Chlamydia Trachomatis Associated Reactive Arthritis: A Urinary PCR Based Study

Abstract

Background and Objective: *Chlamydia trachomatis* is increasingly being associated with reactive arthritis (ReA). The present study was undertaken to assess the role of *C. trachomatis* in patients with ReA since such data is lacking from the Indian population. **Materials and Methods:** PCR using specific primers for *C. trachomatis* was carried out from urine samples of 65 patients with ReA, 20 of other inflammatory arthritis, and 20 healthy controls. **Results:** *C. trachomatis* DNA was detected from urinary samples with PCR in 24 (36%) of 65 ReA patients. PCR was negative in the patients of other inflammatory arthritis as well as in normal healthy control group. Out of the 24 patients with urinary PCR positivity, 14 (58.33%) were males and 10 (41.66%) were females. **Conclusion:** Urinary PCR plays an important role in rapid diagnosis of ReA associated with *C. trachomatis*.

Keywords: *Major outer membrane protein, nucleic acid amplification tests, polymerase chain reaction on a urine sample, post-venereal reactive arthritis*

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Introduction

Reactive arthritis (ReA) is a form of inflammatory arthritis following certain gastrointestinal and genitourinary infections. ReA is characteristically an asymmetric arthritis mainly involving the lower limbs. Most patients of ReA have oligoarthritis or monoarthritis.[1,2] Such pattern of joint involvement is seen in nearly 50% of the patients presenting to the clinic with early arthritis,[3] thus making ReA an important differential diagnosis. The postdysenteric form of ReA has been associated with members of Salmonella, Shigella, Campylobacter, and Yersinia while the postvenereal form has been classically associated with genital infection by *Chlamydia trachomatis* (Ct).[1]

The reported incidence of ReA in the general population following genital infection (4.6–13/100,000) is similar to that following dysenteric infection (5–14/100,000),[4] suggesting that the burden of reported Ct-induced ReA (CIA) is similar to ReA caused by all other enteric pathogens put together. However, it still appears to be a gross underestimation of CIA burden.[5] Although the world has witnessed a decrease in dysenteric illnesses, Ct continues to be the most common sexually transmitted disease with 115 million new cases every year worldwide.[6] In addition, unlike dysentery, the preceding symptoms of Ct-related urethritis are vague and may not even be present in 80% of the cases.[7] Further, there is a dearth of data on CIA from India, though high incidence of genital infections with Ct continues to be reported from India.[8]

The pathogenesis of CIA involves acquisition of Ct from the entry site followed by its dissemination within monocytes through the bloodstream into the joints where an inflammatory response is generated.[9] Ct has been demonstrated within the mononuclear cell fraction of peripheral blood from patients with CIA[10] and also within the synovial fluid of inflamed joints.[11]

A wide variation in clinical severity, lack of disease definition, or specific diagnostic criteria, make the diagnosis of ReA challenging.[5,12] While culture technique is time consuming, costly, needs technical expertise and stringent precautions, serology lacks sensitivity, and specificity.[12,13] Nucleic acid amplification tests (NAATs) such as polymerase chain reaction (PCR),

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targeting Ct-specific antigens in the synovial fluid, have shown promising results in the diagnosis of CIA.[14,15] However, due to ongoing inflammation in the joint, it is not always practical to draw synovial fluid for testing. One alternative could be to use a urine sample for diagnosing CIA. The only study utilizing urine sample for detecting Ct by PCR was carried out by Kuipers *et al.*[16] way back in 1995 by spiking the urine samples with serially diluted load of Ct. To our knowledge, no study has yet evaluated PCR detection of Ct directly from the urine samples of ReA patients. The present study was therefore planned to assess the role of urinary PCR for *Chlamydia trachomatis* in patients with ReA since such data is lacking from the Indian Population.

Materials and Methods

Study design

105 urine samples received in the department of microbiology between 1/4/2009 and 31/3/2011 were included in the study. They were divided into three groups according to the following criteria:

Group I – ReA group (n = 65); patients diagnosed on the basis of diagnostic criteria laid down by third international workshop on ReA.[17] Informed consent was obtained and detailed history was recorded on a formed performa.

Group II – n = 20; patients of other inflammatory arthritis such as rheumatoid arthritis, ankylosing spondylitis, gout, and septic arthritis.

Group III – n = 20; healthy individuals without any illness.

Sample processing

About 10 ml of first void urine was collected in a sterile container from all the patients and the control groups. Out of this 2 ml was transferred to 2 ml microcentrifuge tube. It was centrifuged at 12,000 rpm for 20 min, supernatant was discarded and pellet was washed in 5 ml of sterile water. It was centrifuged again at 2000 rpm for 10 min. This pellet so formed was aliquoted into two. The first aliquot was used for DNA extraction and the second was stored at -20° C for future reference. All the samples were coded and randomly distributed to blind the investigator. DNA extraction was carried out using commercial kit (Qiagen, Hilden, Germany), as per manufacturer's instructions.

PCR for Chlamydia trachomatis

DNA was amplified using primers against major outer membrane protein (mOMP) of Ct (band size–180 bp). The targets used were CT-1:5/GCC GCT TTG AGT TCT GCT TCC 3/and CT-2: 5/GTC GAA AAC AAA GTC ACC ATA GTA 3/. Briefly, for a 50 µl reaction, PCR buffer10X, dNTPs 10 mM (1.0 µl), CT-1 (10 pm) (1.0 µl), and CT-2 (10 pm) (1.0 µl), Taq polymerase (0.5 µl), DNA template (10.0 µl), and molecular grade water (MGW) were added to an eppendorf. After initial denaturation at 94°C for 5 min, 40 cycles of amplification were carried out with annealing at 60°C for 1.5 min, extension at 72°C for 1 min, and final extension at 72°C for 10 min. Positive control DNA of *C. trachomatis* (obtained from Dr. A. Mittal) and negative control (MGW) were used for amplification with each run. The amplified products were stored at -4°C till detection. Amplified products were run on 1.5% agarose gel stained with ethidium bromide and were examined under UV light. DNA giving positive amplification band at 180 bp (corresponding to the positive control) were labelled as positive for *Chlamydia trachomatis* [Figure 1].

Statistical analysis

Standard formulae were used for calculation of sensitivity, specificity, positive and negative predictive value. Mann-Whitney's U test was used to compare the age of patients with urinary PCR positivity and Chi-square test was used to compare the differences with respect to gender. P value of <0.05 was considered significant.

Results

Detection of C. trachomatis in patients of ReA

Out of 65 clinically diagnosed cases of ReA, urinary PCR was positive for *C. trachomatis* in 24 (36.9%) patients. None of the samples from patients of group II (non-ReA arthritis) and III (healthy controls) were positive for Ct. The positive and negative controls produced

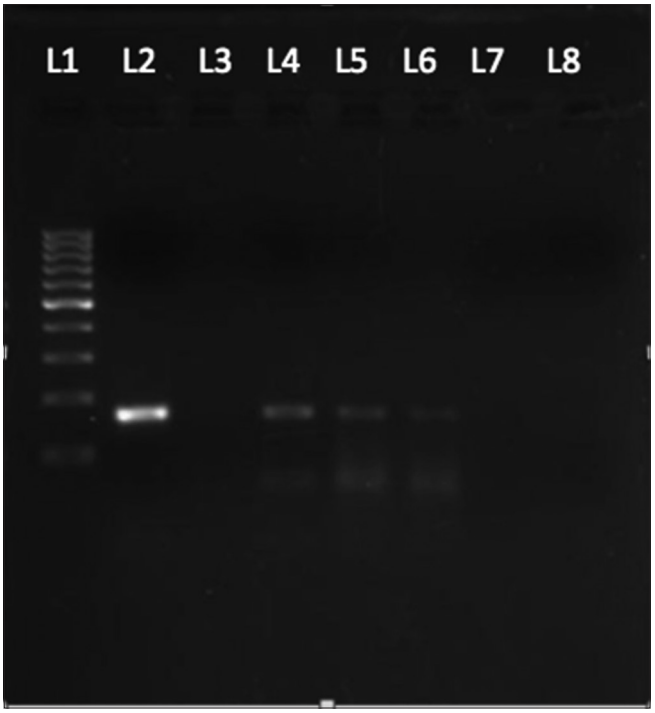

Figure 1: Gel picture of urinary PCR for *Chlamydia trachomatis*. L1 - Molecular Marker (100 basepair), L2 - positive control, L3 - Negative control, L4-L6 - sample positive for *Chlamydia trachomatis* DNA, L7 -sample of group 2 (disease control), L8 - sample of group 3 (healthy control)

satisfactory results in each run. Urinary PCR for Ct had a sensitivity, specificity, positive predictive value, and negative predictive value of 36.9%, 100%, 100%, and 49.3%, respectively for the diagnosis of ReA. There was a significant difference in the age of patients with positive urinary PCR for Ct as compared to those with negative urinary PCR ($P = 0.00024$). The median age of 24 patients with positive urinary PCR for Ct was 30 (IQR 27–34.5) while that of patients with negative urinary PCR was 40 (IQR 32.5–51). The difference with respect to gender was not significant ($P = 0.83$).

The most common joint involvement was ankle (69.2%) followed by the knee (46.15%), sacroiliac joints (41.5%), toes (41.5%), and hip (24.6%).

Discussion

ReA is one of the first systemic diseases in which the classical process of host-pathogen interaction was recognized.[2] Evidence of postenteric ReA dates back to the fifteenth century when Christopher Columbus, the great explorer, was reported to have arthritis of the lower limbs after a bout of dysentery.[18] However, ReA following venereal infection with *C. trachomatis* has rather remained an obscured entity and a diagnostic dilemma for centuries. After the detection of *Chlamydia* DNA within the synovial fluid of patients with ReA,[19,20] the third international workshop on ReA in 1995 proposed diagnostic criteria.[17] It was rather vague but it did emphasize that "the patient must have the typical peripheral arthritis plus evidence of a preceding infection (clinical diarrhea or urethritis within the preceding 4 weeks or laboratory-evidence of the same)". The fourth international workshop met in 1999 and added the terms acute and chronic ReA with 6 months as the cut-off.[21] No further updates have since been introduced. In view of missing evidence of preceding infection in most cases, the role of Ct as a causative agent was questioned for long and it was conceived as an innocent bystander.

In 2010, a definite causality between Ct and ReA was established by Carter *et al.*[22] in their prospective double-blind, placebo-controlled six-month trial wherein all patients clinically diagnosed of chronic CIA had Chlamydia-specific DNA in their peripheral blood monocytes and/or synovial fluid. Further, 63% of patients undergoing active antimicrobial treatment were responders in contrast to 20% receiving only placebo. This study opened new avenues and sincere efforts were made to detect the presence of Ct at all possible sites involved in its pathogenesis leading to ReA. Consequently, the elementary body of Ct was detected in synovial fluid using Ct-specific PCR[15] and direct immunofluorescence[11] and mRNA from metabolically-active Ct was detected from synovial biopsies of remitting patients.[23] Since the joint involvement has grasped all the attention, synovial samples have been the preferred sites for experiment. However, since Ct travels via the blood route to the joints (as evidenced by its presence in PBMCs),[10] it is expected to be present itself or at least shed its antigens into the urine. This aspect of utilizing urine sample for Ct detection was perceived by Kuiper's *et al.*[16] nearly 25 years ago, but never got the desired importance/popularity in the context of CIA. The present study is a pioneer work wherein a noninvasive sample like urine was used for detection of CIA.

The sensitivity of diagnosing CIA with conventional PCR using urinary sample was 36.9% in the current study. Since there is no study available wherein urine samples from patients have been used for diagnosing CIA, a direct comparison with existing literature cannot be made. Nevertheless, when compared to studies using synovial fluid samples, the sensitivity of detection of CIA using urine samples as reported by us, is higher. While Freise *et al.*[15] reported a sensitivity of 30%, Kumar *et al.*[14] reported a sensitivity of 23.6% for diagnosing CIA using same gene target (outer membrane protein) but from synovial fluid samples. About 2.6% samples of rheumatic arthritis also gave positive results in a study by Kumar *et al.*,[14] thus compromising the specificity. In contrast to this, 100% specificity was obtained in the present study. Further, while Freise *et al.*[15] used alkaline lysis in addition to Qiagen kit for DNA extraction, Kumar *et al.*[14] used seminested and nested PCR format. Contrary to these studies, not only a simple conventional PCR was applied on a noninvasive sample like urine in our study but also higher sensitivity was achieved in diagnosing CIA. Using direct immunofluorescence,[11] the reported sensitivity of 33.3% in diagnosing CIA is lower than 36.9% as reported in the current study.

The limitation of the present study was that no synovial fluid or other relevant sample was simultaneously tested along with the urine sample to evaluate the performance of different sample types. Also, the performance of PCR was not evaluated against other available tests. Moreover, Kuiper *et al.*[16] evaluated the sensitivity of different techniques and different sample types in detecting elementary bodies of Ct. They made serial dilutions of purified elementary body of Ct in synovial fluid, peripheral blood, peripheral blood leukocytes, and urine, and subjected all these sample types to following techniques: outer membrane protein by PCR, outer membrane protein by direct immunofluorescence, chlamydial rRNA by chemiluminescent probe, and chlamydial lipopolysaccharide by enzyme immunoassay. They concluded that PCR was the most sensitive and specific method for Ct detection for all sample types and that urinary-PCR was 100 times more sensitive in detecting Ct that synovial-fluid-PCR. Another limitation was that a repeat testing of urine sample was not conducted after completion of anti-Ct therapy to ascertain whether PCR-positivity decreases once patient goes into remission. A study by Strelic *et al.*[24] had previously reported that decrease in the PCR-positivity of PBMCs was significantly associated with achievement of clinical remission.

To conclude, urinary PCR can serve as a simple and reliable technique in diagnosing *Chlamydia trachomatis*-induced reactive arthritis.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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