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▣ BRIEF COMMUNICATION

Prevalence of *Trichomonas vaginalis* by PCR in Men Attending a Primary Care Urology Clinic in South Korea

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Abstract: *Trichomonas vaginalis*, a causative agent of trichomoniasis, may trigger symptomatic or asymptomatic nongonococcal urethritis and chronic prostatitis in men. Despite the availability of highly sensitive diagnostic tests, such as nucleic acid amplification tests, including PCR, few prospective studies present data on male *T. vaginalis* infection in South Korea. In the present study, the prevalence of *T. vaginalis* and associated clinical conditions were evaluated in 201 male patients from a primary care urology clinic in South Korea. The prevalence of *T. vaginalis* infection in our cohort was 4% (8/201) by PCR. *T. vaginalis* infection was common in men older than 40 years (median age, 52 years). Among the 8 *Trichomonas*-positive patients, 87.5% (7/8) had prostatic diseases, such as prostatitis and benign prostatic hyperplasia, and 25.0% (2/8) and 12.5% (1/8) were coinfected with *Chlamydia trachomatis* and *Mycoplasma genitalium*, respectively. Our results suggest that *T. vaginalis* infection is not rare in men attending primary care urology clinics in South Korea, especially in those older than 40 years, in whom it may explain the presence of prostatic disease. The possibility of *T. vaginalis* infection should be routinely considered in older male patients with prostatic diseases in South Korea.

Key words: *Trichomonas vaginalis*, sexually transmitted disease, diagnosis, PCR, multiplex PCR

Trichomoniasis, a sexually-transmitted disease (STD) caused by *Trichomonas vaginalis* (*T. vaginalis*), is highly prevalent in females. However, men are also infected and represent a risk of transmission to sexual partners as a reservoir of *T. vaginalis* [1-4]. Although the majority of *T. vaginalis* infection in men is asymptomatic, *T. vaginalis* may cause symptomatic or asymptomatic nongonococcal urethritis and chronic prostatitis [5-7]. Thus, early diagnosis and treatment of trichomoniasis, especially asymptomatic *T. vaginalis* infection, are imperative for men as a public health concern as well as for women. The detection of an asymptomatic *T. vaginalis* infection by direct microscopy and culture showed low sensitivity. Nucleic acid amplification tests, including PCR and transcription-mediated amplification, with increased diagnostic performance have been developed (reviewed in [4]) and validated for the detection of *T. vaginalis* in women and men [8-10].

The prevalence of *T. vaginalis* among men attending an STD clinic ranged from 2.8% to 17% [11,12], with the highest prevalence in those aged >40 years [13,14], and was as high as 73% in male partners of women diagnosed with vaginal trichomoniasis [15]. Recently, *T. vaginalis* was detected in only 1 of 435 men who visited a health examination center in South Korea [10]. However, the prevalence of *T. vaginalis* significantly increased to 21.2% in male patients with chronic recurrent prostatitis and urethritis [16]. Few recent studies examined the prevalence of *T. vaginalis* infection in men. Here, we examined 201 urine specimens from a primary care urology clinic in South Korea using PCR assays to determine the prevalence of *T. vaginalis* and associated clinical conditions in men.

From May 2013 to November 2013, 201 male patients from a primary care urology clinic (Top Urology Clinic) in Daegu, South Korea, were screened for trichomoniasis by PCR and for

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bacterial STDs by multiplex PCR. As is required by the Declaration of Helsinki, donor confidentiality was maintained throughout, and the study was approved by the Institutional Review Board with written consent (KNUH 2013-04-051). The bivariate analysis included 201 male patients who completed questionnaires. Demographic characteristics and urogenital symptoms of trichomoniasis were assessed by self-administered questionnaires.

In this study, first-voided urine (VB1) specimens, which were reported to be sufficient for the detection of *T. vaginalis* in men, were used in the PCR assays [16]. VB1 (30-40 ml) samples from male patients were provided in sterile 50-ml screw-cap plastic tubes, immediately frozen at -20˚C, and transported on ice to a laboratory where they were stored at -70˚C. The diagnosis of *T. vaginalis* infection was made using PCR targeting Tvk [17] and the β-tubulin gene [18], while the diagnosis of bacterial sexually-transmitted infections (STIs) was made using multiplex PCR (STD6 ACE Detection kit, SeeGene, Seoul, Korea) [19]. After thawing, the frozen urine specimens were divided into 2 tubes (10 ml each), equilibrated at room temperature, centrifuged at 5,000 g for 15 min, and resuspended in 1 ml of sterile saline.

DNA was prepared from the suspension by 2 different methods; the QIAamp DNA Mini kit (Qiagen, Valencia, California, USA) for multiplex PCR according to the manufacturer's instructions or heat-treating with a chelating resin for PCR [18,20]. Briefly, 1 ml of suspended urine precipitate was centrifuged at 10,000 g for 5 min, suspended in 200 µl of a 5% suspension of chelating resin (Chelex 100; Sigma, St. Louis, Missouri, USA) in 0.01 M Tris buffer (pH 8.0), and incubated at 56˚C for 30 min. Preparations were mixed gently, boiled for 10 min, and centrifuged at 12,000 g for 1 min in a microcentrifuge, and 1 µl of supernatant was directly used as the template for PCR. The PCR conditions were as follows: an initiation step at 95˚C for 5 min; 40 cycles at 90˚C for 1 min, 60˚C for 30 sec, and 72˚C for 2 min; and a final extension step at 72˚C for 10 min. The PCR primer set Tvk 3/7 (Tvk 3, 5'-ATTGTCGAACATTGGTCTTACCCTC-3'; Tvk 7, 5'-TCTGTGCCGTCTTCAAGTATGC-3') [17] amplified the 261 bp products and BTUB 9/2 (BTUB 9, 5'-CATTGATAACGAAGCTCTTTACGA-3'; BTUB 2, 5'-GCATGTTGTGCCGGACATAACCAT-3') [18] amplified a DNA product of 112 bp from *T. vaginalis* genomic DNA. The lower limit of *T. vaginalis* detection by PCR using Tvk 3/7 and BTUB 9/2 was found to be 1 organism per reaction, consistent with a previous report [18]. Multiplex PCR based on the dual priming oligonucleotide (DPO) system (Seegene, Seoul, Korea) was used for the diagnosis of STIs, including *Trichomonas*. This method enables the simultaneous detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, and *T. vaginalis* [19]. In cases of discrepancies between PCR and multiplex PCR results, PCR results (both Tvk 3/7 and BTUB 9/2 PCR-positive) were considered as true positive for trichomoniasis.

The mean age of the 201 male participants was 53.6 years (range; 17-87 years). Though the group was age-diverse (Table 1), 81.6% (164/201) of the participants were older than 40 years. In this cohort of male patients who visited a primary care urology clinic, the prevalence of *T. vaginalis* infection was 4.0% (8/201) by PCR assay with 100% concordance between the Tvk and BTUB primer sets (Table 1). The median age of 8 men with *Trichomonas*-positive specimens was 52 years (range; 28-74 years). As shown in Table 1, the prevalence of *T. vaginalis* infection was 11.1% (1/9) in those aged 20-29 years, 5.7% (2/35) in those aged 40-49 years, 5.7% (3/53) in those aged 50-59 years, 2.1% (1/48) in those aged 60-69 years, and 3.8% (1/26) in those aged 70-79 years, with male patients in the 20-29 age group showing the highest rate of *T. vaginalis* infection (Table 1). However, if we consider the relative proportion of each age group to the total number of *Trichomonas*-infected patients, 12.5% (1/8) of infected patients were < 30 years, 25.0%

Table 1. Analysis of *Trichomonas vaginalis* infection status according to sociodemographic characteristics and urogenital symptoms (N = 201)

Factor	Characteristic	No. of patients	*T. vaginalis* prevalence (%)
Overall		201	8 (4.0)
Age (years)	10-19	2	0 (0.0)
	20-29	9	1 (11.1)
	30-39	26	0 (0.0)
	40-49	35	2 (5.7)
	50-59	53	3 (5.7)
	60-69	48	1 (2.1)
	70-79	26	1 (3.8)
	80-89	2	0 (0.0)
Marital status	Single	28	1 (3.6)
	Married	150	6 (4.0)
	Divorced	4	1 (25.0)
	Missing	19	0 (0.0)
Urogenital symptoms	None	75	3 (4.0)
	Penile discharge	11	0 (0.0)
	Penile tingling	28	1 (3.6)
	Pain during urination	98	4 (4.1)
	Lower abdominal pain	16	0 (0.0)

(2/8) were 40-49 years, 37.5% (3/8) were 50-59 years, 12.5% (1/8) were 60-69 years, and 12.5% (1/8) were 70-79 years, indicating that more than 60% (62.5%) of *T. vaginalis* infections occurred in men older than 50 years.

An examination of marital status showed that 87.5% (7/8) of *T. vaginalis*-infected patients were currently or previously married. According to these results, it is likely that *T. vaginalis* is more prevalent in older ages. However, owing to the small number of young participants (10-40 years), no statistical association ($P=0.920$) was obtained between *Trichomonas* infection and older ages. The analysis of urogenital symptoms reported via self-administered questionnaires indicated that 37.5% of *T. vaginalis*-infected male patients had no penile discharge, odor, or lower abdominal pain, while 12.5% and 50.0% reported penile tingling and pain during urination, respectively. Among the 8 *Trichomonas*-positive male patients, 50.0% (4/8) were diagnosed with prostatitis, 37.5% (3/8) were with benign prostatic hyperplasia (BPH), and 12.5% (1/8) were with urethritis (Table 2). As shown in Table 2, no *Trichomonas*-positive reaction was obtained by multiplex PCR (Table 2). In the 8 samples that were positive by PCR assay, 2 (25.0%) were positive for *C. trachomatis* and 1 (12.5%) was positive for *M. genitalium* by multiplex PCR.

Skerk et al. [21,22] reported that 71.0-74.2% of chronic prostatitis cases were attributable to infection, 10.5-19.0% of which were due to trichomoniasis. Recently, Lee et al. [16] detected *T. vaginalis* in 21.2% of Korean patients complaining of lower urinary tract symptoms, 71.4% of whom had chronic prostatitis. Among 8 *Trichomonas*-positive male patients in our study, 87.5% (7/8) were diagnosed with prostatitis or BPH (Table 2), showing that the vast majority of *Trichomonas*-positive male patients had prostatic diseases, similar to previous reports.

As shown in Table 2, no *Trichomonas*-positive reaction was obtained by multiplex PCR. Although the detection limit for purified *T. vaginalis* genomic DNA from in vitro culture samples was similar for PCR and multiplex PCR (data not shown), their diagnostic performance in urine specimens differed. Previous reports have suggested the possible limitations of multiplex PCR, which include PCR drift by stochastic fluctuation in the interaction of PCR reagents or competitive inhibition by PCR selection [10].

Concurrent STIs, such as *C. trachomatis* or *N. gonorrhoeae*, are frequently detected in persons with trichomoniasis [23]. In the United States, approximately 10% of male partners with trichomoniasis were coinfected with *C. trachomatis* or *N. gonorrhoeae* [15]. In our study, 25.0% and 12.5% of *Trichomonas*-positive male patients were coinfected with *C. trachomatis* and *M. genitalium*, respectively.

As mentioned above, 2 studies conducted in the United States in the early 2000s reported that 12.9% (47/363) [8] and 17.3% (52/300) [12] of men attending an STI clinic were positive for *T. vaginalis* by PCR assay. In Japan, a study conducted in the 2000s using PCR reported that no *T. vaginalis* was detected in 100 Japanese men with and without urethritis [24]. Recently, the prevalence of *T. vaginalis* was reported to be 1.4% (3/215) in men with urethritis and 1.0% (1/98) in men without urethritis [25]. In the present study, 4% (8/201) of male patients attending a primary care urology clinic were *Trichomonas*-positive, indicating that the male prevalence of *T. vaginalis* in South Korea appears to be lower than that in the United States and higher than that in Japan.

In contrast to women with *Trichomonas* infection, the association between *Trichomonas* infection and older ages in men is not fully understood. A United States study conducted in the

Table 2. Detection of STI microorganisms in *Trichomonas*-positive patients

No.	Age	PCR		Multiplex PCR						Disease
		Tvk	BTUB	TV	MH	MG	CT	NG	US	
1	43	+	+	–	–	–	–	–	–	Prostatitis
2	28	+	+	–	–	–	+	–	–	Prostatitis
3	74	+	+	–	–	–	+	–	–	BPH
4	52	+	+	–	–	–	–	–	–	Prostatitis
5	56	+	+	–	–	–	–	–	–	Prostatitis
6	53	+	+	–	–	+	–	–	–	Urethritis
7	46	+	+	–	–	–	–	–	–	BPH
8	67	+	+	–	–	–	–	–	–	BPH

TV, *Trichomonas vaginalis*; MH, *Mycoplasma hominis*; MG, *Mycoplasma genitalium*; CT, *Chlamydia trachomatis*; NG, *Neisseria gonorrhoeae*; US, *Ureaplasma* sp.; BPH, benign prostatic hyperplasia; BTUB, β-tubulin gene from *T. vaginalis*.

late 1990s found that *T. vaginalis* infection was significantly associated with age (0.8%, <30 years versus 5.1%, ≥30 years). However, Wendel et al. [8] recently reported that the prevalence of *T. vaginalis* in men aged ≤28 years (13%, 23/178) was similar to that of men aged >28 years (24/185). In the present study, *T. vaginalis* was significantly more prevalent among older men who had been married (≥40 years, 87.5%) than among relatively younger single men (<40 years, 12.5%). During the study period, the number of patients aged <40 years was small compared to the number of patients aged ≥40 years. Although further studies are required to examine the prevalence of *T. vaginalis* in young men, it can be considered that STIs, including *T. vaginalis* infection are indeed rare in men younger than 40 years or that the majority of this subgroup is asymptomatic and thus seldom visits the Urology Clinic. It appears that >80% of *T. vaginalis* infections are asymptomatic, causing the infection to persist. Here, we found that the majority of *T. vaginalis* infections occurred in men older than 40 years with urethritis or prostatic diseases. Thus, it can be supposed that few men, especially those younger than 40 years, are diagnosed with and treated for trichomoniasis in South Korea. With the exception of male partners of women diagnosed with vaginal trichomoniasis, *T. vaginalis* infection has not been routinely considered in male patients attending primary care urology clinics in South Korea owing to the availability or cost of diagnostic testing. Thus, the early diagnosis and treatment of *T. vaginalis* infection, especially in young men, and rapid point-of-care tests are urgently needed to prevent the increase of this disease burden.

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CONFLICT OF INTEREST

We have no conflict of interest related to this study.

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