



Association between *Chlamydia trachomatis* Infection and Prostate Cancer: A Case-Control Study

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ABSTRACT

Background: Evidence indicating the association of cancers and chronic inflammations is increasing. The importance of urinary tract and sexually transmitted infections (UTIs and STIs) in the development of prostate cancer is still unclear. *Chlamydia trachomatis* (*C. trachomatis*) is one of the most important causes of UTIs and STIs. Here, a case-control study was performed on the Iranian population to assess the association between *C. trachomatis* and prostate cancer (PC). **Materials & Methods:** Paraffin-embedded prostate tissue specimens collected from 62 PC and 62 PBH (benign prostate hyperplasia) (as controls) patients were screened to detect *C. trachomatis* 16srRNA gene using nested polymerase chain reaction (nested PCR) method. A p -value $< .05$ was interpreted as a remarkable difference using SPSS statistical software Ver. 16.

Findings: There was a significant difference regarding the prevalence of *C. trachomatis* ($p < .001$; OR=10.07; 95% CI [2.81-36.001]) between the PC (33.87%) and BPH (4.84%) samples. Furthermore, prostate-specific antigen (PSA) levels were statistically higher ($p < .05$) in *C. trachomatis*-positive patients than in patients with negative *C. trachomatis*.

Conclusion: It could be concluded that patients with a history of *C. trachomatis* infections are more likely to develop PC. Therefore, early diagnosis and treatment of *C. trachomatis* infection may help the prevention of PC. Moreover, nested PCR is a suitable method for *C. trachomatis* detection in paraffin-embedded prostate tissue specimens.

Keywords: Prostate cancer, *Chlamydia trachomatis*, Benign prostatic hyperplasia, Sexually transmitted infections.

CITATION LINKS

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Introduction

Prostate cancer (PC) is one of the most prevalent life-threatening malignancies in men worldwide ^[1]. Nevertheless, the causes of PC are not entirely known ^[2]. Chronic infections are considered to be the cause of at least 20% of all human cancers. For instance, chronic infection with *Helicobacter pylori* could induce gastric cancer ^[3-5].

The prostate gland may be infected by microbial infections, including urinary tract infections (UTIs) and sexually transmitted infections (STIs). Therefore, studies have suggested that chronic inflammations caused by UTIs and STIs may be linked to an increased risk of PC ^[6-10]. On the other hand, some cases of PC have been observed in patients with a history of chronic prostatitis and chronic pelvic pain syndrome (CPPS), which are caused by bacterial infections ^[11]. The most common bacteria detected in PC patients are *Propionibacterium acnes*, *Mycoplasma genitalium*, *Enterobacteriaceae* spp., and *Bacteroides* spp. ^[11].

Chlamydia trachomatis (*C. trachomatis*) is an obligate intracellular bacterium and one of the most common causes of STIs in the world ^[12]. *C. trachomatis* could cause asymptomatic and persistent infections ^[13]. In sexually active men, *C. trachomatis* is the most common cause of nongonococcal urethritis ^[12]. It could also play a role in epididymitis and prostatitis. In women, chronic *C. trachomatis* infections are associated with cervicitis, endometritis, salpingitis, and pelvic inflammatory disease (PID) ^[14]. Furthermore, the role of *Chlamydia* in promoting the host DNA damage and proliferation and subsequently triggering tumorigenesis has been established. Several studies have revealed that chronic *C. trachomatis* infections play an important role in the development of cervical and ovarian cancers ^[15-17]. However, studies on PC are limited and yielded conflicting

results due to the use of different detection methods. In this study, the hypothesis that the presence of *C. trachomatis* is associated with PC was investigated using the standard nested polymerase chain reaction method (nested PCR), especially by examining its role in increasing serum prostate-specific antigen (PSA) levels and Gleason scores (GS).

Objectives: This case-control study was conducted on patients referring to Shohada hospital (Tehran, Iran) to evaluate the possible role of *C. trachomatis* in the etiology of PC. The presence of *C. trachomatis* DNA was compared in prostate tissue of PC (case group) and BPH (control group) patients using the nested PCR method.

Materials and Methods

Study population: The present descriptive case-control study was conducted on formalin-fixed and paraffin-embedded (FFPE) prostate tissue specimens taken from men (50-89 years old) through open prostatectomy, core needle biopsy, and transurethral resection of the prostate (TURP) during 2007-2012 in Shohada hospital in Tehran, Iran. FFPE samples included 62 PC and 62 BPH (controls) cases. The research population characteristics are summarized in Table 1.

Microscopic evaluation of H&E stained pathological slides of PC patients: For each PC patient, there were several FFPE prostate tissue blocks as well as pathological hematoxylin and eosin-stained (H&E) slides that matched with blocks.

FFPE blocks and H&E slides were obtained from different areas of prostate tissue of each PC patient. Therefore, microscopic evaluation of H&E stained slides was performed by an experienced pathologist to identify cancerous areas (cancerous and non-cancerous tissues differentiation). Ultimately, the best block with the highest percentage of neoplastic cells and cancerous

Table 1) The study population characteristics.

Characteristic	Prostate Cancer (Cases)	Benign Prostate Hyperplasia (Controls)
Total number of patients	62	62
Mean age (range, years)	67.8 ± 7.9 (86-50)	68 ± 9.1 (40-89)
< 60	10 (16.13%)	11 (17.74%)
60-69	23 (37.1%)	22 (35.48%)
70-79	28 (45.16%)	24 (38.72%)
> 79	1 (1.61%)	5 (8.06%)
Needle biopsy sampling	22 (35.48%)	4 (6.45%)
TURP ¹ sampling	8 (12.91%)	40 (64.52%)
Prostatectomy sampling	32 (51.61%)	18 (29.03%)
Median Gleason Score (range)	6.92 ± 1.23 (5-10)	-
2-5 (low)	7 (11.29%)	-
6-7 (intermediate)	39 (62.90%)	-
8-10 (high)	16 (25.81%)	-
Presence of PIN ²	11 (17.74%)	-
Mean PSA ³ (ng/mL)	19.19 ± 16.43	4.16 ± 3.8
0-4 (ng/mL)	3 (4.84%)	38 (61.29%)
4.1-10 (ng/mL)	19 (30.65%)	19 (30.65%)
10.1-20 (ng/mL)	22 (35.48%)	5 (8.06%)
> 20 (ng/mL)	18 (29.03%)	0 (0%)
¹ Transurethral resection of the prostate.		
² Prostatic intraepithelial neoplasia.		
³ Prostate-specific antigen		

tissue was selected from the blocks of each PC patients for future analysis.

Record of medical laboratory information: Demographic and medical information, including age, clinical symptoms (symptomatic or asymptomatic), Gleason score, presence of prostatic intraepithelial neoplasia (PIN), and serum PSA level (ng/mL), were collected from patients’ medical records. This information is displayed in Table 1.

DNA extraction: Genomic DNA was extracted from FFPE prostate tissue samples using G-spin™ Total DNA Extraction Kit (iNtRON Biotechnology, Korea) according to the manufacturer’s instructions. Briefly, FFPE tissues were cut into thin sections using a sterile razor blade. To eliminate DNA cross-contamination between the samples, razor blades were changed for each specimen. Sections (up to 25 mg) were deparaffinized using xylene. Absolute

ethanol and 70% ethanol were used for xylene elimination and tissue hydration, respectively. After enzymatic digestion with proteinase K, bacterial DNA was extracted from homogenized tissue.

DNA concentration and quality were measured using a NanoDrop (Biochrom WPA Biowave II, UK). DNA samples were maintained at -20 °C.

Nested PCR: The standard nested PCR assay was carried out to amplify and identify *C. trachomatis* using *C. trachomatis* nested PCR detection kit (Pars Tous Co., Iran) according to the manufacturer’s instructions. The reaction mixtures with a final volum of 20 µL for the first-round PCR contained 14.6 µL of PCR Master Mix 1, 0.4 µL of HS-Taq DNA polymerase, and 5 µL of DNA template. Also, 5 µL of PCR-grade water and 5 µL of standardized positive controls were used as negative and positive controls, respectively. The PCR amplification was performed

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Table 2) Comparison of demographic, clinical, and pathological features between positive and negative *C. trachomatis* infections in prostate cancer patients

Characteristic	Prostate Cancer with Positive <i>C. trachomatis</i> (n = 21)	Prostate Cancer with Negative <i>C. trachomatis</i> (n = 41)	P-Value
Mean age (range, years)	68.7 ± 7.2	67.7 ± 8.7	> .05
Clinical features			
Symptomatic	14 (66.67%)	29 (70.73%)	> .05
Asymptomatic	7 (33.33%)	12 (29.27%)	
Gleason score (range)			
2-5 (low)	3 (14.29%)	4 (9.76%)	> .05
6-7 (intermediate)	13 (61.90%)	26 (63.41%)	
8-10 (high)	5 (23.81%)	11 (26.83%)	
Presence of PIN ¹	6 (28.57%)	5 (12.20%)	> .05

¹ Prostatic intraepithelial neoplasia.

under the following conditions: an initial denaturation step at 94 °C for 10 min, followed by 40 cycles of denaturation at 94 °C for 30 s, 55 °C for 40 s, and 72 °C for 30 s and a final extension step at 72 °C for 5 min. In the second step of amplification, 5 µL of the first step products, 14.6 µL of PCR Master Mix 2, and 0.4 µL of HS-Taq DNA polymerase were used. The annealing temperature for the second step was set at 57 °C. After electrophoresis of PCR products, the presence of 250 base pairs (bp) fragments was considered positive for *C. trachomatis*.

Statistical analysis

Data were analyzed using SPSS statistical software Ver. 16 by employing Chi-square test and *t*-test. *P*-values <.05 were regarded as a statistically remarkable difference.

Findings

In the present study, *C. trachomatis* was detected in 21 (33.87 %) PC (the case group) and 3 (%4.84) BPH (the control group) samples (Figure 1), and this difference in the prevalence rate of *C. trachomatis* between the two groups was significant [*p*< .001; OR=10.07; 95% CI (2.81-36.001)]. In this study, the association between the

presence of *C. trachomatis* and age, clinical and pathological features, and PSA levels was investigated in PC patients. The mean PSA value was 17.36 ± 16.07 ng/mL in *C. trachomatis*-positive PC patients and 10.31 ± 13.28 ng/mL in patients with negative *C. trachomatis*. There was a significant (*p*< .05) association between the presence of *C. trachomatis* and increased PSA levels in PC patients (Figure 2). However, there was no significant (*p* > .05) difference between *C. trachomatis*-positive and *C. trachomatis*-negative patients in terms of age, symptomatic or asymptomatic clinical signs, Gleason score, and PIN presence (Table 2). In PC patients, the highest rate of *C. trachomatis* infection was detected in TURP specimens; 50% of TURP specimens were *C. trachomatis* positive. In addition, 36.6 and 28% of needle biopsy and of prostatectomy specimens were positive, respectively. However, there was no significant difference between different types of sampls regarding the presence of *C. trachomatis* in PC patients.

Discussion

Since PC has been proposed to be associated with chronic inflammation ^[18], various studies have confirmed STIs (such as

gonorrhea and mycoplasma infections) as a risk factor for PC [19-22]. However, some studies have found null associations between the two [23, 24]. On the other hand, *Chlamydia* as a common cause of STIs could also induce chronic and persistent prostate infections [6, 25]. Therefore, there is growing evidence of the role of *C. trachomatis* in PC development.

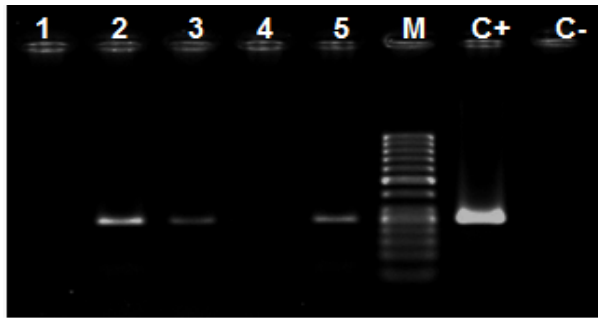


Figure 1) The results of agarose gel electrophoresis using the nested-PCR method for the detection of 16S rRNA gene in *C. trachomatis*. Numbers 1 to 5 indicate nested PCR products of patients (negative, positive, positive, negative, and positive *C. trachomatis* DNA samples, respectively). Sign "M" indicates a 50 bp ladder. "C+" and "C-" indicate positive control (250 bp fragment) and negative control, respectively.

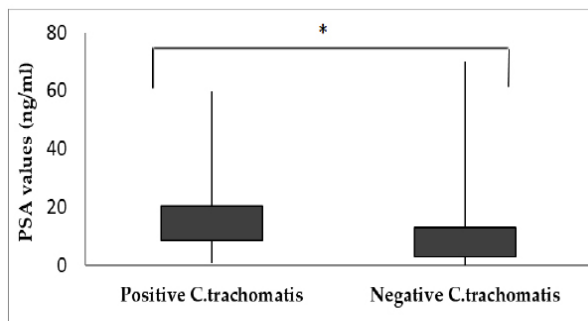


Figure 2) The association between the presence of *C. trachomatis* and PSA values. PSA levels were higher in *C. trachomatis*-positive PC patients than in PC patients without *C. trachomatis* infections. The symbol (*) indicates a significant difference ($p < .05$). A p -value $\leq .05$ was regarded as a significant difference between the case (PC) and control (BPH) groups. *C. trachomatis*: *Chlamydia trachomatis*; PSA: prostate-specific antigen; PC: prostate cancer; BPH: benign prostate hyperplasia.

In this case-control study, the prevalence of *C. trachomatis* was investigated in PC and BPH specimens as the case and control

groups using the nested-PCR method. The statistical analysis results showed the higher prevalence rate of *C. trachomatis* in PC specimens (33.87%) than in BPH specimens (4.84%). Therefore, the association of *C. trachomatis* infection and PC was confirmed in this study. In the present study, PSA levels were significantly higher in *C. trachomatis*-positive PC patients than in those with negative *C. trachomatis*. These findings indicate that *C. trachomatis* in prostate tissue could induce PSA production and increase serum PSA levels.

In a similar study in Japan in 2019, 45 PC and 33 BPH FFPE tissue specimens were screened for the presence of seven STI agents, including *C. trachomatis*, using PCR. In contrast to the present study result, no significant association was found between *C. trachomatis* and PC; however, *M. genitalium* was shown to be associated with PC [26]. Recently, a molecular and cell cultur study showed that *C. trachomatis* could efficiently proliferate in a PC cell line and also affect the expression of cytokines related to tumor progression in these cells [27]. However, in a study by Yow et al. (2014), no *C. trachomatis* was detected in 195 FFPE specimens obtained from PC patients using PCR [28]. In another study in 2008, only 0.5 % *C. trachomatis* was detected in frozen PC biopsies using PCR [29].

A positive association between PC and *C. trachomatis* was also reported in a cohort self-report study in 2002–2003 (follow-up period 2006) on 1658 PC cases collected from men of different races in California, including African-American, Asian-American, Latino, and white. Their race/ethnicity-stratified analysis showed that PC was associated with a history of chlamydial infection among Asian-American and Latin-American men in California [6]. However, the interpretation of self-report studies results is challenging for some reasons as follows:

first, chlamydial infections are frequently asymptomatic in men. Therefore, people are sometimes unaware of their disease. Furthermore, conventional methods are less sensitive in detecting asymptomatic *C. trachomatis* infections. Second, self-report studies usually lack accurate and complete information due to some bias.

Most serological studies have not been successful in confirming the positive association between PC and *C. trachomatis*. In addition, in some retrospective and prospective case-control studies (in Europe and the USA) conducted using serological methods such as micro-immunofluorescence, the prevalence of *C. trachomatis* in serum samples of PC patients hasn't been reported to be significant compared with control samples [30-33]. Therefore, this discrepancy between the present and other studies results could be attributed to the use of different detection methods. Immunological mechanisms might explain the lack of a positive association in serological methods. First, prostate tissue secretes proteases, and semen fluid contains large amounts of prostaglandins. These immune-inhibiting molecules may inhibit humoral immune responses and antibody production even in the presence of *C. trachomatis* in prostate tissue [30, 34, 35]. Second, adenocarcinoma is a common histological type (about 95%) of PC, and the presence of *C. trachomatis* in prostate adenoid cells prevents the induction of antibody production. In confirmation of this fact, studies have reported an association between increased chlamydial antibody with cervical and lung squamous cell carcinomas, but not with adenocarcinoma types [16, 21]. Third, antibodies produced in older men (those at higher risk of PC incidence) may not be as significant as in young men due to decreased immune function [36]. Fourth, the sensitivity of different markers in detecting chlamydial infections in prostate tissue

may vary. For example, a large case-control prospective study in 2007 on white and black races showed that there was an association between PC in black and the presence of *C. trachomatis* IgA antibodies (anti-HSP60), but not IgG (anti-OMP) [7]. According to the current study results, the nested-PCR method could be considered as a sensitive assay for detecting *C. trachomatis* in FFPE prostate tissue.

In the present research, the highest prevalence rate of *C. trachomatis* infection in PC patients was observed in samples taken through TURP (50% of TURP, 36.6% of needle biopsy, and 28% of prostatectomy samples were positive); however, there was no significant difference regarding the prevalence rate of *C. trachomatis* between different sample types. This result suggests that TURP specimens might be contaminated with *C. trachomatis* due to sampling through the urethra. The urethra is an excretory tube of the urinary bladder and therefore may be infected with *C. trachomatis*.

Finally, various factors could affect the results of studies, including the type of study (self-report, laboratory), detection method (molecular, serological), samples (fresh biopsy, FFPE), and sampling (TURP, prostatectomy, needle biopsy). Therefore, the evidence is still unclear and insufficient, and further studies are required.

Conclusion

The present study detected a significant difference in terms of *C. trachomatis* prevalence in FFPE prostate tissues between Iranian PC and BPH patients using the nested-PCR method. Therefore, it is suggested that *C. trachomatis* could be involved in PC development. The global prevalence of *C. trachomatis* as a cause of STIs and UTIs, anatomical location of the prostate, and the importance of inflammation in cancer development could support this result.

Therefore, early diagnosis and treatment of *C. trachomatis* infections may be useful in the prevention of PC.

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Ethical Permission: All ethical principles to protect participants' dignity, rights, safety, and well-being were the primary considerations of this research project and were performed according to Shahid Beheshti University of Medical Sciences guidelines.

Conflicts of interests: None.

Authors Contribution: Conceptualization: GS, NB; Methodology: NB, GS, ZG, HZh; Software: AA; Validation: NB, GS, ZGh; Formal analysis: AA; Investigation: NB, HZh; Writing and original draft preparation: NB; Writing, review, and editing: GS; Supervision: GS; Adviser: ZGh.

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