Detection of Chlamydia trachomatis Using polymerase chain reaction (PCR)

Shatha T. Ahmed
Baghdad University / Science college for women/ Biology Department.

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ABSTRACT

Chlamydia trachomatis now is one of the most prevalent bacteria sexually transmissible diseases (STD), and as such, constitutes a serious public health problem. Diagnosis of Chlamydial infections is based on isolation of bacteria in tissue culture media that requires at least 48 to 72h.

The aim of this study was the detection of Chlamydia trachomatis, using polymerase chain reaction (PCR), because its a sensitive and specific method for detection of small quantity of bacterial DNA in clinical samples.

From May to December 2008, a total of 147 specimens of endocervical swabs were collect from 100 women were attending the Gynecology Departments of Women Health Center at Al-Elwyia Obstetrics Hospital in Baghdad and 47 women attending to Al-Samarrai infertility Hospital were examined by a specific PCR for the Chlamydia plasmid (KL1 and KL2) genes with a PCR product of 241bp. The PCR detection revealed that Chlamydia trachomatis in 39(26.5%) o f the infected women.

The results of this study indicate that PCR technique is a useful method for detecting C. trachomatis taken from endocervical area.

Key words: Chlamydia trachomatis , PCR,STD

INTRODUCTION

C. trachomatis is one of the most common sexually transmitted pathogens of humans, with an estimated 90 million chlamydial infections are detected annually worldwide [1]. Most chlamydial infections are asymptomatic both in men and women. However, if not treated properly, it can lead to severe sequelae in women, such as pelvic inflammatory disease, ectopic pregnancy, and tubal infertility [2]. Taking in account that chlamydiae are sensitive to antibiotics, it is
crucial to have an accurate method for the diagnosis of infections. Detecting chlamydial genital infection and preventing transmission and spread of this infection to the upper reproductive tract are challenges for both clinicians and laboratory workers [3]. *Chlamydia trachomatis* has 15 serovars (A-K, L1, L2, L3 and Ba). The growth of serovars D to K seems restricted to epithelial columnar and transitional cells, while serovars L1, L2 and L3 cause systemic disease (lymphogranuloma venereum - LGV). The location of the infection determines the nature of the clinical disease [4]. Diagnosis of *C. trachomatis* infection is frequently based on bacterial isolation in tissue culture media. This method requires careful specimen collection and stringent transport condition and requires at least 48 to 72 h to be performed [5]. Molecular genetics techniques are useful for the identification of microorganisms that are difficult to cultivate, such as *C. trachomatis*, and for those that grow slowly [6]. Polymerase chain reaction (PCR) has recently been introduced for detection of *C. trachomatis* and studies have reported its superior sensitivity in comparison with culture[7,8] enzyme-linked immunosorbent assay[6,9] or direct fluorescent-conjugated antibody (DFA) staining [10]. Information regarding incidence and prevalence of laboratory confirmed *C. trachomatis* infection of the genital tract in Iraq is limited to a few studies because of lack of proper laboratory facilities. Though PCR technique has been successfully used in clinical specimens elsewhere. In view of the sensitivity and broad applicability, as there have been no previous PCR studies of *C. trachomatis* infection in Iraq, in this PCR testing was used for detecting *C. trachomatis* from endocervical samples. Attempted to set up, a PCR based method to detect the *C. trachomatis* infection among the women population using individual single cervical swab samples.

**MATERIALS AND METHODS**

*Specimens:* Endocervical swab samples were obtained from 147 women from May to December 2008, out them 100 women were attending the Gynaecology Departments of Women Health Center at Al-Elwyia Obstetrics Hospital in Baghdad and the rest 47 women were attending to Al-Samarrai infertility Hospital.

*Preparation the samples for PCR.* In the present study, each clinical specimens was suspended in 1 ml phosphate buffered saline and vortexed for one minute to release the material from swab. The swab was removed and the suspension centrifuged for five minutes at 10,000 rpm/min to pellet cells. After removing the supernatant by aspiration, the cells were resuspended in 400 µl TE buffer(10 mM Tric-Hel, 1 mM EDTA ) with non-ionic detergent Triton X 100 1% and proteinase K (200 µg/ml). Cell suspension was incubated at 55°C for one hour, then
heated to 95°C for ten minutes to inactivate the proteinase K[11]. **Primers:** One pair of oligonucleotide primers specific for a region of the *C. trachomatis* gene coding for the 7.5 ORF cryptic plasmid was selected. The sequences from 5′ to 3′ of these oligonucleotide primers were as follows: The primers KL1-F (5′ TCCGGAGCGAGTTACGAAGA 3′); KL2 R(5′AATCAATGCCCCGATGGT 3′) were used to amplify 241 bp fragment of chlamyidal plasmids (KL1 and KL2) [11,12].

**PCR mixture and amplification** For PCR, Thermocycler (Thermo, USA) PCR reagents from promega, U.S.A. with GoTaq DNA polymerase were used. The mixture of final volum (50µl) for each sample contained 0.5µM each primer(forward & reverse); 200µM each dNTPs; 1X PCR buffer pH 8.3; 2.5 mM MgCl2; and 1.5 units of Taq DNA polymerase and 9µl of sample DNA was vortexed and subjected to 40 cycles of amplification. Each cycle was composed of 95°C for 5 minute for DNA pre denaturation and sequential incubations of 94°C for 1 minute for denaturation, 55°C for 1 minute for annealing primer to these templates, and 72°C for 2 minutes for DNA chain extension. At the end of 40 cycles, samples were kept for another 7 minutes at 72°C for completion of extension of DNA chain. **Amplified product detection:** Visualisation of amplified product was carried out by agarose gel electrophoresis. A 10µl of post-PCR mixture was subjected to electrophoresis on 2% agarose gel in presence of ethidium bromide. A DNA ladder (100bp) was also run simultaneously to confirm size of the amplified product.

**RESULTS AND DISCUSSIONS**

DNA was extracted from all 147 collected endocervical swab specimens, PCR amplification using these DNA samples showed that, of the 147 samples, 39(26.5 %) produced the specific 241bp DNA fragment (Figure.1), 25 of 100 sample from cervicitis women and 14 of 47 sample from infertile women.
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Shatha

**Fig. -1:** Detection of *C. trachomatis* from cervical specimens by PCR (cryptic plasmid primers). 2% agarose gel, 70 volt for 1 hour. Lane M, DNA size markers 100bp(promega, USA); lane 1 and 2, negative patients for *C. trachomatis*; lane 3 - 5 positive patients for *C. trachomatis,* and lane 6, negative control.

Culture was earlier considered the gold standard, but PCR studies suggest that the sensitivity of the culture even is as low as 75% to 85%. It is universally accepted that culture can no longer serve as a reference method in the evaluation of diagnostic tests for *C. trachomatis.*[13]. Recently, tests based on the amplification of nucleic acids have been developed as alternatives to conventional methods for the diagnosis of sexually transmitted diseases, such as culture, Direct Immuno Fluorescence and Enzyme Immuno Assay. So far PCR and Ligase Chain Reaction (LCR) are the two most frequently used DNA-based methods [14,15,16,17]. The cryptic plasmid have several regions of highly conserved nucleotide sequences, which have been used for primer selection.[11,12,14,18,19,20] In the present study, these primer pairs also identified the 241 sequence of KL1&KL2 gene of *C. trachomatis* infection present in individual cervical swab sample. PCR was carried out for 40 cycles, so that any sample with low number of *C. trachomatis* genome could be optimally amplified for visualisation. Present observation demonstrates that *C. trachomatis* can be detected by this PCR-based method, from cervical swabs in properly collected samples using an appropriate cycle number. It offers hope for more accurate diagnosis, which would lead to a better control of this infection and to a better prevention of pelvic inflammatory disease, ectopic pregnancies and tubal infertility. *Chlamydia trachomatis* is a highly prevalent bacteria in many regions, and it seriously affects public
health; therefore it needs effective epidemiological control, starting with an adequate method for correct and effective diagnosis. There are several studies that determined the frequency of \textit{C. trachomatis} in women with cervicitis. A study in Iran determined the prevalence of 15\% by using PCR [21]. In Manaus-AM, Brazil in endocervical smear of sexually active women is 20.7\% [11]. Another study in Papua New Guinea is 26\% [17]. Another study in Senegal showed that prevalence of \textit{C. trachomatis} in Dakar commercial sex workers is 29\% [22]. Although it is consistent with result of current study (26.5\%), the high-risk behavior of that study population can explain such a high prevalence of \textit{C. trachomatis} genital infection. The sharp worldwide increase in the incidence of PID during the past two decades has led to secondary infertility and ectopic pregnancy. \textit{Chlamydia} PID is the most important preventable cause of infertility and adverse pregnancy outcomes. The proportion of tubal factors infertility among all infertility cases ranges from less than 40\% in developed countries to up to 85\% in developing countries [23]. Punnonen \textit{et al.,} 1979 [24] published the first study linking past \textit{Chlamydia} infection and tubal factor infertility. Paavonen and Eggert-Kruse, 1999 [25] reported that, based on the available evidence, approximately 4\% develop chronic pelvic pain 3\% infertility, and 2\% adverse pregnancy outcomes. Watson \textit{et al.} [26] reported that Screening women for lower genital tract infection with \textit{Chlamydia trachomatis} is important in the prevention of pelvic inflammatory disease, ectopic pregnancy and infertility they also demonstrated that nucleic acid amplification techniques are superior to other methods for detecting asymptomatic chlamydial infection in a young and sexually active population. The test most often used to detect this sexually transmitted infection, result of this study revealed that 14 (35.5\%) infertile women. For this reason screening women for prevention of pelvic inflammatory disease, ectopic pregnancy and infertility is necessary in Iraq. \textbf{Conclusions} In summary, nucleic acid amplification methods, such as PCR, are significantly sensitive and should therefore be used in preference to other tests for the detection of genital \textit{C. trachomatis} infection.

\textbf{REFERENCES}

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