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Detection of *Trichomonas vaginalis* Infection in Chronic Prostatitis/Chronic Pelvic Pain Syndrome Patients by Rapid Immunochromatographic Test

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Abstract

This study aims to evaluate associations between the immunochromatographic rapid test technique and *Trichomonas vaginalis* (TV) infection in patients with chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) in Taiwan. All patients received post-prostate massage urine (VB3) Trichomonas rapid tests. The demographic characteristics and urogenital symptoms of CP/CPPS were recorded. Routine urinalysis of VB3 was also performed, and laboratory examination results of semen were recorded if available. A total of 29 patients with TV infection and 109 without TV infection were enrolled, which reflected that the prevalence in patients with TV infection was approximately 21%. Patients with TV infection displayed a significantly higher frequency of suprapubic/lower abdominal pain ($p = 0.034$), semen leukocyte > 5/high-power field (HPF) ($p = 0.020$), and an inflammatory type (category IIIA) ($p = 0.005$) than patients without TV infection. A higher prevalence of TV infection was found in patients with category IIIA (47.37%). No significant difference was found in the symptom duration and other clinical symptoms. In conclusion, the high prevalence of TV infection was revealed in CP/CPPS patients using the VB3 rapid Trichomonas test, especially in CP/CPPS patients with category IIIA. Thus, rapid TV testing might be vital for CP/CPPS patients in the hospital.

K e y w o r d s: chronic prostatitis, epidemiology, *Trichomonas vaginalis*

Introduction

Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) is categorized as a prostatitis syndrome by the National Institute of Health (NIH) (category III) (Krieger et al. 1999). It is defined as a urologic pain or discomfort in the pelvic region and is associated with urinary symptoms and fertility alterations (Motrich et al. 2018). Besides, it accounts for more than 90% of prostatitis-like symptoms in men (Magistro et al. 2016). The estimated prevalence of CP/CPPS ranges from 2.2–9.7%, resulting in a substantial number of physician visits and related medical costs (Krieger et al. 2008). CP/CPPS causes pain and harms life quality, causing stress, depression, and other psychological responses (Shoskes and Nickel 2013). Although many patients are affected, the complex and heterogeneous etiology of CP/CPPS is poorly understood. It has been proposed that infection, intra-prostatic urinary reflux, cytokines, pelvic floor spasms, and psychological traits may all play some role in the pathophysiology of CP/CPPS (Shoskes and Nickel 2013). The variable syndrome of CP/CPPS infection has been confirmed, and a multimodal therapeutic approach addressing the individual clinical phenotypic profile was suggested rather than monotherapy for management (Magistro et al. 2016).

Trichomonas vaginalis (TV), a protozoan parasite, is the etiological agent of trichomoniasis, the most prevalent non-viral sexually transmitted disease (STD) worldwide (Meites et al. 2015). The prevalence of TV in men was estimated to be 1% in a recent WHO report and possibly as high as 3–17% in men visiting

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STD clinics (Poole and McClelland 2013). More than 142 million new cases of trichomoniasis occur annually. TV infection was common in sexually active young women with symptoms and signs of vaginitis. However, more than three-quarters of male genitourinary tract with trichomoniasis are generally asymptomatic and might accompany mild urethritis, epididymitis, and prostatitis. The role of TV infection in chronic prostatitis, benign prostate hyperplasia, and prostate cancer is an emerging field of interest.

Diagnostic testing of TV in men is rarely performed in a clinical setting for several reasons. TV infection in men is usually asymptomatic, and the traditional testing methods have lower sensitivity in men because of a lower organism load (Edwards et al. 2016). PCR-based assays for TV diagnosis provided a more sensitive form of testing than the traditional wet mount and culture methods (Gaydos et al. 2017). Besides point-of-care tests using immunochromatographic capillary flow dipstick technology is a simple technique that allows for a rapid diagnosis of trichomoniasis and may help for an early diagnosis and treatment (Meites et al. 2015). A new immunochromatographic rapid test has been discovered and reported to have a sensitivity of 100% and specificity of 88% using on urogenital specimens compared with the wet mount and PCR method (Wu et al. 2013). In this study, the above immunochromatographic rapid test was used to diagnose TV infection in patients with CP/CPPS to determine the prevalence of TV and the associated clinical characteristics. Besides, this study aims to reinforce the importance of a rapid TV test as a detection tool to confirm TV infection in CP/CPPS. Potential targets for testing TV infection were identified through recording the detailed patient characteristics adapted from the concept of UPOINTS (Urological, Psychosocial, Organ-specific, Infection, Neurological, Tenderness, Sexual domain) phenotype of CP/CPPS (Shoskes and Nickel 2013).

Experimental

Materials and Methods

From January 2013 to September 2015, patients with characteristic pelvic pain and urinary complaints compatible with CP/CPPS as defined by the NIH diagnostic criteria were enrolled in the Department of Urology, Chang Gung Memorial Hospital (Krieger et al. 1999). Patients with symptom duration less than three months or the following potentially significant urological causes of pain were excluded: the presence of lower genitourinary tract cancer, active urolithiasis, gastrointestinal disorders, radiation or chemical cystitis, acute urethritis/epididymitis/orchitis, functionally significant urethral stricture disease or neurological disorders affecting the bladder. The Internal Review Board of our institution reviewed and approved the study protocol with the IRB number 104-1533B. All participating subjects provided signed informed consent to participate in the study.

Routine physical examinations and digital rectal examinations were performed on all patients. Midstream and post-prostate massage urine (VB3) samples from all patients were collected for a urinalysis and bacterial culture. The JD TV test (Jei Daniel Biotech™, China), a US FDA-registered immunochromatographic strip test using specific antibodies to detect Trichomonas protein antigens, was performed to detect TV (Hobbs and Seña 2013). The post-massage urine specimen was collected with the first 10 ml of the patient's urine after prostate massage and performed test according to the manufacturer's instructions (Jei Daniel Biotech™, China). Briefly, 0.5 ml of the urine sample was mixed in 0.5 ml of test buffer (0.01% Tris-HCl and 0.05% NaN_3, PH 7.5) for 10 seconds. The JD's Trichomonas V® test strip was placed in the mixture buffer, and the result was read visually after 15 minutes. A positive result was indicated by the presence of both red test and control lines, whereas only a red control line was visible in a negative result.

The demographic characteristics and urogenital symptoms of all enrolled patients were recorded. The presence of depression or stress was recorded. Any pelvic pain symptom was recorded from each patient's chief complaint and could be more than one. Any lower urinary tract symptom was recorded from the patient report. Lower urinary tract syndrome (LUTS) included storage and voiding symptoms, and dysuria was recorded by physician inquiry. Seminal analyses were also recorded if available. Hematospermia was recorded from the patient report and RBC > 5 in the high-power field (HPF) in semen analysis. Inflammatory chronic prostatitis was defined as semen leukocyte > 5/HPF or WBC > 10/HPF in post-massage urine. Any sexual dysfunction symptom was recorded from the patient report and physician inquiry without a questionnaire. This retrospective study was approved by the Human Ethics Committee of the hospital. Data were examined by descriptive analysis.

Baseline characteristics were compared between groups using chi-squared/Fisher's exact tests and independent *t*-tests to detect differences in the categorical and continuous demographic variables, respectively. Data are expressed as number (percentage) for categorical variables and mean ± SD for continuous variables. A two-sided p-value of < 0.05 was regarded as statistically significant. Data management and statistical analyses were conducted using SAS version 9.4 software (SAS Institute, Inc.).

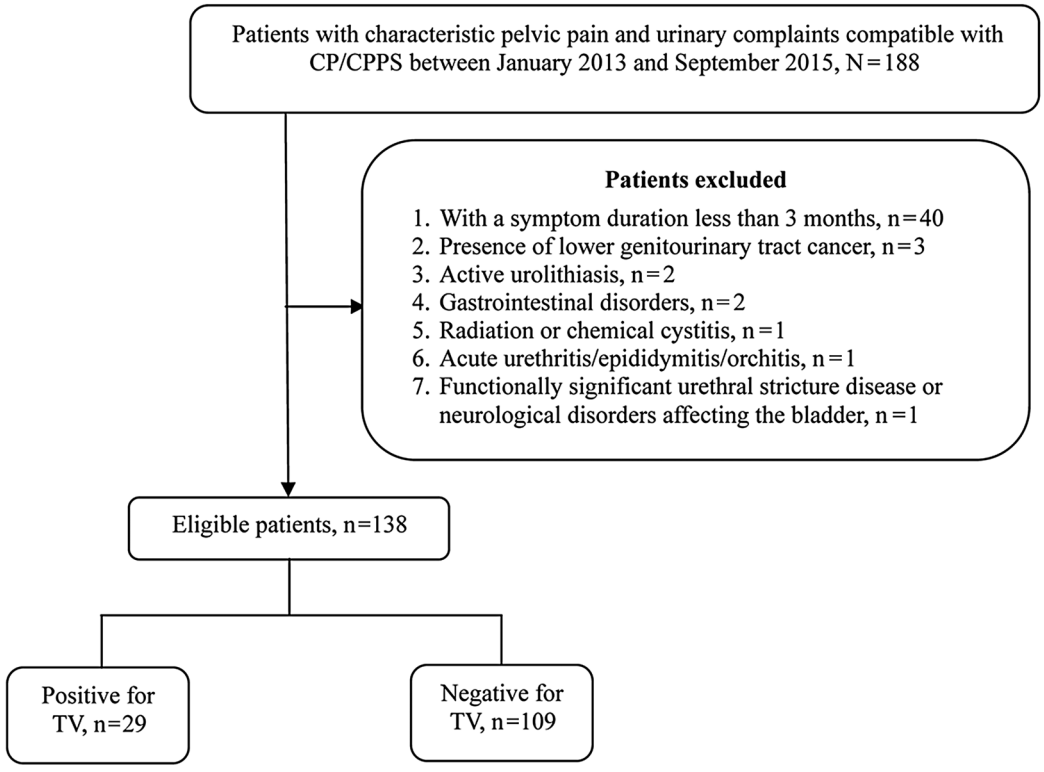

Fig. 1. Flowchart of the study population.

Results

A total of 29 patients with TV infection and 109 without TV infection were enrolled after applying the inclusion and exclusion criteria (Fig. 1). Midstream urine samples were all negative for pyuria and bacterial culture. Among patients with TV infection, 86.2% had been treated with metronidazole 500 mg twice daily for 4 weeks. Of these, 92% had received follow-up TV tests, with one patient showing positive results, while the other 22 patients were negative. The baseline characteristics and clinical symptoms of patients with and without TV infection are shown in Table I. Patients with TV infection displayed a significantly higher frequency of suprapubic/lower abdominal pain (44.83% vs. 24.77%, $p=0.034$), semen leukocyte >5/HPF (17.24% vs. 3.67%, $p=0.020$) and inflammatory type (category IIIA) (31.03% vs. 9.17%, $p=0.005$). Besides, the prevalence of hematospermia in patients with TV infection was approximately 21%, nearly six times higher than in patients without TV infection. There appeared to be no significant difference between these groups in the symptom duration and other clinical symptoms.

The difference in baseline characteristics and clinical symptoms between non-inflammatory type (category IIIB) and category IIIA are presented in Table II. Nineteen patients were identified as category IIIA CP/CPPS, and 119 patients as category IIIB CP/CPPS. Patients with category IIIB CP/CPPS were more likely to have a higher marriage rate (69.75% vs. 31.58%, $p=0.001$) and a lower prevalence of TV infection (16.81% vs. 47.37%, $p=0.005$). Besides, no significant difference was observed in other baseline characteristics.

Discussion

The prevalence of TV in the cases with CP/CPPS was 21% in this study, and the mean age and symptom duration of these patients are similar to a previously reported cohort study (Clemens et al. 2015). The patients with symptoms of suprapubic or lower abdominal pain were more likely to have TV infections. Besides, CP/CPPS patients with self-reported or lab-confirmed hematospermia, semen leukocyte >5/HPF, or category IIIA were also more likely to have TV infection.

Inflammation was thought to contribute to the symptoms associated with CP/CPPS (Sung et al. 2014). The new NIH classification for category III defined these patients as either category IIIA or category IIIB based on the presence of significant WBCs in prostatic-specific specimens such as EPS, VB3, and semen (Sung et al. 2014). Besides, no difference in outcome was shown in the two groups in previous studies (Nickel et al. 2001; Kim et al. 2011; Sung et al. 2014). However, in this study, patients with category IIIA CP/CPPS were more likely to have a significantly higher prevalence of

Table I
Baseline characteristics and clinical symptoms between CP/CPPS patients with TV and without TV infection.

Characteristic	Negative for TV (n = 109)	Positive for TV (n = 29)	*p*-value
Age, year	44.58 ± 12.03	43.90 ± 11.47	0.785
Symptom duration, month	22.23 ± 31.25	26.93 ± 25.78	0.458
Married	70 (64.22%)	19 (65.52%)	0.897
Smoking			0.206
Never-smoker	72 (66.06%)	24 (82.76%)	
Current smoker	32 (29.36%)	5 (17.24%)	
Ex-smoker	5 (4.59%)	0 (0%)	
Alcohol drinking	27 (24.77%)	6 (20.69%)	0.647
Betel nut chewing	8 (7.34%)	0 (0%)	0.204
Comorbidity			
Diabetes	10 (9.17%)	1 (3.45%)	0.458
Hypertension	17 (15.60%)	4 (13.79%)	1.000
Hyperlipidemia	32 (29.36%)	9 (31.03%)	0.861
Kidney stone	3 (2.75%)	3 (10.34%)	0.107
Depression/stress	22 (20.18%)	8 (27.59%)	0.390
Digital rectal exam			
Prostate enlargement			0.337
Non-enlarged	39 (35.78%)	9 (31.03%)	
Mild	66 (60.55%)	17 (58.62%)	
Moderate	4 (3.67%)	3 (10.34%)	
Prostate tenderness	22 (20.18%)	4 (13.79%)	0.434
Prostate-specific antigen level, ng/ml	1.27 ± 1.22	2.57 ± 3.24	0.068
Any pelvic pain	104 (95.41%)	28 (96.55%)	0.789
Scrotal	25 (22.94%)	7 (24.14%)	0.892
Perineal	49 (44.95%)	12 (41.38%)	0.730
Suprapubic/lower	27 (24.77%)	13 (44.83%)	**0.034**
Abdominal			
Inguinal	17 (15.60%)	4 (13.79%)	1.000
Urethral/penile	34 (31.19%)	8 (27.59%)	0.708
Any lower urinary tract symptoms	68 (62.39%)	19 (65.52%)	0.756
Urgency	8 (7.34%)	0 (0%)	0.204
Frequency	36 (33.03%)	13 (44.83%)	0.238
Nocturia	15 (13.76%)	3 (10.34%)	0.764
Incomplete emptying	24 (22.02%)	3 (10.34%)	0.159
Dysuria	12 (11.01%)	6 (20.69%)	0.213
Small stream	17 (15.60%)	3 (10.34%)	0.568
Hesitancy	17 (15.60%)	1 (3.45%)	0.121
Hematospermia	4 (3.67%)	6 (20.69%)	**0.006**
Semen leukocyte > 5/HPF	4 (3.67%)	5 (17.24%)	**0.020**
WBC > 10/HPF in post-massage urine	7 (6.42%)	5 (17.24%)	0.129
Inflammatory type (category IIIA)	10 (9.17%)	9 (31.03%)	**0.005**
Total testosterone, ng/ml	4.23 ± 2.01	4.32 ± 1.36	0.880
Free testosterone, pg/ml	10.20 ± 4.01	10.52 ± 4.61	0.837
Any sexual dysfunction	49 (44.95%)	14 (48.28%)	0.750
Erectile dysfunction	39 (35.78%)	13 (44.83%)	0.372
Premature ejaculation	19 (17.43%)	2 (6.90%)	0.245

Data are presented as means ± SD or n (%). Significant values are showing in bold.
TV – *Trichomonas vaginalis*, HPF – high-power field, WBC – white blood cells

Table II
Baseline characteristics and clinical symptoms between patients with category IIIB and category IIIA.

Characteristic	Category IIIB (n = 119)	Category IIIA (n = 19)	*p*-value
Age, year	45.15 ± 11.72	39.97 ± 12.16	0.077
Symptom duration, month	23.22 ± 30.89	23.21 ± 25.78	0.999
Positive for TV	20 (16.81%)	9 (47.37%)	**0.005**
Married	83 (69.75%)	6 (31.58%)	**0.001**
Smoking			0.719
Never-smoker	81 (68.07%)	15 (78.95%)	
Current smoker	33 (27.73%)	4 (21.05%)	
Ex-smoker	5 (4.20%)	0 (0%)	
Alcohol drinking	28 (23.53%)	5 (26.32%)	0.776
Betel nut chewing	8 (6.72%)	0 (0%)	0.598
Comorbidity			
Diabetes	9 (7.56%)	2 (10.53%)	0.648
Hypertension	18 (15.13%)	3 (15.79%)	1.000
Hyperlipidemia	37 (31.09%)	4 (21.05%)	0.374
Kidney stone	5 (4.20%)	1 (5.26%)	1.000
Depression/stress	24 (20.17%)	6 (31.58%)	0.367
Digital rectal exam			
Prostate enlargement			0.254
Non-enlarged	44 (36.97%)	4 (21.05%)	
Mild	70 (58.82%)	13 (68.42%)	
Moderate	5 (4.20%)	2 (10.53%)	
Prostate tenderness	24 (20.17%)	2 (10.53%)	0.527
Prostate-specific antigen level, ng/ml	1.53 ± 1.94	1.95 ± 2.26	0.485
Any pelvic pain	115 (96.64%)	17 (89.47%)	0.192
Scrotal	25 (21.01%)	7 (36.84%)	0.147
Perineal	51 (42.86%)	10 (52.63%)	0.426
Suprapubic/lower Abdominal	35 (29.41%)	5 (26.32%)	0.782
Inguinal	16 (13.45%)	5 (26.32%)	0.169
Urethral/penile	36 (30.25%)	6 (31.58%)	0.907
Any lower urinary tract symptoms	75 (63.03%)	12 (63.16%)	0.991
Urgency	8 (6.72%)	0 (0%)	0.598
Frequency	39 (32.77%)	10 (52.63%)	0.093
Nocturia	17 (14.29%)	1 (5.26%)	0.466
Incomplete emptying	24 (20.17%)	3 (15.79%)	1.000
Dysuria	15 (12.61%)	3 (15.79%)	0.715
Small stream	17 (14.29%)	3 (15.79%)	1.000
Hesitancy	15 (12.61%)	3 (15.79%)	0.715
Hematospermia	8 (6.72%)	2 (10.53%)	0.628
Total testosterone, ng/ml	4.26 ± 1.87	4.19 ± 1.70	0.907
Free testosterone, pg/ml	9.76 ± 3.69	12.83 ± 5.52	0.101
Any sexual dysfunction	54 (45.38%)	9 (47.37%)	0.872
Erectile dysfunction	45 (37.82%)	7 (36.84%)	0.935
Premature ejaculation	18 (15.13%)	3 (15.79%)	1.000

Data are presented as means ± SD or n (%). Significant values are showing in bold.
Category IIIB – non-inflammatory type, Category IIIA – inflammatory type, TV – *Trichomonas vaginalis*

TV infection than category IIIB CP/CPPS (47.37% vs. 16.81%, $p = 0.005$), which revealed that category IIIA might be a vital factor of CP/CPPS patients.

Skerk et al. (2004) reported 1,442 patients with chronic prostatitis, of whom 151 (10.5%) tested positive for TV by EPS and VB3 urine cultures. However,

more than half (58.6%) of the patients had bacterial infections and had chronic bacterial prostatitis rather than CP/CPPS. Given the low sensitivity of traditional cultures, the true prevalence of TV in CP/CPPS patients with PCR is likely to be higher than that reported by Skerk et al. (2004). Lee et al. (2012) reported 33 patients with CP/CPPS, of whom seven tested positive for TV by PCR, yielding a prevalence rate of 21.2%, similar to the present report (21%). Although PCR-based assay displayed higher sensitivity and specificity and has more potential to be the gold standard laboratory method for confirmation of TV infection (Gaydos et al. 2017), the immunochromatographic strip test can be an alternative method due to the easy operation, cheap, and immediate reporting of results. This study's findings support the efficacy of strip tests with urine samples to detect TV in patients with CP/CPPS. Besides, considering previous studies and this study, the prevalence of TV in patients with CPPS is approximately 21%.

Patients with chronic prostatitis syndrome were reported to detect the infection with several pathogens, such as *Chlamydia trachomatis*, *Ureaplasma urealyticum*, or TV, with normal WBC count in EPS or VB3 (Hobbs and Seña 2013). However, further statistical analysis of their raw data showed that the patients with TV infection were more likely to have leukocytes in EPS or VB3 (66.2%) compared to those infected with *C. trachomatis* (32.5%) and *U. urealyticum* (44.4%, $p < 0.001$, calculated using raw data from the study of Skerk et al. (2004). In this study, patients with WBCs in their semen, but not post-massage urine, were associated with an increased risk of TV infection, reflecting the inflammatory process involved with the TV. Inflammatory or infectious conditions in the genitourinary tract, including the prostate, have been reported to be the most common causal factors of hematospermia (Stefanovic et al. 2009). Except for bacterial infections, Herpes simplex virus (HSV), *C. trachomatis*, *Enterococcus faecalis*, and *U. urealyticum* have all been reported to be causes of hematospermia (Lee 2015). TV is also a possible cause of hematospermia; however, the link has never been reported before. This study's finding highlights the importance of screening patients with CP/CPPS combined with hematospermia.

The underlying mechanisms of sexual dysfunction in CP/CPPS remain unclear. Vasculogenic, endocrine, neurogenic, and psychological factors may all play some roles in the pathogenesis of sexual dysfunction in these patients. A recent meta-analysis of 24 studies involving 11,189 patients reported an overall prevalence of erectile dysfunction and premature ejaculation in men with CP/CPPS were 0.29 (95% CI 0.24–0.33) and 0.40 (95% CI 0.30–0.50), respectively (Li and Kang 2016). A similar prevalence of erectile dysfunction (27.5%) was also founded, but a much lower prevalence of premature ejaculation (7.2%), hence a lower prevalence of overall sexual dysfunction. A possible explanation is that men with CP/CPPS were reluctant to engage in sexual activity due to painful ejaculation or decreased sexual desire. In this study, the patients with positive TV did not have a higher prevalence of sexual dysfunction than negative TV (48.28% vs. 44.95%, $p = 0.75$), while a similar trend was also observed in those with a depressive or stressful status. It is further evidence that the underlying pathophysiology of sexual dysfunction caused by CPPS is more psychological than pathological.

There are several limitations of this study. Firstly, the sensitivity and specificity of the VB3 rapid Trichomonas test for TV infection in this study were not assessed due to the lack of a control group and the PCR-based gold standard laboratory method. Secondly, the patients diagnosed with CP/CPPS were not undergoing transperineal biopsy or voided bladder-1 (VB1) test to exclude urethritis or urethral contamination. Thirdly, the UPOINTS clinical phenotype was incorporated in the detailed history but not the validated questionnaire, NIH CPSI, so the severity of the symptoms could not be investigated. Fourthly, the small and imbalanced case numbers between patients with and without TV infection and between patients with category IIIB and IIIA might cause statistical bias. Fifthly, the symptoms of sexual dysfunction were obtained by inquiry and not a diagnostic questionnaire, leading to some deviation. A large-scale prospective trial containing the VB3 rapid Trichomonas test and PCR-based assay is still needed to confirm this finding.

Conclusion

This study revealed the high prevalence of TV infection in patients with CP/CPPS category IIIA using the VB3 rapid Trichomonas test. Besides, suprapubic or lower abdominal pain, hematospermia, leukocyte in semen, and category IIIA were significantly higher in patients with TV infection than without TV infection. If the above clinical symptoms are found, the VB3 rapid immunochromatographic Trichomonas test can be used to establish a stronger correlation with TV infection at the time of diagnosis.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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