Original Article

pISSN: 2287-4208 / eISSN: 2287-4690
World J Mens Health 2019 Sep 37(3): 355-363
https://doi.org/10.5534/wjmh.180081

The World Journal of Men's Health

Roles of *Ureaplasma* Species in Idiopathic Chronic Prostatitis: A Case-Control Study

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Purpose: Because of the inconsistent symptoms associated with *Ureaplasma* infections, their clinical significances in genitourinary tracts are under debate. Therefore, we evaluated the presence of *Ureaplasma urealyticum* (UU) and *Ureaplasma parvum* (UP) in urine samples and examined their associations with chronic prostatitis (CP) through a case and control study.
Materials and Methods: We included 696 nonchlamydial nongonococcal (NCNG) urine samples from men; 350 were categorized into non-inflammatory CP, 88 in inflammatory CP, and 258 in non-CP group. We amplified a region in the *Ureaplasma* urease areas from these samples and determined their biovars using the Sanger method.
Results: Among the NCNG population, the rates of UU, UP, and non-UU/UP were 3.88%, 6.46%, and 89.66%, respectively. The overall infection rates of non-CP, inflammatory CP, and non-inflammatory CP groups were 4.15%, 6.10%, and 3.65% in UU (p=0.612) and 6.85%, 7.22%, and 6.50% in UP (p=0.968), respectively. UU infection increased the risk of white blood cell (WBC) counts (≥5) in urine (p=0.005). In contrast, UP infections did not increase the risks of urethritis. Re-analysis from the 633 men who were excluded from urethritis effects did not reveal the associations between UU infection and the clinical characteristics of CP. Furthermore, the profiles from the National Institutes of Health-Chronic Prostatitis Symptom Index questionnaire and WBC counts in expressed prostatic secretion were similar among the non-CP and the two CP groups in each *Ureaplasma* infection.
Conclusions: We found that UU may induce male urethritis. However, *Ureapalsma* species in urine were not definitively associated with the occurrence of CP.

Keywords: Chronic prostatitis; Infection; Prostate; *Ureaplasma parvum*; *Ureaplasma urealyticum*

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INTRODUCTION

Ureaplasma species, including *Ureaplasma urealyticum* (UU) and *Ureaplasma parvum* (UP), are classified as small sized bacteria [1,2]. The simple genomic structures and limited metabolic activity impose a close and long-term parasitic interaction between the bacteria and the infected cells [1,2]. In *Ureaplasma*-related infections, clinical symptoms and signs are often mild or even asymptomatic, making it very difficult to characterize their pathogenicity. Similarly, their parasitic nature and indolent course in infected host cells suggest the possibility of *Ureaplasma* species as causal pathogens for enigmatic chronic genitourinary infections.

Chronic prostatitis (CP) is one of the most common urological disorders [3]. Some sexually transmitted

Received: Sep 6, 2018 **Revised:** Dec 17, 2018 **Accepted:** Dec 30, 2018 **Published online** Feb 12, 2019
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infections (STIs) may contribute to the pathogenesis of CP [3-5]. For example, *Chlamydia trachomatis* that harbor similar genomic simplicity and clinical characteristics as *Ureaplasma* are found in prostate tissues in patients with idiopathic CP [4]. Through a case and control study, the chlamydial infected men reveal increased leukocyte counts in expressed prostatic secretion (EPS) and exacerbated CP-associated pain severity in men with CP. These findings clearly suggest the association of urinary chlamydia infections with CP and consider the possibility of *Ureaplasma* species associated CP [5].

Generally, urologists employ multiplex-nucleic acid amplification tests (NAAT) from voided urine samples to reveal pathogens for urethritis and CP in men [6]. Based on the results of these examinations, clinicians must determine the pathogenicity of these bacteria and whether antibiotics are to be prescribed.

While the presence of UU in EPS or in first voided urine sample from CP patients may support the association between *Ureaplasma* and CP [7,8], *Ureaplasma* microbes are not found in the perineal prostate biopsy tissues from idiopathic CP patients [4]. Furthermore, only a handful of case-control studies that estimate unique pathogenicity of *Ureaplasma* infections in the prostate are currently available [7-10]. In addition, we could not find well designed case-control studies with strict diagnostic criteria in defining idiopathic CP [3]. To understand the specific role of *Ureaplasma* species in CP and to design a strict case-control study, we first excluded patients with gonococcal and chlamydia infections through a multiplex NAAT, as well as patients with cystitis or bacterial prostatitis based on a lower urinary localization test. We had also removed the effects of urethral inflammation form the overall data and re-analyzed the subtracted data to enhance statistical power for CP. Finally, we aimed to evaluate the potential role of *Ureaplasma* species as causal pathogens in men with CP and clinical features of *Ureaplasma* infected CP patients.

MATERALS AND METHODS

1. Study design and ethics statement

Informed consents were obtained from all study participants, and the research protocol was reviewed and approved by the Institutional Review Board of the Dankook University Hospital (IRB No. 201303014). The study was performed in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, and Declaration of Tokyo, 1975, as revised in 1983).

A total of 810 first voided urine samples were collected from men who visited Dankook University Hospital Urogenital Infection and Prostate Diseases Clinic from September 2009 to March 2015. During the visit, first voided urine samples were collected into 15 mL centrifuge tubes and were stored at -70°C until NAAT was performed. The remaining samples were delivered to the central laboratory office to obtain white blood cell (WBC) counts.

The causes for the visit to the clinic included lower urinary tract symptoms, CP-like symptoms, symptoms with lower urinary tract infections, and annual prostate check-up. We excluded 49 cases of chronic bacterial prostatitis by ordinary aerobic prostatic culture methods [11] and 63 chlamydial infected cases by NAAT methods [5]. Other exclusion criteria prior to enrollment were as follows: presence of genitourinary cancer, urinary stone diseases, acute epididymitis, urinary culture-proven cystitis, traumatic urethral stricture, a history of prostate surgery within the past 3 months, and acute urinary retention [3]. Two combined cases of UU and UP were also excluded from the data analysis (Fig. 1).

Clinical information included the age of participants and the presence of CP-like symptoms according to the results from the Korean version of the National Institutes of Health-Chronic Prostatitis Symptom Index (NIH-CPSI) questionnaire. The results of WBC counts in the remaining first voided urine samples were reviewed and classified into the WBC count categories of 0–1, 2–4, and ≥5 per high-power field (HPF). We also reviewed the results of the WBC counts in the EPS from the digital rectal massage, which were also classified into three categories of 0–4, 5–14, and ≥15 WBCs per HPF [5].

Finally, the 696 participants were classified into the following groups according to the results of NIH-CPSI questionnaire and EPS evaluation: the non-CP group (control group), inflammatory CP group (≥15 WBCs per HPF in EPS), and non-inflammatory CP group (<15 WBCs per HPF in EPS) [5]. Here, CP group is defined as men with the presence of pelvic pain or discomfort conditions that had lasted at least 3 months [3]. Three hundred fifty men were categorized into the

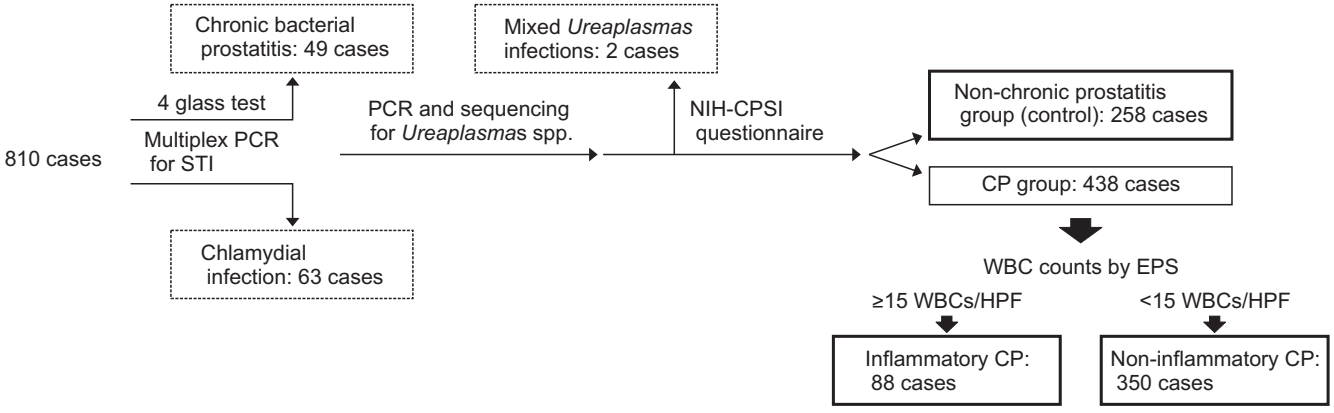


Fig. 1. Schematic drawing of the study design. A total of 810 men underwent basic chronic prostatitis (CP) evaluation tests including a lower urinary localization test and an in house multiplex polymerase chain reaction (PCR) test for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. Among this population, 49 were diagnosed with chronic bacterial prostatitis and 63 were diagnosed with urinary chlamydial infection; these 112 men were excluded from the study. Two men with mixed *Ureaplasma* infections in their urine were also excluded from this study. The remaining 696 men were classified into either the control group (258 cases) or CP positive group (438 cases). The compatible CP patients were further classified into inflammatory CP (88 cases) or non-inflammatory CP (350 cases) based on the white blood cell (WBC) counts in expressed prostatic secretion (EPS). Dotted box: excluded cases. Solid box: included cases. HPF: high-power field, NIH-CPSI: National Institutes of Health-Chronic Prostatitis Symptom Index, STI: sexually transmitted infection.

non-inflammatory CP group and 88 as patients with inflammatory CP. The non-CP group, which included 258 men, was defined as individuals who visited the clinic for annual prostate checkup. Participants in the control group did not report of symptoms of urethral discharge and pain or discomfort items of NIH-CPSI questionnaire.

2. In-house nucleic acid amplification tests

DNA was extracted from the stored urinary sample [5]. From the stored DNA samples, a urease complex area of *Ureaplasma* species were amplified with a set of consensus primers (forward; 5'-CAA TCT GCT CGT GAA GTA TTA C-3', reversed 5'-ACG ACG TCC ATA AGC AAC T-3'). The biovars of DNA sequences were determined by the Sanger method (Solgent, Daejeon, Korea) [12]. The sequences were compared with the reference genotypes in the GenBank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

3. Statistical methods

We used nonparametric Mann–Whitney U-test to evaluate the difference in the ordinal scores between each *Ureaplasma* infection and the non-UU/UP group. Pearson chi-square test and Fisher's exact tests were used to evaluate the difference in categorical data. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated with logistic regression analysis. Two-sided null hypotheses of no difference were rejected if p-values were less than 0.05. All analyses were performed using IBM SPSS ver. 23 software for Windows (IBM Corp., Armonk, NY, USA).

RESULTS

1. Sequencing results

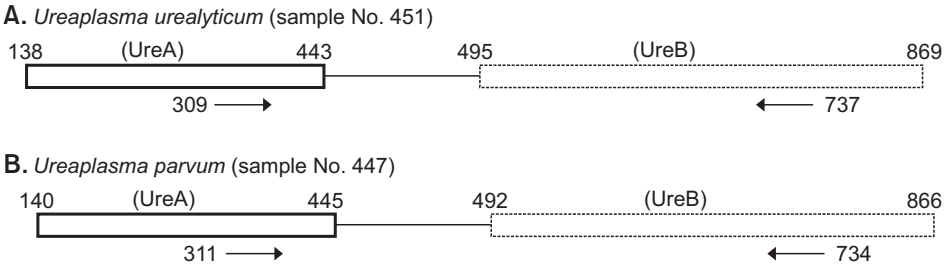
We amplified a partial segment of UreA and UreB regions of *Ureaplasma* species (Fig. 2A, 2B). Because of the clear dissimilarity in DNA sequences between two amplified regions, we could easily distinguish UU infections from UP infections (Fig. 2C).

2. Infection rates of *Ureaplasma urealyticum* and *Ureaplasma parvum* in the nonchlamydial nongonococcal setting

The infection rates of UU, UP, and non-UU/UP in nonchlamydial nongonococcal (NCNG) setting were 3.88% (27/696), 6.46% (45/696), and 89.66% (624/696), respectively (Table 1, 2). Furthermore, the mean age± standard deviation (range of patient age) of the UU, UP and non-UU/UP groups was 42.73±12.73 years (18–72 years), 46.35±11.83 years (19–81 years), 45.99±12.42 years (18–79 years) (p>0.05), respectively.

3. Urethral inflammation by *Ureaplasma urealyticum* and *Ureaplasma parvum*

Urinary WBC counts were significantly different among UU, UP, and non-UU/UP groups (p=0.002).



C. Comparison between the >451 (*U. urealyticum*) and the >447 (*U. parvum*)

CLUSTAL 2.1 multiple sequence alignment

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451     CAATCTGCTCGTGAAGTATTACGTGTTGATCAAGTTATGGAAGGTGTAGATACAATGGTT
447     CAATCTGCTCGTGAAGTACTACGTGTTGATCAAGTTATGGAAGGTGTAGATACAATGGTT
        ****************** ****************************************

451     GGTATAATCCAAGTTGAAGTTACTTTCCCAGATGGTACTAAACTAGTTTCTGTACACAGC
447     AGTATAATTCAAGTTGAAGTTACTTTCCCTGATGGTACTAAACTAGTTTCTGTACACGAT
        .******* ********************:***************************..

451     CCAATTTACAAATAATTTTTACAATTCGTAAAATCAATTTTAATTTTATAAGGAGATAAT
447     CCAATTTACAAATAACATTTACAATTCG-----TAAAATTTTTTTATAAAAGGAGATAAT
        *************** :***********      *.**:***::**:**:***********

451     GATTATATGTCAGGATCATCAAATCAATTCACTCCAGGTAAATTAGTACCAGGAGCAATT
447     GATTATATGTCAGGATCATCAAGTCAATTTAGTCCAGGTAAATTAGTACCAGGGGCAATT
        **********************.****** * *********************.******

451     AACTTCGCTGAAGGCGAAATTGTGATGAACGAAGGTAGAGAAGCAAAAGTAATCAGCATT
447     AATTTCGCTAGTGGTGAAATTGTGATGAATGAAGGTAGAGAGGCAAAAGTAATTAGTATT
        ** ******..:** ************** ***********.*********** ** ***

451     AAAAATACTGGTGACCGTCCTATCCAAGTTGGATCACATTTCCACTTATTTGAAACAAAT
447     AAAAATACTGGGGACCGTCCTATACAAGTTGGATCACATTTTCACTTGTTTGAAGTGAAT
        *********** ***********.***************** *****.******. .***

451     AGTGCATTAGTATTCTTTGATGAAAAAGGAAACGAAGACAAAGAACGTAAAGTTGCTTAT
447     AGTGCATTAGTATTTTTTGATGAAAAAGGAAATGAAGATAAAGAACGCAAAGTTGCTTAT
        ************** ***************** ***** ******** ************

451     GGACGTCGT
447     GGACGACGT
        *****:***
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Fig. 2. (A, B) We amplified partial segments of UreA and UreB regions of *Ureaplasma* species, and found genotypes of *U. urealyticum* and *U. parvum*. (C) Because of the clear dissimilarity in DNA sequences between *U. urealyticum* and *U. parvum* in the amplified region, the two genotypes could be distinguished clearly. The solid box of each organism contains the amplified DNA in the partial UreA gene, while the dotted box of each organism represents the partial UreB gene. The numbers in boxes represent DNA sequence loci based on AF085733 for *U. parvum* and AF085721 for *U. urealyticum*. Primers for polymerase chain reactions are indicated by the arrows.

Specifically, the rates of UU infection according to urinary WBC counts of 0–1, 2–4, and ≥5 HPF groups were 3.78% (22/581), 1.92% (1/52), and 22.22% (4/18) (p=0.001), respectively (Table 1). Compared to the non-UU/UP group, the UU infected group reported frequent dysuria or pain during urination (p=0.035) (Table 1). Based on multivariable analysis, patients with UU infections had significantly higher probability of ≥5 WBC counts/HPF in urine (adjusted OR [$_{adj}$OR], 7.522; 95% CI, 1.687–30.300; p=0.005) in the NCNG clinical setting (Table 1). However, there were no significant differences in the symptoms and signs of urethritis between 45 men in the UP positive and 624 men in the non-UU/UP group (Table 2).

4. Chronic prostatitis by *Ureaplasma urealyticum* and *Ureaplasma parvum*

The overall infection rates in the control, inflammatory, and non-inflammatory CP groups were 4.15% (10/241), 6.10% (5/82), and 3.65% (12/328) in UU (p=0.612), and 6.85% (17/248), 7.22% (6/83), and 6.50% (22/338) in UP (p=0.968), respectively (Table 1, 2). To remove the effects of urethral inflammation by UU infection, we excluded 18 cases of ≥5 WBC counts /HPF in urine and re-analyzed the remaining 633 case to enhance statistical power. There were no clinical associations in clinical characteristics of pure idiopathic CP cases and the UU infections; the subtracted infection rates in the control, inflammatory, and non-inflammatory CP groups were 3.78% (9/238), 4.17% (3/72), 3.40% (11/323) in UU (p=0.941), respectively.

In addition, each UU and UP positive group had similar NIH-CPSI questionnaire profiles compared to the non-UU/UP group (Table 1, 2). Similarly, we could not find any significant difference between the UU or UP infected group and the non-UU/UP group in the pain scores, urination domain, and impact of symptom scores from the NIH-CPSI questionnaire according to nonparametric Mann–Whitney U-test (Table 3). WBC counts in EPS were not statistically associated with the *Ureaplama* infections (UU, p=0.549 and UP, p=0.778) (Table 1, 2).

Table 1. Clinical characteristics of UU infection

Variable	UU	Non-UU/UP[a]	Odds ratio (95% confidence interval) from logistic regression	
			Univariable	Multivariable
Age (y)	42.73±12.73	45.99±12.42	Not included	Not included
16–35	8 (29.62)	116 (18.58)	1	
36–55	15 (55.55)	388 (62.18)	0.561 (0.232–1.355)	0.604 (0.240–1.520)
≥56	4 (14.81)	120 (19.23)	0.483 (0.142–1.649)	0.525 (0.146–1.892)
WBCs in urine[b]				
0–1	22 (81.48)	559 (89.58)	1	
2–4	1 (3.70)	51 (8.17)	0.498 (0.066–3.772)	0.506 (0.065–3.933)
≥5	4 (14.81)	14 (2.24)	7.260 (2.208–23.865)*	7.522 (1.687–30.300)*
WBCs in EPS				
0–4	20 (74.07)	403 (64.58)	1	
5–14	2 (7.40)	89 (14.26)	0.453 (0.104–1.972)	0.422 (0.094–1.899)
≥15	5 (18.51)	119 (19.07)	0.847 (0.311–2.304)	0.491 (0.157–1.532)
Missing	0	13 (2.08)		
Pain[c]				
Perineum				
No	23 (85.18)	510 (81.73)	1	
Yes	4 (14.81)	114 (18.27)	0.778 (0.264–2.293)	0.556 (0.177–1.739)
Testicles				
No	18 (66.66)	488 (78.20)	1	
Yes	9 (33.33)	136 (21.79)	1.794 (0.788–4.083)	1.677 (0.704–3.993)
Tip of penis				
No	20 (74.07)	540 (86.53)	1	
Yes	7 (25.92)	84 (13.46)	2.250 (0.923–5.484)	1.371 (0.478–3.937)
Below waist				
No	19 (70.37)	447 (71.63)	1	
Yes	8 (29.62)	177 (28.36)	1.063 (0.457–2.473)	0.732 (0.287–1.865)
Pain during urination				
No	16 (59.25)	480 (76.92)	1	
Yes	11 (40.74)	144 (23.07)	2.292 (1.040–5.049)*	2.175 (0.892–5.305)
Ejaculatory pain				
No	20 (74.07)	511 (81.89)	1	
Yes	7 (25.92)	113 (18.10)	1.583 (0.654–3.833)	1.409 (0.541–3.671)
NIH classification				
Control	10 (37.03)	231 (37.02)	1	Not included
IIIA[d]	5 (18.51)	77 (12.24)	1.500 (0.497–4.525)	Not included
IIIB[e]	12 (44.44)	316 (50.64)	0.877 (0.373–2.065)	Not included

Values are presented as mean±standard deviation or number (%).
EPS: expressed prostatic secretion.
[a]Negative for *Ureaplasma urealyticum* (UU) and *Ureaplasma parvum* (UP) (624 men). [b]We reviewed the results of white blood cell (WBC) count in the first-voided urine sample and have classified them into the WBC count categories of 0–1, 2–4, and ≥5 per high-power field. [c]Pain characteristics were measured using the Korean version of National Institutes of Health-Chronic Prostatitis Symptom Index (NIH-CPSI) questionnaire. [d]Inflammatory prostatitis. [e]Noninflammatory prostatitis. *Statistically significant at p<0.05.

DISCUSSION

STIs remain one of the major health issues, causing serious and adverse effects on reproductive health [13]. Recently developed multiplex NAAT methods for STIs can simultaneously detect multiple causative organisms [6,14]. Various scales of multiplex NAAT assays that range from detection of 2–3 to 7 or more STI pathogens have been developed, and the medical practices for executing multiplex NAAT assays have been

Table 2. Clinical characteristics of UP infection

Variable	UP	Non-UU/UP[a]	Odds ratio (95% confidence interval) from logistic regression	
			Univariable	Multivariable
Age (y)	46.35±11.83	45.99±12.42	Not included	Not included
16–35	8 (17.77)	116 (18.58)	1	
36–55	31 (68.88)	388 (62.18)	1.159 (0.518–2.590)	1.066 (0.462–2.460)
≥56	6 (13.33)	120 (19.23)	0.725 (0.244–2.154)	0.689 (0.224–2.119)
WBCs in urine[b]				
0–1	39 (86.66)	559 (89.58)	1	
2–4	3 (6.66)	51 (8.17)	0.843 (0.252–2.824)	0.973 (0.286–3.315)
≥5	3 (6.66)	14 (2.24)	3.071 (0.847–11.141)	3.740 (0.931–15.025)
WBCs in EPS				
0–4	28 (62.22)	403 (64.58)	1	
5–14	8 (17.78)	89 (14.26)	1.033 (0.459–2.328)	1.260 (0.548–2.897)
≥15	8 (17.78)	119 (19.07)	1.337 (0.483–3.700)	0.828 (0.353–1.939)
Missing	1 (2.22)	13 (2.08)		
Pain[c]				
Perineum				
No	38 (84.44)	510 (81.73)	1	
Yes	7 (15.55)	114 (18.27)	0.824 (0.359–1.892)	0.816 (0.340–1.957)
Testicles				
No	39 (86.66)	488 (78.20)	1	
Yes	6 (13.33)	136 (21.79)	0.552 (0.229–1.331)	0.542 (0.219–1.339)
Tip of penis				
No	41 (91.11)	540 (86.53)	1	
Yes	4 (8.88)	84 (13.46)	0.627 (0.219–1.796)	0.536 (0.176–1.631)
Below waist				
No	32 (71.11)	447 (71.63)	1	
Yes	13 (28.88)	177 (28.36)	1.026 (0.526–2.000)	1.061 (0.519–2.167)
Pain during urination				
No	36 (80.0)	480 (76.92)	1	
Yes	9 (20.0)	144 (23.07)	0.833 (0.392–1.771)	0.910 (0.404–2.051)
Ejaculatory pain				
No	35 (77.77)	511 (81.89)	1	
Yes	10 (22.22)	113 (18.10)	1.292 (0.622–2.686)	1.455 (0.666–3.180)
NIH classification				
Control	17 (37.77)	231 (37.02)	1	Not included
IIIA[d]	6 (13.33)	77 (12.33)	1.059 (0.403–2.781)	Not included
IIIB[e]	22 (48.88)	316 (50.64)	0.946 (0.491–1.822)	Not included

Values are presented as mean±standard deviation or number (%).
EPS: expressed prostatic secretion.
[a]Negative for *Ureaplasma urealyticum* (UU) and *Ureaplasma parvum* (UP) (624 men). [b]We reviewed the results of white blood cell (WBC) count in the first-voided urine samples and have classified them into the WBC count categories of 0–1, 2–4, and ≥5 per high-power field. [c]Pain characteristics were measured using the Korean version of National Institutes of Health-Chronic Prostatitis Symptom Index (NIH-CPSI) questionnaire. [d]Inflammatory prostatitis. [e]Noninflammatory prostatitis.

financially supported by the Korean National Medical Insurance to suppress the spread of STIs [6]. Consequently, many microbes that are certainly undetected by conventional diagnostic tests have now become recognized by the public health systems.

The overall positive rates for various STIs with multiplex NAAT-based STI tests range from 30% to 50%, depending on study designs [6,14]. In particular, previous studies consistently report that the most prevalent microbe detected by such methods is UP and UU [6,14].

Table 3. Continuous variables of genitourinary symptoms and *Ureaplasma* infection

Variable	Positive		Non-UU/UP[c]
	UU[a]	UP[b]	
NIH-CPSI pain score			
Item 1	1.03±1.05	0.66±0.79	0.81±0.89
Item 2	0.66±0.78	0.42±0.54	0.41±0.60
Item 3	1.37±1.39	0.97±1.13	1.35±1.45
Item 4	2.88±2.91	2.55±2.35	2.80±2.73
Sum of items 1–4	5.96±5.69	4.62±4.02	5.39±4.90
NIH-CPSI urination score			
Item 5	2.29±1.98	1.35±1.20	1.63±1.58
Item 6	1.70±1.45	1.91±1.32	1.81±1.44
Sum of items 5 and 6	4.00±3.25	3.26±2.32	3.45±2.64
NIH-CPSI impact of symptom score			
Item 7	0.83±1.00	0.68±0.76	0.98±1.03
Item 8	1.25±1.07	1.20±0.99	1.41±1.01
Item 9	2.70±1.66	2.86±1.63	3.15±1.58
Sum of item 7–9	4.87±3.34	4.75±3.06	5.55±3.12
Sum of NIH-CPSI scores 1–9	14.80±11.16	12.64±8.10	14.45±8.84

Values are presented as mean±standard deviation.
We could not find any difference between each *Ureaplasma urealyticum* (UU) or *Ureaplasma parvum* (UP) infected group and the negative group using nonparametric Mann–Whitney U-test.
NIH-CPSI: National Institutes of Health-Chronic Prostatitis Symptom Index.
[a]27 UU infected men were included. [b]45 UP infected men were included. [c]Non-UU/UP group, 624 men were included.

As *Ureaplasma* species are frequently found in coinfections with *Neisseria gonorrhea* or *C. trachomatis* in urine samples, combinations of these well-known STIs may alter the clinical characteristics of infected individuals and therefore must be ruled out to evaluate the unique characteristics of *Ureaplasma* species. Accordingly, cases of *N. gonorrhea* and *C. trachomatis* infections were excluded from this study. Our study revealed that the infection rate for *Ureaplasma* species in the sample population was close to 10%, and UP infections were more frequently found than UU in the NCNG settings.

N. gonorrhea and *C. trachomatis* microbes can induce male urethritis or CP, therefore they are eradicated by administering specific antimicrobials [5,9,15]. However, urethral inflammatory response after *Ureaplasma* infections is significantly lower than that of *Mycoplasma genitalium* or *C. trachomatis* [16]. In other words, clinical symptoms or signs such as urethral discharge and increased WBC counts in voided urine samples are usually inconsistent following *Ureaplasma* infections [9]. Unfortunately, well designed case-controls studies that may differentiate the role of *Ureaplasma* species as causative or bystanders in genitourinary infections are currently not available. Although there is a controversy regarding the pathogenicity of *Ureaplasma* species in genitourinary tracts, some physicians conventionally prescribe antibiotics regardless [17]. Consequently, *Ureaplasma* species may be exposed to increased evolutional pressure induced by such frequent medications. Indeed, many *Ureaplasma* species harbor resistance to conventional antibiotics [18,19]. Therefore, the clinical significance or pathogenicity of *Ureaplasma* infections in male genitourinary organs must be appropriately evaluated to avoid unnecessary treatments.

Ureaplasma species are genetically classified into UU and UP. Although conventional researches in genitourinary *Ureaplasma* infections have been based on NAAT methods, currently there are no Food and Drug Administration-approved clinical tests to differentiate UU from UP infection in the United States. Therefore, sequencing-based *Ureaplasma* detection and differentiation methods may be employed as a gold standard until properly-evaluated NAATs are established.

Our results suggest that UU may be associated with NCNG urethritis because UU infections increased the risk of higher WBC counts in urine (≥5 HPF) ($_{adj}$OR, 7.522; 95% CI, 1.687–30.300). Furthermore, UU infected men had reported more frequently of dysuria or pain during urination than the non-UU/UP group (p=0.035). But, we found that UP was not a pathogen in male urethra. Therefore, UP infections were not justified with antibiotic treatments.

Intracellular incorporating pathogens can also gain a foothold in the genitourinary tract and act as a trigger for chronic inflammation or recurrent epithelial damage, ultimately aggravating chronic genitourinary pain or induce CP [1,2]. Prostate can harbor various microorganisms that are not detectable by traditional culture approaches but rather are confirmed by prokaryotic DNA amplification and cloning methods [4,20]. With the abilities of *Ureaplasma* species to elicit chronic infection in host cell, the possibility of *Ureaplasma* species-associated CP has been under debate over the last several decades [1,2,7,8,10].

Although many urologists have been directed to find laboratory biomarkers for CP, we still lack definitive criteria for diagnosis of the disease [3]. Therefore,

patients must be basically evaluated with the NIH-CPSI questionnaire and a lower urinary tract localization test with the Meares–Stamey 4 glass to define CP [3]. In addition, a proper diagnostic workup for CP must rule out other mimicking conditions, similar to our exclusion criteria. While many STI organisms can contribute to the pathogenesis of CP, typical cases of urethritis must be excluded in CP case-control study. Therefore, we removed the 18 cases of urethral inflammation and re-calculated clinical significances in the remaining 633 cases. Nevertheless, even after adjusting for cases of idiopathic CP, statistical significances between clinical characteristics of CP and the UU infections were not found. The frequencies of each item in the pain domain of NIH-CPSI questionnaire were not different between the adjusted UU infected men *versus* non-infected men.

It has been suggested that WBC counting in EPS is the most accurate method in evaluating prostatic inflammation. Therefore, the variations in EPS WBC concentration is a deciding factor for discriminating inflammatory CP from non-inflammatory CP cases. We found that the 4.73% (20/423) of those with WBC counts in their EPS in the range of 0–4 HPF, 2.19% (2/91) in 5–14 HPF group, and 4.03% (5/124) in ≥15 HPF group had UU infections (p=0.549). Even if the effects of urethral inflammation were excluded, the adjusted data analysis revealed the similar results; 4.31% (18/417) of those with WBC counts in EPS in the range of 0–4 HPF, 2.22% (2/90) in the 5–14 HPF, and 2.65% (3/113) in the ≥15 HPF group (p=0.512). Furthermore, we did not observe a significant difference in the response to the NIH-CPSI questionnaire between the UU/UP positive groups *versus* the non-UU/UP group. Therefore, we hypothesized that the presence of UU or UP in NAAT from CP patients is not related to the manifestations of CP. Additionally, urinary UU or UP may not be able to invade and trigger inflammation in the prostate because WBC counts in EPS were not statistically different [3,5]. Therefore, we advised against antibiotic treatment in UU or UP infections in patients with CP.

CONCLUSIONS

Based on the case-control study, our results suggest that urinary presences of UU or UP in patients with CP are not associated with its occurrence. Accordingly, treatment with antimicrobial agents is not reasonable in the cases of *Ureaplasma* species associated CP. UU is a pathogen because it is associated with male urethral infection, while UP is not a pathogen but a bystander in male urethra.

ACKNOWLEDGEMENTS

This work was supported by a Research Fund of Dankook University in 2017.

Disclosure

The authors have no potential conflicts of interest to disclose.

Author Contribution

Research conception & design: Lee GH. Performing the experiments: Lee GH, Park H. Data acquisition: Lee GH, Park H. Data analysis and interpretation: Lee GH, Park H. Statistical analysis: Lee GH. Drafting of the manuscript: Lee GH, Park H. Critical revision of the manuscript: Lee GH, Park H. Receiving grant: Lee GH. Approval of final manuscript: all authors.

Data Sharing Statement

The data analyzed for this study have been deposited in HARVARD Dataverse and are available at https://doi.org/10.7910/DVN/QFH9O0.

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