ORIGINAL ARTICLE

Role of *Mycoplasma genitalium* and *Ureaplasma urealyticum* in non-gonococcal urethritis in Hong Kong

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Objective To determine the association of *Mycoplasma genitalium* and *Ureaplasma urealyticum* in symptomatic male patients presenting with non-gonococcal urethritis in a sexually transmitted infection clinic in Hong Kong.

Design Cross-sectional study.

Setting A sexually transmitted infection clinic, Department of Health, Centre for Health Protection, Hong Kong.

Patients A cohort of consecutive new male patients attending the government sexually transmitted infection clinic.

Main outcome measures Prevalence of *Mycoplasma genitalium* and *Ureaplasma urealyticum* among symptomatic male patients with non-gonococcal urethritis and asymptomatic patients without non-gonococcal urethritis.

Results Specimens of 22 and 10 patients tested positive by polymerase chain reaction for *Ureaplasma urealyticum* and *Mycoplasma genitalium* respectively, among the symptomatic non-gonococcal urethritis group (n=98). In the asymptomatic control group (n=236), corresponding patient numbers whose specimens tested positive were 47 and 5. There was no statistically significant difference between the two groups, in terms of the proportion of patients infected with *Mycoplasma genitalium* (P=0.799) or *Ureaplasma urealyticum* (P=0.535).

Conclusions In our study, demonstration of *Mycoplasma genitalium* and *Ureaplasma urealyticum* by polymerase chain reaction was not associated with symptomatic non-gonococcal urethritis in male patients attending a Hong Kong government clinic for sexually transmitted infections.

Key words
Mycoplasma infections; Polymerase chain reaction; Sexually transmitted diseases; *Ureaplasma* infections; Urethritis

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Introduction

Urethral inflammation is the most common presentation among male patients attending government sexually transmitted infection (STI) clinics in Hong Kong. In 2006, among male attendees of Social Hygiene Clinic (SHC), 9572 were newly diagnosed to have an STI; among these 1413 (14.8%) and 4540 (47.4%) patients respectively, were found to have gonorrhoea and non-gonococcal urethritis (NGU).[1] Urethritis is classified as gonococcal or non-gonococcal depending on the presence or absence of *Neisseria gonorrhoeae* isolated as the aetiological agent. In the past, gonorrhoea was the commonest STI in Hong Kong, but more recently, it has been replaced by NGU. The most frequent cause of NGU is *Chlamydia trachomatis* (CT),[1] whilst other possible associations are identified less frequently and include *Mycoplasma genitalium* (MG),[2-9] *Ureaplasma urealyticum* (UU),[10,11] *Trichomonas vaginalis*,[12] *Neisseria meningitidis*, *Herpes simplex* virus, *Candida* species, bacterial urinary tract infection, urethral stricture, and foreign bodies.

Chlamydia trachomatis has been well established as a pathogen responsible for NGU in Hong Kong, but other aetiological agents are not well defined. The objective of this study was to determine the association of MG and UU in patients presenting with NGU in a government STI clinic in Hong Kong, by comparing the proportion of patients testing positive for these organisms among symptomatic patients with NGU and asymptomatic controls (without NGU).

生殖支原體和解脲支原體與非淋菌性尿道炎的關係：香港的情況

目的 確定在一家性病診所具非淋菌性尿道炎症狀的男性病人中，生殖支原體(*Mycoplasma genitalium*)和解脲支原體(*Ureaplasma urealyticum*)與非淋菌性尿道炎的關係。

設計 橫斷面研究。

安排 香港衛生署衛生防護中心的一家性病診所。

患者 到性病診所求診的男性病人所組成的連續病例。

主要結果測量 患非淋菌性尿道炎並具症狀的男性病人，以及沒有患非淋菌性尿道炎又不具症狀的病人，身體帶有生殖支原體和解脲支原體的普遍程度。

結果 在非淋菌性尿道炎並具症狀一組（共98人），22位病人的樣本對解脲支原體的聚合酶鏈反應測試呈陽性反應，10位病人的樣本對生殖支原體的聚合酶鏈反應測試呈陽性反應。在不具症狀的對照組（共236人），對測試呈陽性反應的樣本分別有47個和5個。非淋菌性尿道炎並具症狀一組與不具症狀的對照組之間，病人受生殖支原體(P=0.799)或解脲支原體(P=0.535)感染的比例並沒有統計學上的顯著差異。

結論 對香港一所政府性病診所的男性病人所做的聚合酶鏈反應測試研究顯示，生殖支原體和解脲支原體與具症狀的非淋菌性尿道炎並無相關性。

Methods

A cross-sectional study was conducted in a government STI clinic (Yung Fung Shee SHC, Kowloon), where consecutive new male patients were recruited to participate in the study from November 2005 to May 2006 (7 months). The study was carried out with the approval from the ethics committee of the Department of Health and informed consent of the patients. Patients under the age of 18 years, those who had received antibiotic therapy within 1 month prior to presentation, or had structural abnormality of the urogenital system were excluded. Those who were homosexual, or unable to establish effective and meaningful communication due to cognitive impairment, psychiatric illness, language barrier, or physical disability were also excluded.

A questionnaire was used to record the history and in particular symptoms of urethral discharge, dysuria, penile irritation, frequency, and testicular discomfort. In addition, the patient's age, ethnicity, age of first sexual intercourse, as well as history of prior STIs, casual sex in the past 12 months, history of new sexual partner in the past 3 months, and frequency and correct use of condoms during casual sex were recorded.

A full urogenital examination was performed to detect any STIs and the presence or absence of urethral discharge was specifically noted. When there was no visible discharge, the patient would be asked to massage the urethra and express any possible exudate. For every patient, the first author obtained the history and conducted the physical examination. Symptomatic patients were defined as those who presented with either: frequency, dysuria, discharge, urethral discomfort, or testicular discomfort or they had an observable urethral discharge upon physical examination with or without 'milking'. Asymptomatic patients were defined as those who did not complain of either of these symptoms, and had no observable urethral discharge upon physical examination.

For each recruit, the time they had last voided urine was noted. A urethral smear was obtained by a sterile inoculation loop and directly plated onto the specimen slide for Gram stain and Thayer-Martin medium for gonococcal culture. Only two designated research assistants at Yung Fung Shee SHC performed the microscopy in order to limit the inter-operator variability. The number of polymorphonuclear leukocytes (PMLs) with or without intracellular gonococci under a high-power (x1000) microscopic field over an average of five fields were recorded as <1, <5, 5-9, and 10-29. Patients were defined as having NGU if their (i) urethral smear for Gram stain showed five or more PMLs per high-power microscopic field, and (ii) their smears were negative for Gram-negative diplococci and cultures negative for *N gonorrhoeae*. Whereas, gonococcal urethral infection was diagnosed if Gram staining of the urethral smear revealed intracellular Gram-negative diplococci or were culture positive for *N gonorrhoeae*.

Subsequently, a thin cotton-tipped swab was inserted into the urethra to collect further exudates, and the swab was then shaken vigorously in the transit medium, which was then subjected to nucleic acid amplification testing for *Chlamydia* (COBAS Amplicor, Roche Diagnostic Systems, Inc, Branchburg, NJ). A first catch urine sample was obtained and tested for MG and UU by a technique involving real-time polymerase chain reaction (PCR). The DNA was extracted from 1 mL of urine using the NucliSens mini-MAG (Magnetic extraction) System (bioMérieux, The Netherlands). Two real-time PCR assays were used to differentially detect UU and MG. Real-time PCR assay of UU was specifically designed to target the urease gene by using two TaqMan minor groove binder primers and probe sets. Then MG was detected by a TaqMan minor groove binder real-time PCR assay, targeting a conserved region of the MgPa adhesion gene. Exogenous internal positive control (Applied Biosystems, CA) was added into both real-time PCR assays for monitoring PCR inhibition. All recruited patients were also tested for syphilis and human immunodeficiency virus. If STIs were detected, the patients were managed accordingly with appropriate treatment protocols and health counselling.

TABLE 1. Demographic and sexual behaviour data for the symptomatic non-gonococcal urethritis (NGU) group and asymptomatic controls

	Symptomatic NGU group (n=98)	Asymptomatic control group (n=236)	P value
Mean age (range) [years]	38 (18-75)	41 (18-87)	0.048*
Age of sexual debut (years)	20	21	0.027*
No. with a history of sexually transmitted infections	60 (61%)	59 (25%)	<0.001†
No. with a history of casual sex in the past 12 months	80 (82%)	173 (73%)	0.106†
No. with a history of new partner(s) in the past 3 months	69 (70%)	127 (54%)	0.005†
Always used a condom during casual sex in the past 3 months	43%	57%	0.064†
No. of correct use of condom	31 (32%)	90 (38%)	0.260†

* *t* test
† χ^2 test

TABLE 2. Numbers of patients with organisms detected by polymerase chain reaction in the non-gonococcal urethritis (NGU) group and asymptomatic controls

Organisms	Symptomatic NGU group (n=98)	Asymptomatic control group (n=236)	P value (with logistic regression)
Chlamydia trachomatis	49 (50%)	14 (6%)	<0.001
Ureaplasma urealyticum	22 (22%)	47 (20%)	0.535
Mycoplasma genitalium	10 (10%)	5 (2%)	0.799

By comparing the numbers and proportions of persons testing positive for MG and UU among NGU patients and those without, the significance of these two factors as causative agents in NGU could be explored. Data were entered and stored in Microsoft Access and analysed using the Statistical Package for the Social Sciences (Windows Version 13, SPSS Inc, Chicago, US). Proportions were compared using Pearson's Chi squared test. Logistic regression with confounding factors adjustment was used to test the association of CT, MG, and UU with NGU. Unpaired independent *t* tests were utilised for comparing continuous variables.

Results

A total of 507 patients were recruited into the study from 543 consecutive patients. Among the patients who were excluded, 20 had taken antibiotics in the previous month, five were homosexuals, six had indeterminate PCR results secondary to the presence of inhibitors, four posed a language barrier, and one had bladder extrophy.

The mean age of the patients was 40 (median 38; range, 18-87) years; 99% of whom were Chinese. There were 98 symptomatic patients (with NGU) and 236 asymptomatic controls; their mean ages were 38 and 41 years respectively. The mean age at first sexual intercourse for the symptomatic NGU group was 20 years, whereas for the asymptomatic controls, it was 21 years. In the symptomatic NGU group, a significantly greater proportion of patients than asymptomatic controls had a history of prior STIs and admitted to having a new partner in the past 3 months. In all, 72 and 138 patients respectively had casual sex within the past 3 months. Among the patients who had such casual sex in the past 3 months, 31 (43%) within the NGU group and 78 (57%) within the asymptomatic controls used a condom consistently. A summary of the demographic data and sexual behaviour pertaining to the two groups is shown in Table 1.

Within the symptomatic NGU group, the number of patients testing positive by PCR for CT, UU, and MG were 49, 22, and 10 respectively. Among these patients, 10 were co-infected with CT and UU and three were co-infected with CT and MG. Among the asymptomatic controls, the number of patients testing positive by PCR for CT, UU, and MG were 14, 47, and 5 respectively; and among these, seven were co-infected with CT and UU, one was co-infected with CT, MG, and UU. There was no statistically significant difference in the proportion of patients infected with MG (P=0.799) or UU (P=0.535) in the symptomatic NGU and asymptomatic control groups (Table 2).

Discussion

Chlamydia trachomatis is well established as a cause of NGU and this was confirmed again in the current study in which there was a significant difference between the symptomatic NGU and asymptomatic control groups (P<0.001). However, the relationship and association of NGU with UU and MG is still debated. In 1977, two volunteers self-inoculated UU onto their urethra and this produced symptomatic urethritis with PML in the urine and provided evidence that UU was pathogenic.[13] In the current study, among 98 patients with symptomatic NGU, UU

TABLE 3. Summary of recently published articles on the association of *Ureaplasma urealyticum* (UU) with non-gonococcal urethritis (NGU)

Paper	Symptomatic NGU cases	Asymptomatic controls	Detection of UU (symptomatic cases vs controls)
Present study, 2007, Hong Kong	98, ≥5 PML* on smear	236, <5 PML on smear	22% vs 20% (P=0.535)
Morency et al,[3] 2001, Central African Republic	136, ≥5 PML on smear	100	44% vs 48% (P>0.05)
Totten et al,[14] 2001, Seattle, US	121, ≥5 PML on smear	117, <5 PML on smear	35% vs 42% (P=0.34)
Pepin et al,[4] 2001, West Africa	659 with urethral discharge	339	26% vs 28% (P>0.05)
Deguchi et al,[10] 2004, Japan	317, ≥5 PML on smear	141, <5 PML on smear	16% vs 8% (P=0.025)
Bakare et al,[15] 2002, Nigeria	154 with NGU	Not specified	14% of cases (P<0.001)

* PML denotes polymorphonuclear leukocytes per high-power field

TABLE 4. Summary of recently published articles on the association of *Mycoplasma genitalium* (MG) with non-gonococcal urethritis (NGU)

Paper	Symptomatic NGU cases	Asymptomatic controls	Detection of MG (symptomatic cases vs controls)
Present study, 2007, Hong Kong	98, ≥5 PML* on smear	236, <5 PML on smear	10% vs 2% (P=0.799)
Leung et al,[9] 2006, UK	302, ≥5 PML on smear	378	11% vs 1% (P<0.001)
Mena et al,[2] 2002, New Orleans, US	97, ≥5 PML on smear	184	21% vs 8% (P<0.002)
Morency et al,[3] 2001, Central African Republic	136, ≥5 PML on smear	100	42% vs 15% (P<0.001)
Totten et al,[14] 2001, Seattle, US	121, ≥5 PML on smear	117, <5 PML on smear	22% vs 4% (P<0.01)
Pepin et al,[4] 2001, West Africa	659, urethral discharge	339	10% vs 9% (P=0.05)
Johannisson et al,[5] 2000, Sweden	115, ≥5 PML on smear	118, <5 PML on smear	15% vs 1% (P<0.001)
Busolo et al,[6] 1997, Italy	52, urethritis	44	12% vs 0% (P=0.03)
Janier et al,[7] 1995, Paris	100, with discharge	96, no discharge	29% vs 9% (P<0.001)
Horner et al,[8] 1993, UK	103, ≥5 PML on smear	53, <5 PML on smear	23% vs 6% (P=0.006)

* PML denotes polymorphonuclear leukocytes per high-power field

was found in 22%, whereas in the 236 asymptomatic patients without NGU, UU was found in 20%. More recently, findings from a number of studies[3,4,10,14,15] investigating the possible association of UU with NGU using PCR have been reported; their results were conflicting, but could not be compared directly as the selection of controls differed (Table 3).

Mycoplasma genitalium is the smallest free-living organism, widespread in nature and was first isolated in 1980 from two of 13 men with NGU.[16] Since the development of sensitive and specific PCR assays in the early 1990s, around the world a number of clinical studies[2-9,14] have shown a strong association between MG and NGU, independent of CT (Table 4). In the current local study, five (2%) of the 236 asymptomatic patients without NGU tested positive for MG, compared to 10 (10%) of the 98 symptomatic patients with NGU. However, this difference was not statistically significantly different when they were compared by logistic regression analysis (P=0.799; Table 2). As the prevalence of MG was relatively low in Hong Kong, a type II error may have been inadvertently introduced, so a larger study might help to clarify any possible association of MG with NGU.

One limitation of the current study was the considerable difference in the size of the symptomatic NGU and asymptomatic control groups, which was due to consecutive patient recruitment (the strategy adopted to reduce selection bias). It may have been more advantageous to compare groups of similar size and with similar sexual demographics. In addition, a larger study population would be more likely to compensate for several possible confounding factors. For example, the patients in the symptomatic NGU group were younger and had an earlier age at first sexual intercourse. They also tended to engage in more risky sexual behaviour, being more likely to have a new partner in the preceding 3 months and a history of prior STIs. In a sense, these differences were only to be expected, as this study involved a selected group of symptomatic patients with confirmed NGU to compare with asymptomatic patients without NGU. Men who had sex with men were not included in the current study to avoid further complicating the issues.

In conclusion, the current study did not demonstrate an association between MG or UU and NGU among symptomatic male patients attending a government STI clinic in Hong Kong. Nevertheless,

the use of condom which gives significant protection against other STIs like CT should be emphasised to the at-risk population.[17] Larger local studies to investigate NGU among male patients and their female sexual partners are needed in the future to better define the pathogenicity of MG and UU.

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