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

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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[1,4,7,8]. 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 cervisitis, 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

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Table 1: Distribution of patients and controls with and without specific genitourinary symptoms and positive *T. vaginalis* and *M. genitalium*

Patient group (mean age y^{-1})	Sex		Positive *T. vaginalis* N (%)	Positive *M. genitalium* N (%)	Total
	Males N (%)	Females			
Symptomatic [a] (42)	99 (53)	89 (47)	3 (1.6)	17 (9.0)	188
Asymptomatic (40)	102 (52)	93 (48)	1 (0.5)	7 (3.6)	195
Total	201 (52)	182 (48)	4 (1.0)	24 (6.2)	383

[a]: Each patient complained of one or more specific genitourinary symptoms (urinary frequency, dysuria, suprapubic /pelvic pain, presence of vaginal/urethral discharge)

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 symptomatic 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 pellet was examined microscopically for the presence of the 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). The culture media were incubated at 37°C up to 7 days and the media was checked daily by microscopic examination for the presence of lived, motile Trophozoits of *T. vaginalis*[13].

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`ATTGTCGAACATTGGTCTCCTC 3`) and TVK7 (5` TCTGTCCCGTCT TCAAGTATGC 3`)[13]. For *M. genitalium*, MgPa 1 (5'-AGTTGATGAAACCTTAACCCCTTGG-3') and MgPa3 (5'-CCGTTGAGGGGTTTTCCATTTTTGC-3') primers were used for PCR amplification[16]. PCR reaction mixture of a total volume of 25 uL was composed of 15 uL of PCR Master Mix (Promega, USA) 2.5 uL of 10 uM each primer and 5 uL 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), where 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.

Gel electrophoresis: 15 uL of amplified product was electrophoresed on a 2% agarose gel containing 0.5 ug mL^{-1} ethidium bromide and viewed on a gel documentation system (UVP, USA). Samples containing a 300 and 290bp fragments were considered positive for *T. vaginalis* and *M. genitalium*, respectively[14,17].

Statistical analysis: Statistical significance was determined using χ^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,

Table 2: Clinical and epidemiological features of 3 symptomatic patients and one asymptomatic control person with positive with *T. vaginalis*

Patients					
Sex	Age	Marital status	Specific symptoms	WBC count in urine/ HPF	Laboratory results
M	55	Married	Dysuria, pelvic pain, orchitis	15-20	+ve PCR -ve Microscopy -ve Culture
M	60	Married	Dysuria Recurrent urination,	0-2	+ve PCR -ve Microscopy -ve culture
M	55	Married	Not present	0-2	+ve PCR -ve Microscopy -ve culture
F	36	Married	Dysuria, pelvic pain	6-8	+ve PCR +ve Microscopy +ve culture

Table 3: Clinical and epidemiological features of 17 patients with positive *M. genitalium*

Patients	No. (%)
Total No.	17 (100)
Mean Age	39.9 years
Sex	
Males	11 (65)
Females	6 (35)
Marital status	
Married	13 (76)
Single	4 (24)
Urine microscopy	
Numerous WBCs (> 5/ HPF)	7 (41)
Few WBCs (1-4/ HPF)	10 (59)
Specific symptoms[a]	
Urinary frequency	13 (76)
Dysuria	10 (59))
Suprapubic/pelvic pain	6 (35)
Urethral discharge	1 (6)

[a]: Most patients (13/17, 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.

DISCUSSION

Information on the incidence and spectrum of clinical features associated with fastidious organisms like *C. trachomatis, M. genitalium and T. vaginalis*, is still rarely reported in Jordan and in most Middel 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[12-15].

Most symptomatic patients infected with *M. genitalium* (76%) have at least two specific symptoms; urinary frequency and dysuria, whereas about one third of the patients (36%) suffered from pelvic or suprapubic pain and only one patient has urethral discharge (Table 3). In addition, presence of few pus cells (59%, p>0.05) has be been detected more often than numerous pus cells (41%) in urine of infected symptomatic patients (Table 2 and 3). The spectrum of genitourinary symptoms and signs among our patients shows that clinical diagnosis and confirmation of both infections of *M. genitalium* and *T. vaginalis* require full clinical and laboratory investigations. A recent study performed in England, has shown that both urethritis and the presence of a urethral discharge and/or dysuria are significantly associated with the detection of *M. genitalium*[19]. This study also found like other studies that detection of *T. vaginalis* using first void urine specimens and PCR performed better than wet mount microscopy and culture, particularly in men with NGU[1,13,14,20].

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.

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