Original Article

Detection of Chlamydia Trachomatis Infection in Female Partners of Infertile Couples

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Abstract

Background: The prevalence of infertility is about 10-15% among the couples overally. Several factors can affect fertility ability of men and women. Chlamydia is a non-motile gram negative obligatory interacellular pathogenic organism. It can cause infections in females as cervicitis, urethritis, endometritis, pelvic inflammatory disease also prostatitis and epidydidimitis in male as well. The aim of this survey is to mention the frequency of infection with Chlamydia in infertile female who were treated in Yazd Research & Clinical Center for Infertility.

Materials and Methods: A questionnaire containing some demographic information and clinical features related to the infection was completed for each infertile woman. Specimen of vaginal discharge was collected by well trained nurses using sterile cotton swap from 91 women. Elisa test was done on blood serum. DNA extraction for Chlamydia was carried out using low salt method and PCR was done using MOMP and plasmid primers. DNA sequencing was performed on two PCR products using Chromas LITE ver.2.01 and analyzed by BLAST.

Results: Of 91 blood samples collected in this survey, none of them was positive by ELISA. Also there was no positive PCR result. Four PCR products showed a questionable band which was not in the range of Chlamydia. The products underwent DNA sequencing and there were not any finding related to any other micro-organism.

Conclusion: However, it is well known that Chlamydia as an infection plays a role in infertility. Nevertheless, there was not evidence of this organism in these infertile patients. It is necessary to design such a survey in larger populations of infertile patients especially on infertile women with tubal infertility and their husbands as well.

Keywords: Chlamydia Infection, Female Infertility, PCR, Chlamydia Screening

Introduction

The prevalence of infertile couples differs according to the definition of couple infertility. About 10-15% of the couples do not achieve pregnancy after one year of sexual intercourse and at the end of their reproductive age, 2-7% of couples remain childless (1).

The causes of infertility can be divided into four major categories: 1. the female factor, 2. the male factor, 3. combined factors and 4. Unexplained infertility. It is difficult to assign exact percentage to each of these categories; however, it is generally reported that in approximately 35% of the cases, infertility is mainly due to the female factor, in 30% to a male factor, in 20% to the abnormalities detected in both partners and in 15% of the cases, no diagnosis can be made after a complete investigation and this group is known as unknown cause infertility.

Infection is one of the causes of infertility either in men or in women. *Chlamydiaceae* are Gram-negative coccoid microorganisms that have a unique, obligately intracellular development cycle. The family contains a single genus, *Chlamydia*. The genus

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Chlamydia is subdivided into four species *including* C. *trachomatis*, which is pathogenic in humans and causes a variety of clinical diseases such as; trachoma, genital infections and lympho-granuloma venereum (LGV). *C. trachomatis* has been subdivided into 15 serotypes, which are grouped according to their clinical expression. The serotypes A-C are associated with ocular disease, while serotypes D–K with genital infections and L1–L3 with lympho-granuloma venereum (LGV).

Chlamydia trachomatis is one of the most common sexually-transmitted pathogens of humans, with an estimation of 92 million new cases occurring worldwide each year (2). Worldwide prevalence of genital *Chlamydia trachomatis* infection is reported to be 8 to 40% and it has focused the attention as being one of the commonest genital tract infections, causing endometritis and salphingitis, which may be asymptomatic in more than 50% of the cases (3). Risk of tubal infertility or ectopic pregnancy depends on the number of episodes and severity of infection. There is twofold increase in ectopic pregnancy after *Chalamydia trachomatis* infection (4).

In Iran, the true prevalence of *C. trachomatis* infection and its relation to infertility is not really known. Few studies have reported the prevalence of *C. trachomatis* between 7-37% in different populations of women (5-7).

The objective of the present study was to assess the prevalence of infection, caused by *C. trachomatis*, among infertile women attending to the Yazd Research & Clinical Centre for Infertility for their infertility treatment.

Materials and Methods

The study was carried from November 2006 to April 2007 at Yazd Research & Clinical Centre for Infertility. A total of 91 consecutive women from infertile couples, who were referred for their infertility problem to the Centre, were enrolled in this study. Some of the patients had clinical signs of genital tract infection and, apart from their infertility problem, were healthy individuals. These infertile couples were potential candidates for assisted reproductive techniques (ART). They were requested to answer a questionnaire concerning the history of previous infections (including PID and cervicitis) as well as some epidemiological data (age, duration of infertility, history and sign of infection, kind of medicine used for infection) and the infertility evaluation was performed afterwards. All patients received counseling before blood collection. Ethical committee of the centre approved the study and all the patients read and signed the informed consent.

The blood serum was taken from each patient for ELISA using the Trinity BioTech Capita Chlamydia IgG, Bray, Ireland. In addition, from each infertile woman, an expert nurse collected a vaginal discharge sample by using a sterile cotton swab. The sample was placed in 1 ml medium (phosphate-buffered saline, PBS 1X, pH 7.2 and SDS 0.001%, Dygene) and transported to the genetic laboratory. DNA was extracted by phenol/chloroform extraction method; according to the protocol routinely used in our lab as described by Sheikhha et al. (8). PCR amplification was performed on 100 ng of genomic DNA, in a reaction mixture, with the total volume of 30 μL that contained 2 mM $MgCl_2$ and 12.5 pM of each of forward and reverse primers of major outer membrane protein (*MOMP*), plus β-*globin* primers (Table 1).

Table 1: Primer sequences for *Chlamydia trachomatis*

Gene	Nucleotide sequences of primers	BP
MOMP	F: 5'CCTGTGGGGAATCCTGCTGAA3' R: 5'GTCGAAAACAAAGTCACCATA GTA3'	144
plasmid	F: GGACAAATCGTATCTCGG R: GAAACCAACTCTACGCGT	517
b-globin	F-GAAGAGCCAAGGACAGGTAC R-CCACTTCATCCACGTTCACC	

F: forward primer, R: reverse primer, BP; Base pair

The reaction was carried out using 30 cycles at 94 °C for 1 min, 62°C for 1 min, and 72°C for 1 min. The PCR product was

run in 2% (w/v) agarose gel and the bands were visualized with UV light. DNA from the line with positive *MOMP* and β-*globin* alleles yielded 149 bp, and 268 bp products, respectively (9). To search any further information, related to the *Chlamydia* sp, whole DNA was amplified and sequenced from genomic DNA obtained from the indexed patients (with non-specific bands by *MOMP*) and searched in BLAST data bank, for significance of the sequences. Statistical analysis of the data was carried out by using ANOVA with the help of SPSS for Windows software. $p<0.05$ was considered to be significant.

Results

The mean age of female patients was 30.22±4.8 years (range 24-41) and the average infertility period was 3.6 years (range 2–12). According to the previous history of infection signs, 61.5% of the studied women had no history of infection , 9 % had disuria ,12% had urinary frequency and 16.5% had both problems. Considering the infertility factors: 50 patients (55%) were presented with female factor; 23 (25.3%) with male factor; and 12 (13%) with the combination of male and female factors. In 6 patients, (6.6%) the infertility was idiopathic. All the patients were tested for *C. trachomatis* by ELISA or PCR (using MOMP). These tests did not show any positive signs of *Chlamydia trachomatis* infection. However, there were some non-specific bands, ranged above 255 bp, as shown in Figure 1.

We used plasmid primer again and there was no positive band for *C. trachomtais* infection or non-specific bands, which were already seen in the four samples using MOMP (Fig 2). The absence of MOMP (in the presence of β-globin PCR product) was the indicator of the respective null genotype for each (Fig 1). Co-amplification of human β-globin served as a positive control, to ensure that a null genotype was attributed to the absence of the respective gene and not because of a PCR failure (9).

Because there were some debates on the non-specific bands, two samples from MOMP were tested by DNA sequencing and the result was searched in BLAST data bank but there were no significant sequences for any microorganism infection (Fig 3).

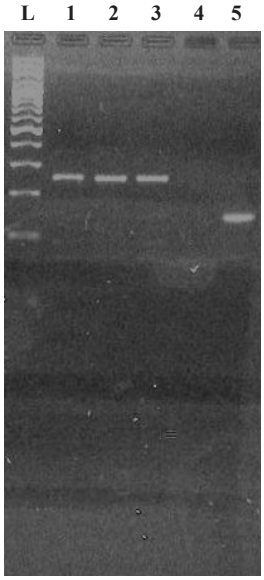

Fig 1: PCR products of MOMP and β-globin.
A 2% (w/v) agarose gel showing the PCR product. DNA from patients with negative MOMP, and β-globin alleles yielded 149bp, and 268 bp products, respectively. The absence of MOMP (in the presence of β-globin PCR product) indicates the respective null genotype. Samples, positive for all three PCR products, were considered 'Non-specific bands (1-3). Line 4: negative control, L: Molecular weight marker and line 5: positive control 144 bp.

Fig 2: PCR products of plasmid
A 2% (w/v) agarose gel showing the PCR product.
DNA from patients with negative plasmid and 268 bp products, respectively. The absence of plasmid indicates the respective null genotype. Samples were previously positive for all three PCR products as 'Non-specific bands with MOMP. Line 4: negative control, L: Molecular weight marker and line 5: positive control 144 bp.

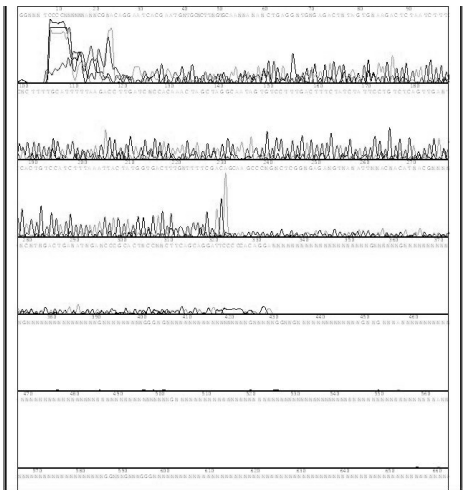

Fig 3: The automatic DNA sequence of the patients samples with non-specific band using MOMP primer (Chlamydia Trachomatis) were searched in BLAST data bank and there were no significant sequences for any microorganism. *Chlamydia trachomatis* infection was not detected. The coloured line indicated for each base as; A=green, T=red, C=blue, G=dark green, unknown=pin

Discussion

One of the most prevalent sexually transmitted pathogens is *C. trachomatis*, therefore it is important to determine the prevalence of infection by this microorganism in infertile couples.

It is concluded that infected women are usually asymptomatic and because of the serious morbidity of these infections, *Chlamydia* programs have traditionally focused on screening the women (10).

It is well known that all forms of vaginitis may include cervicitis, leading to a change in cervical mucus pH, which may impair the fertilization ability of the sperms. Infection by different bacteria can affect the fertility potential of couples in the reproductive age. *Chlamydia trachomatis* is one of the common infectious microorganisms, which is potentially able to cause fertility problem. It is an important associative risk factor for developing the subsequent tubal infertility in women.

Since the symptoms of *C. trachomatis* infection are not specific, the laboratory methods are required for a definitive diagnosis of the infection. Witkin (11) concluded that the individual, with asymptomatic infection, may shed fewer organisms, and in order to make tests easier and with higher specificity, nucleic acid amplification tests (NAATs) such as; PCR may be the techniques of choice for *C. trachomatis* assessment in asymptomatic male partners of infertile couples. Genital and urine specimens are known to contain several factors that inhibit DNA polymerase. PCR technology has been successfully employed for the diagnosis of several microorganisms. Different studies have been carried out, related to *C. trachomatis* infection prevalence, using PCR from cervical scrapers or urine sample (12-15), obtaining different results during different subject of the study. The problem with NAATs samples is the presence of potential inhibitors in clinical specimens that show false negative results (16).

During the present study, we did not find any infection by *Chlamydia* in the group of infertile women who were evaluated by either ELISA or PCR as routine protocols for the investigation before assisted reproduction technology procedures (ART). This finding was in agreement with Deeb et al. (17). Although, the prevalence of transmitted infection was 1.2% but no case with *Chlamydia* infection was detected. In a study of 400 infertile women by using two different techniques; enzyme immunoassay (EIA) and Ligase chain reaction (LCR), evaluated negative results in the first group and 1.9% in second group (18).

In addition, several studies on the subjects different from infertile women have shown a high prevalence of infection. Santus et al. worked on a population of 121 sexually active women in Brazil by using the plasmid. The data showed about 21% positive results for *Chlamydia* infection (19). In addition, in a study from Australia, the prevalence of *Chlamydia* infection was high (45%) in the infertile group and the number of infertile patients, who suffered from other types of infertility problems, was fewer than the women with tubal occlusion (20). Finally, in India, *Chlamydia trachomatis*

was detected in 28% of the infertile women (21).

Conclusion

Regarding the religious belief of Muslim community, which concerns women with a partner, the incidence of sexually transmitted diseases (STD) is low as compared to the western society. In addition, because many of the infertile couples seek health care for their fertility problem, this improves their health condition and their general knowledge on STD, therefore, most of them have a history of using antibacterial medications and this could explain detecting no case with *Chlamydia* infection in our study.

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