

Detection of *Chlamydia trachomatis* in Endocervical Smears of Women with Abortion

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Abstract: *Chlamydia trachomatis* is now one of the prevalent bacteria found in classic Sexually Transmissible Diseases (STD) and as such, constitutes a serious public health problem. *Chlamydia trachomatis* infections are known to manifest in a variety of syndromes in both men and women when left undiagnosed and untreated. While the clinical presentations in men include urethritis, epididymitis, etc., women suffer more serious complications such as mucopurulent cervicitis, Pelvic Inflammatory Disease (PID), ectopic pregnancy and tubal infertility. It is important that in the most cases infection with these bacteria is asymptomatic. In this study from 145 women with abortion and 75 healthy women (control group), endocervical samples were obtained. After collection of samples and patients records, samples DNA were extracted and PCR test with *Chlamydia trachomatis* specific primers (KL1, KL2) that based on amplification of 241bp *Chlamydia trachomatis* fragment were done and PCR product electrophoresis on agarose gel. From 145 women with abortion in 31 (21.37%) and in healthy women (control group) from 75 women in 3 (4%) *Chlamydia trachomatis* were detected, which difference is significant ($p < 0.05$). Women with positive PCR test were in 35-40 age and have a multi abortion. *Chlamydia trachomatis* one of the bacteria that causes many genital disorders. It is important that, although effect of these bacteria confirm on different genital disease, but mechanism of abortion and infertility that produce is unclear. According the results detection of these bacteria in women with abortion and infertile women can be important.

Key words: Abortion, *Chlamydia trachomatis*, PCR

INTRODUCTION

Chlamydia trachomatis infections are the most prevalent sexually transmitted bacterial infections in the world. According to the World Health Organization, there are 90 million chlamydial infections detected globally each year. In women, genital tract infections can lead to the serious sequelae of infertility, ectopic pregnancy and persistent pelvic pain following Pelvic Inflammatory Disease (PID). In men, they can cause urethritis and rarely epididymitis (João Gomes *et al.*, 2005; Marius Domelka *et al.*, 2000; Martin *et al.*, 2005; Schachter *et al.*, 1982).

Strains of *C. trachomatis* are classified into 18 serological variants (serovars) by monoclonal antibody typing of the major outer membrane protein, the immunodominant surface protein of this pathogen. *C. trachomatis* serovars have a propensity for different tissue types although the underlying mechanism for this tropism is not understood. Serovars A, B, Ba and C infect ocular tissue and rarely, Ba and C have also been isolated from the genital tract. Serovars D, Da, E, F, G, H, I, Ia, J and K are responsible for urogenital infections but can also cause a selflimited conjunctivitis and serious respiratory tract infections in both infants and adults.

Serovars L1, L2, L2a and L3 are responsible for lymphogranuloma venereum, which is a more invasive disease with ulceration and lymphadenitis (João Gomes *et al.*, 2005; Nelba *et al.*, 2005; Erol Keles *et al.*, 2005; Cook *et al.*, 1999).

Several laboratory methods are used for the diagnosis of *C. trachomatis*, these include cytological tests for the detection of intracytoplasmic inclusions, cell culture, immunoassay enzyme analysis, direct immunofluorescence, DNA hybridization techniques and DNA amplification-Polymerase Chain Reaction (PCR) (Cristina Santos *et al.*, 2002; Suzanne *et al.*, 2000).

MATERIALS AND METHODS

Collection and preparation of the samples for PCR:

One hundred and five women with abortion (1-5 abortion) and 75 healthy women, between the ages of 15- 45, studied in these investigation. Endocervical smears were collected in 500 μ L of TE. Each sample was supplemented with 5 μ L triton 10% and 5 μ L proteinase K, followed by incubation at 56°C for 90 min and then at 95°C for 30 min. The samples were maintained at -20°C, until used (Cristina Santos *et al.*, 2002).

PCR: The primers KL1-5'-TCCGGAGCGAGTTACGAAGA-3' and KL2- 5'-AATCAATGCCCGGGATTGGT- 3' were used to amplify a chlamidial plasmid 241 bp fragment (Cristina Santos *et al.*, 2002).

A typical PCR reaction containing a final volume of 50 µL was composed of 5 µL of the DNA sample, 25 mM of MgCl₂, 25mM dNTP, 1mM of each primer KL1, KL2 and 1.5 U of Taq polymerase. The amplification was made in a thermocycler Ependorf, using the following 30 cycle's program: denaturation at 92°C for 1 min, annealing at 62°C for 1 min and polymerization at 72°C for 1 min, followed by a final PCR extension at 72°C for 4 min. The PCR products were analyzed by electrophoresis in a 1.5% agarose gel. In this study, sterile dH₂O and *Chlamydia trachomatis* (ATCC 434) species were used as negative and positive controls, respectively, for both extraction of DNA and PCR analysis. All procedures were applied for both the samples and negative and positive controls.

RESULTS

From 145 women with abortion in 31 (21.37%) and in healthy women in 3 (4%) *Chlamydia trachomatis* were detected (Table 1), that these differants is significant (p<0.05). There was a 241 bp DNA band in agarose gel electrophoresis in Fig. 1.

From 31 positive patients in women with abortion, 12 women have a 4 abortion, 8 women have 3 abortions, 9 women have 2 abortion and 2 women have a one abortion in the medical history.

Table 1: PCR results in women with abortion and healthy group

Groups	PCR	Results	Total
Women with abortion	PCR positive	31* (21.37%)	145
	PCR negative	114	
Healthy women	PCR positive	3 (4%)	75
	PCR negative	72	

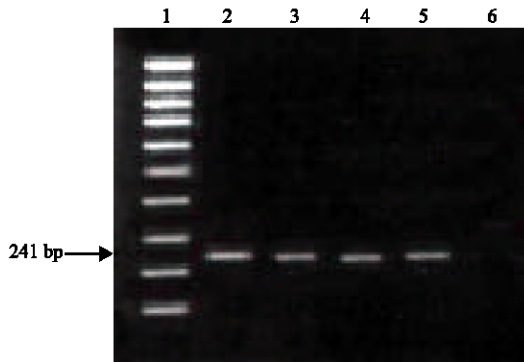


Fig. 1: PCR product in agarose gel. 1-DNA size marker, 2-control positive sample, 3, 4, 5 clinical positive sample, 6-control negative sample

Table 1: PCR results in patient and healthy women according to their ages

Groups	PCR	15-25	26-35	36-45	Total
Women with abortion	Positive	8	14	9	31
	Negative	31	44	39	114
Healthy women	Positive	21	22	29	72
	Negative	0	1	2	3

DISCUSSION

The members of the order Chlamydiales are obligately intracellular bacteria that are proven or suspected pathogens of vertebrates. Recent studies from the United States and Europe report that the prevalence of Chlamydia trachomatis ranges from 5 to 20% in sexually active persons (Domeika *et al.*, 1994; Claas *et al.*, 1991). Among all women infected with CT, 75-80% are infected at the cervix. Seventy to eighty of cervical infections are without associated symptoms or signs. When present, symptoms are non-specific. About 20-30% have specific signs of mucopurulent cervicitis including mucopurulent endocervical discharge, edematous cervical ectopy with spontaneous or easily induced endo-cervical bleeding. Among women, the consequences of the disease include pelvic inflammatory disease, ectopic pregnancy and infertility, sequelae often accompanied by a substantial economic impact. This study provided a PCR method that can be used as a rapid and inexpensive procedure for detection of Chlamydia. According the other studies different frequency such as 17, 19, 29, 37% and etc of Chlamydia frequency were reported. In all studies presence of these bacteria due to many genital disorders (Karin *et al.*, 1999). According our study frequency Chlamydia in women with abortion (3 or 4 abortion) group is highly than others and it seems that these bacteria are an important role in abortion produce. According our results the most women with positive test is in age with 26- 35 (Table 2).

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