Chlamydial Epididymitis Diagnosed by Genetic Detection of *Chlamydia trachomatis* from Epididymal Aspirate by Polymerase Chain Reaction

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**Introduction**

When it is difficult and/or it takes a long time to isolate and identify pathogens by culture, a DNA probe method, which is a rapid, simple and specific detection system, is helpful for diagnosis and management of the infections. In addition to their advantages, a system using polymerase chain reactions (PCR) possesses a high sensitivity in detection of pathogens. PCR has been already applied for detection of *C. trachomatis* in urethritis and cervicitis and its usefulness has been demonstrated.

We applied PCR for diagnosis of acute chlamydial epididymitis. We reported a case in which chlamydial epididymitis was justified by detection of *C. trachomatis* from epididymal aspirate by PCR.

**Case Report**

A 38-year-old male had contact with a prostitute in February 1991. He consulted our clinic in March 1991, because he had noticed bilateral scrotal pain. Physical examination revealed tender swellings of the lower poles of bilateral epididymis. There were no pathological findings in the spermatic cords or testis. Although urethral discharge was not seen, urinalysis of the midstream urine showed pyuria without bacteriuria. Routine bacterial culture of the urine was negative. The urinary sediment and the urethral sample collected by a swab were examined by an enzyme-immunoassay (Chlamydiazyme®) and PCR for detection of *C. trachomatis*. Under local anesthesia with lidocaine in the left spermatic cord, 0.5 ml of sterile saline was injected into the swollen lower pole of the left epididymis and then aspirated with a syringe. The epididymal aspirate was also examined by Chlamydiazyme® and PCR for detection of *C. trachomatis*. The symptoms ameliorated after a one-week treatment with minocycline, but the tender swellings of the bilateral epididymis remained. The urinalysis was normalized. The urethral sample was collected by a swab for Chlamydiazyme® and PCR. After a two-week treatment with minocycline, indurations were still felt in bilateral epididymis, but were not tender. The aspirate was collected from the left epididymis, in the same manner as described above, for detection of *C. trachomatis* by PCR. The urethral sample and the urinary sediment were treated with 1 ml of the lysis buffer in Chlamydiazyme® kit following the protocol. Two hundred μl of the lysates and the epididymal aspirate were used for Chlamydiazyme® and 70 μl of them were used for DNA preparation. Chlamydiazyme® for detection of *C. trachomatis* was carried out following the manufactory protocol. For DNA preparation, 10 μl of 5% Tween-20, 10 μl of 5% NP-40 and 10
μl of 1 mg/ml proteinase K were added to 70 μl of the lysate of the urethral sample or the urinary sediment or to 70 μl of the epididymal aspirate, the mixture was incubated for 60 min at 55°C and then in boiling water for 10 min. Two μl of the mixture were used as template DNA for PCR. Two primer oligonucleotides (CT-A1; 5’GATAGCGGACCAAGAGACTA3’, CT-B1: 5’CCATAGATAACCCATACGCATGCTG3’) were complementary to the sequence within major outer membrane protein gene. The PCR solution consisted of 10 μl of 10X PCR buffer (500 mM KCl, 100 mM Tris-HCl, pH 8.3, 15 mM MgCl₂, 0.1% gelatin), 5 μl of a deoxynucleotide mixture (2 mM each of dATP, dCTP, dTTP and dGTP), 5 μl each of oligonucleotide primer (4 μM), 1 μl of Taq DNA polymerase (1 unit/μl), 2 μl of the clinical sample DNA solution and 72 μl of distilled water. In the positive control, C. trachomatis E/UW-5/CX DNA was used as template DNA instead of clinical sample DNA. In the negative control, no template DNA was added to the PCR solution. Finally, 100 μl paraffin oil was added to prevent evaporation. Thirty-two of the following incubation cycles were performed, as follows: 30-sec denaturation step at 95°C, 20-sec annealing step at 55°C and 30-sec extension step at 72°C. The amplified product was electrophoresed on a 2% agarose gel with molecular size markers (φ X174/Hind I digest). After electrophoresis, the gel was stained with ethidium bromide and then photographed under short-wave UV illumination. In the urethral sample collected at the first consultation, chlamydial antigen was detected by Chlamydiazyme® and a DNA fragment of 242-bp specific for C. trachomatis was amplified by PCR (Fig. 1). In the urinary sediment of the midstream urine and the epididymal aspirate collected at the first consultation, chlamydial antigen was not detected by Chlamydiazyme®, but a DNA fragment of C. trachomatis was amplified by PCR (Fig. 1). After a one-week treatment, chlamydial antigen in the urethral swab was negative by Chlamydiazyme® and a DNA fragment of 242-bp was not amplified by PCR. After a two-week treatment, no DNA fragment was amplified by PCR in the epididymal aspirate. Therefore, we concluded that the epididymitis in this case was caused by C. trachomatis.

**Discussion**

Primers-directed enzymatic amplification of DNA, PCR, has been shown to offer a number of potential advantages for genetics, microbiology, oncology and so on. PCR has been applied for diagnosis of viral and bacterial infections. The system for detection of C. trachomatis using PCR has been reported in urethritis and cervicitis and demonstrated to be as useful as culture method and enzyme-immunoassays.

The PCR procedure with the primers of CT-A1 and CT-B1 used in this report has been shown to amplify 242-bp DNA which is specific for C. trachomatis and common among 15 serovars of C. trachomatis by our previous studies. In this case, 242-bp DNA specific for C. trachomatis was amplified by PCR with

![Agarose gel electrophoresis of PCR products. M, DNA molecular size markers (φ X174/Hind III digest); P, positive control; N, negative control; 1, urethral sample at the first consultation; 2, urinary sediment at the first consultation; 3, epididymal aspirate at the first consultation.](image-url)
CT-A₁ and CT-B₁ from the epididymal aspirate. After treatment with minocycline, *C. trachomatis* DNA was not detected in the epididymal aspirate by PCR and the clinical findings ameliorated. Therefore, the diagnosis of chlamydial epididymitis was justified. This would be the first report in which chlamydia epididymitis was diagnosed by genetic detection of *C. trachomatis*.

*C. trachomatis* has been considered to be a major pathogen causing “nonbacterial” epididymitis¹³,¹⁴. Although the diagnosis of chlamydial epididymitis has been done by *Chlamydia* culture from direct aspiration of the epididymis or determining serum antibodies to *C. trachomatis*¹³,¹⁴, the diagnosis would be practically presumptive in most cases. In this report, we demonstrated that the rapid, sensitive and specific detection method of *C. trachomatis* using PCR could be used for diagnosis of chlamydial epididymitis as well as other chlamydial infections. This application could substantially improve the medical capabilities for diagnosis and management of patients with chlamydial epididymitis and help to understand its epidemiology.

**References**

Polymerase chain reaction 法により精巣上体吸引物より Chlamydia trachomatis を検出し得たクラミジア性精巣上体炎の 1 例

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急性クラミジア性精巣上体炎の 1 例を報告した。Chlamydia trachomatis に特異的のプライマーを用いた polymerase chain reaction (PCR) 法にて尿道障害物、尿沈渣、避孕した精巣上体からの吸引物から C. trachomatis DNA の増幅が認められた。ミノサイクリンによる治療により精巣上体炎の症状は改善し、尿道障害物と精巣吸引物からの PCR による C. trachomatis DNA の増幅は認められなくなった。精巣上体吸引物を検出材料として DNA 診断がなされたクラミジア性精巣上体炎の最初の報告と思われた。