sensitivity and specificity in *Chlamydia* detection. In another study by Churakov et al. (2005), they compared PCR and DFA for detection of *Chlamydia*. Their study revealed higher positive results by PCR method in comparison with DFA. In Krepel et al. (1995) study, 55963 endocervical swabs were examined by PCR and DFA, from which 134 cases had definite positive results by DFA, while 38 other cases had suspicious results, 33 of which were positive in PCR method. In another study by Forstl et al. (2005), where 2563 cervical swabs were performed on non-patient women evaluated by DFA as air-dried slides, 14.1% were positive for *Chlamydia* infection. However, this rate was 14.5% in PCR method. In a study by Ostergaard et al. (1991), 336 cases positive for *C. trachomatis* by enzyme immunoassay, were re-evaluated by DFA and PCR methods. A total of 119 out of the 336 were positive by both DFA and PCR methods, where 6 cases were positive only in PCR method and 241 cases were negative in both methods. In Balabanov et al. (2006) study done on men with sterile pyuria, the *Chlamydia* detection rate was 13.6 and 13.44% by PCR and IF, respectively. According to Krepel (1995) study, 33 cases out of 38 suspicious cases in DFA method proved to be positive by PCR, but in this study this measure was null.

In the present investigation, the detection rate of *Chlamydia* by DFA and PCR methods is 3.8 and 4.6%, respectively, which is very low than the rates of previously

mentioned studies which have been reported to be between 12 and 20% (Balabanov et al., 2006; Churakov et al., 2005; Fatholahzadeh et al., 2004; Fedorova et al., 2007; Förstl et al., 2005; Hashemi et al., 2007; Krepel, 19951; Nazer et al., 2008; Ostergaard et al., 1991; Santos et al., 2003; Sohrabi et al 2007).

In addition, this study was performed on women with vaginal symptoms, while the previously mentioned studies were mostly performed on women who were referred to clinics for other tests such as pap smear or in totally asymptomatic women.

Conclusion

With respect to the results of this study and the previous studies mentioned already, it can be concluded that *C. trachomatis* infection rate in the present study is low. This point shows that this infection is not a major concern in society health care and it could be as a result of monogamy based on religious concerns in the studied cases.

Chlamydia detection findings of PCR and DFA in the present study are similar to the ones of the previously mentioned studies but in this study, PCR results showed that there were no positive results in DFA suspicious cases. Based on the results of this study, Giemsa staining is not a suitable and reliable technique for *Chlamydia trachomatis* detection.

Abbreviations

PID, Pelvic inflammatory disease; **ELISA,** enzyme-linked immunosorbent assay; **PBS,** phosphate buffer saline; **PCR,** polymerase chain reaction; **DFA,** direct immunofluorescence; **EIA,** enzyme immunoassay.

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