Research Journal of Biological Sciences 3 (3): 342-344, 2008
ISSN: 1815-8846

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

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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* fragmant 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, endocervical smears, detection

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

Several laboratory method 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 *et al*., 2002; Suzanne *et al*., 2000).

MATERIALS AND METHODS

Collection and preparation of the samples for PCR: One hundred and forty 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 μL of TE. Each sample was supplemented with 5 μL triton 10% and 5 μL proteinase K, followed by incubation

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at 56°C for 90 min and then at 95°C for 30 min. The samples were maintained at -20°C, until used (Cristina *et al*., 2002).

PCR: The primers KL1-5'-TCCGGAGCGAGTTAC GAAGA-3' andKL2-5'-AATCAATGCCCGGGATTGGT-3' were used to amplify a chlamidial plasmid 241 bp fragment (Cristina *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 cycles 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.

RESULTS AND DISCUSSION

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.

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 5-20% in sexually active persons (Domeika *et al*., 1994; Claas *et al*., 1991). 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).

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

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

*p< 0.05

Table 2: 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

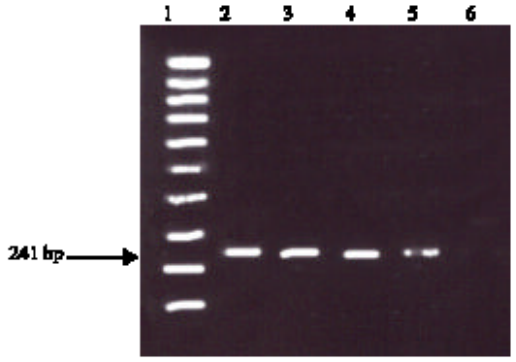

Fig. 1: PCR product in agarose gel. 1- DNA size marker, 2- control positive sample, 3, 4, 5 clinical positive samples, 6- control negative sample

REFERENCES

Claas, H.C.J., J.H.T. Wagenvoordt and H.G.M. Niesters, 1991. Diagnostic value of the polymerase chain reaction for Chlamydia detection as determined in a follow-up study. J. Clin. Microbiol., 29: 42-45.

Cook, R.L., G. Kirsten and M. Lassak, 1999. Screening for Chlamydia trachomatis infection in college women with a polymerase chain reaction assay. CID., 28: 1002-1007.

Cristina, S., T. Fabiane, V. Ana and A.F. Spartaco, 2003. Detection of *Chlamydia trachomatis* in endocervical smears of sexually active women in Manaus-AM, Brazil by PCR. Brazilian J. Infect. Dis., 7: 91-95.

Domeika, M. and M. Bassiri Mardh, 1994. Diagnosis of genital *Chlamydia trachomatis* infections in asymptomatic males by testing urine by PCR. J. Clin. Microbiol., 32: 2350- 2352.

Erol Keles, Yasemin Bu, K. Irfan, K. Turgut and Y. Sinasi *et al*., 2005. Identification of *Chlamydia trachomatis* with Polymerase Chain Reaction in Middle Ear Fluid in Otitis Media with Effusion. Indian Pediatr., 42: 686-691.

Karin, D.E. Everett and Arthur A. Andersen, 1999. Identification of nine species of the Chlamydiaceae using PCR-RFLP. Int. J. Sys. Bacteriol., 49: 803-813.

Marius Domelka and Odeta Drulyte, 2000. Use of PCR for the detection of genital *Chlamydia trachomatis* infection on self obtained mailed vaginal samples. Acta Obstet. Gynecol. Scand., 79: 570- 575.

Martin, Ingegerd Gu, H. Bjorn and E. Lars, 2005. Real-time PCR for pharmacodynamic studies of *Chlamydia trachomatis*. J. Micribiol. Methods, 61: 361-367.

Nelba, T.Á., ARnoldoz, B. Judith and J.G. Willem Melchers, 2005. *Chlamydia trachomatis* and genital human papillomavirus infections in females. universities students in Honduras. Am. J. Trop. Med. Hyg., 73: 50-53.

Schachter, J., E.C. Hill and E.B. King, 1982. *Chlamydia trachomatis* and cervical neoplasia. J. Am. Med. Assoc., 248: 2134- 2138.

Suzanne, M., T. Sepehr, H. Julene and Shujun Ch, 2000. Termination of pregnancy by PCR and LCR in women presenting for prevalence *Chlamydia trachomatis* Assessment. Sex. Transm. Inform., 76: 173-176.