Detection of Mycoplasma genitalium and Trichomonas vaginalis Infections in General Jordanian Patients

A.A. Shehabi, Z.M. Awwad, M. Al-Ramahi, E. Charvalos and L.F. Abu-Qatouseh

1Departments of Pathology and Microbiology
2Special Surgery-Urology
3Obstetric and Gynecology
Jordan University Hospital, Amman, Jordan
4Institute of Microbiology, University of Greece, Athena, Greece

Abstract: Problem statement: Both M. genitalium and T. vaginalis were recognized as important cause of sexually transmitted infections in developed countries. This study investigated the prevalence of M. genitalium and T. vaginalis in general Jordanian patients and their role of causing genitourinary tract diseases. Approach: A cross sectional study of 383 Jordanian adult patients aged between 19-78 years were investigated for presence of M. genitalium and T. vaginalis at the urology and obstetric-gynecology clinics at the Jordan University Hospital in Amman. First voided urine specimens were tested using urine microscopy, PCR for M. genitalium and T. vaginalis as well as culture for T. vaginalis. Results: The incidence of M. genitalium was higher and statistically significant (17/188, 9%, p = 0.022) than T. vaginalis (3/188; 1.6%) among patients diagnosed with specific urinary symptoms and signs, while this incidence was less but also significant in asymptomatic patients (7/195, 3.6% versus 1/195, 0.5%, p = 0.031), respectively. M. genitalium infection was frequently observed with urinary frequency (76%) and dysuria (59%) among symptomatic patients and more common in men than women (65% versus 35%, p = 0.51) and in married than singles (76% versus 24%, p = 0.59). Dual infection with both organisms was not recognized. Conclusion: Infection caused by M. genitalium and T. vaginalis was associated with higher incidence rate in patients with symptomatic genitourinary disease. Therefore, screening for their occurrence in such patients is important.

Key words: M. genitalium, T. vaginalis, urine PCR, Jordan

INTRODUCTION

Nongonococcal Urethritis (NGU) caused by Chlamydia trachomatis Mycoplasma genitalium and Trichomonas vaginalis are common cause of symptomatic and asymptomatic infections in both men and women in developed countries[1,4] but their prevalence and pathogenesis in most developing countries including Jordan are still limited reported[5,6].

Recently, M. genitalium has been recognized as a common infection associated with symptomatic urethritis and with a high prevalence of infected sexual partners supporting its role as a sexually transmitted infection[1,4,5,8]. First voided urine appeared to be a better diagnostic specimen than the urethral swab for detection M. genitalium in men using PCR[8-10]. In women, M. genitalium cause cervicitis, urethritis, pelvic disease and recently found more commonly in cervical canal of infertile women and its infection can be also detected with high sensitivity by using urine specimen and PCR[7,9-11].

Epidemiologically, T. vaginalis infection is often associated with vaginosis and commonly transmitted with other STDs, whereas its prevalence and spectrum of disease in men are less characterized[12,13]. Diagnosis of T. vaginalis is usually made using wet mount of vaginal swabs and direct microscopy, which are not highly sensitive, while culture method gives results that are more positive but it is less performed. Recent studies showed that detection of T. vaginalis using urine or urethral swab specimens and PCR showed high sensitivity and positive results[13-15].

The purpose of this study was to determine the rate of infection with M. genitalium and T. vaginalis and their association with common specific genitourinary features in general Jordanian patients.

MATERIALS AND METHODS

Patients: A total of 383 Jordanian patients aged between 19-78 years, including 201 (52%) men with
mean age of 42-year and 182 (48%) women with mean age of 40-year were admitted at the urology and obstetric-gynecology clinics at the Jordan University Hospital (JUH) in Amman, over the period May-October 2006. All patients were examined for the presence of any of the following specific urinary symptoms: urinary frequency, dysuria, suprapubic/pelvic pain and presence of vaginal/urethral discharge. Of these, 188 were characterized as asymptomatic patients with one or more specific genitourinary symptoms and signs and the rest 195 subjects were free of any specific urinary symptoms and have been included as controls (Table 1). All Patients gave their written consent to be included in this study and the clinical data of each enrolled patient were reported in a special designed form for the study.

Urine specimen and culture of *T. vaginalis*: First void urine specimens were collected in sterile leak proof containers from all patients included in the study. Urine specimens were transported to the microbiology research laboratory for investigation with 2 h. at the department of pathology and microbiology, Faculty of Medicine, University of Jordan. Ten mL of urine specimens were centrifuged at 2000 g for 10 min and the pellets were examined microscopically for the presence of motile trophozoit of *T. vaginalis* and to count White Blood Cells (WBCs) per High Power Field (HPF). The pellet was then resuspended in 1 mL of Phosphate Buffer Saline (PBS) and 0.5 mL of the sample kept at -70°C for later PCR investigation, whereas the second 0.5 mL was inoculated in a vial containing 5 mL of Trichomonas medium No.2 (Oxoid, UK), where the positive control of *T. vaginalis* which was grown in Trichomonas medium No.2 (Oxoid, UK), the positive control of *M. genitalium* consisted of DNA provided as lyophilized compound supplied from Institute of Microbiology, University of Greece in Athena (E. Charvalos). Negative control was made of distilled water. PCR amplification was performed in two separate tubes for both organisms as follows: 30 cycles of 1 min at 90°C, 30s at 60°C and 2 min at 72°C. After amplification, there was additional extension step at 72°C for 7 min and then samples were cooled to 4°C.

DNA extraction and PCR: DNA extraction was performed according to the instructions provided in the Genomic Wizard DNA extraction Kit (Promega, USA). *T. vaginalis* specific primers TVK3 (5'-ATTTGCAATCTTGGTTCCCTC 3') and TVK7 (5'-CTCTGCCCGTCTCAAATAG 3') were used for PCR amplification[16]. PCR reaction mixture of a total volume of 25 μL was composed of 15 μL of PCR Master Mix (Promega, USA) 2.5 μL of 10 μM each primer and 5 μL of extracted DNA of urine specimens of each patient. The master mix was composed of 3uM MgCl$_2$, 500 uM dNTPs each, 1U Taq DNA polymerase and Taq buffer. Positive and negative controls were included in all PCR runs. The positive control of *T. vaginalis* was composed of DNA extracted from our clinical isolate of *T. vaginalis* which was grown in Trichomonas medium No.2 (Oxoid, UK). Negative control was made of distilled water. PCR amplification was performed in two separate tubes for both organisms as follows: 30 cycles of 1 min at 90°C, 30s at 60°C and 2 min at 72°C. After amplification, there was additional extension step at 72°C for 7 min and then samples were cooled to 4°C.

**Statistical analysis**: Statistical significance was determined using $\chi^2$ and Fisher's exact tests. Results were considered statistically significant if the p value was <0.05.

**RESULTS**

The incidence of *M. genitalium* was higher and statistically significant (17/188, 9%, $p = 0.022$) than *T. vaginalis* (3/188, 1.6%) among symptomatic patients with specific urinary symptoms and signs, whereas this incidence was less but also statistically significant in asymptomatic patients (7/195, 3.6% versus 1/195,
DISCUSSION

Information on the incidence and spectrum of clinical features associated with fastidious organisms like *Chlamydia trachomatis*, *M. genitalium* and *T. vaginalis*, is still rarely reported in Jordan and in most Middle East countries. One study reported that *T. vaginalis* has been detected in 0.9% of women using cervical stained smears over a period of 3.5 years [6], while a study from Egypt showed that trichomoniasis symptomatic cases were detected more by PCR (91.3%) than by culture (72.9%) or other routine methods [17]. This study indicated that *M. genitalium* is more prevalent and significant than *T. vaginalis* (9% versus 1.6%, *p* = 0.022) among Jordanian patients with symptomatic genitourinary infections, respectively, while the incidence of both organisms in a symptomatic control group was less but also significant (3.6 and 0.5%, *p* = 0.031), respectively. Dual infection has been not diagnosed and males to female infection ratio was approximately 2:1 for both organisms. These results indicate that the incidence of *M. genitalium* in our male and female patients is similar to some extent to recent studies from northern European countries which have reported a range of 6-12% among their population [7,8,18,19]. The overall incidence of *T. vaginalis* infection in Jordanian population either symptomatic (1.6%) or a symptomatic (0.5%) is much less than that reported from most developed countries using similar clinical specimens and PCR techniques [16,18].

Most symptomatic patients infected with *M. genitalium* (76%) have at least two specific genitourinary symptoms 0.5%, *p* = 0.031), respectively (Table 1). Dual infection was not recognized and men to female infection ratio was approximately 2:1 (65% versus 35% *p* = 0.51) for both organisms and was more common in married patients (76% versus 24%, *p* = 0.59) (Table 2 and 3).

Table 2 shows characteristics of *T. vaginalis* infection and detection methods in 3 symptomatic patients and one asymptomatic. Table 3 shows the incidence and characteristics of 17 symptomatic patients associated with positive *M. genitalium* infections. Urinary frequency 13/17 (76%) and dysuria 10/17 (59%) were frequently recognized as common symptoms among these patients.

### CONCLUSION

Infection with *M. genitalium* and *T. vaginalis* is associated with higher incidence rate in patients with symptomatic genitourinary disease than in asymptomatic. Therefore, screening for their presence in symptomatic patients is important.
ACKNOWLEDGMENT

This study was supported with a grant (No. 5/3/1/1927) from the deanship of academic research, University of Jordan, Amman, Jordan

REFERENCES