Agents of Non-Gonococcal Urethritis in Males Attending an Israeli Clinic for Sexually Transmitted Diseases

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Abstract

Background: Non-gonococcal urethritis is the most common clinical diagnosis in men seeking care at clinics for sexually transmitted diseases.

Objective: To identify the pathogens involved in NGU among males attending an Israeli STD clinic.

Methods: During 19 months spanning September 1996 to July 1998 we investigated a cohort of 238 male patients attending the Bnai Zion Medical Center STD clinic with a clinical presentation of urethritis. Intraurethral swab specimens were tested for *Neisseria gonorrhea, Ureaplasma urealyticum, Mycoplasma hominis*, and *Trichomonas vaginalis* by culture and for herpes simplex virus by antigen detection. First voiding urine for *C. trachomatis* was done by polymerase chain reaction. The specific seropositivities of HSV types 1 and 2 were tested by enzyme-linked immunosorbent assay.

Results: From among 238 males with dysuria or urethral discharge an etiology for urethritis was found for 71 (29.8%). *N. gonorrhea* was recovered in only three men (4.2%). In the remaining 68 NGU patients *Chlamydia trachomatis* (35/68, 51.5%) and *U. urealyticum* (31/68, 45.6%) were the most common infecting and co-infecting pathogens ($P < 0.0001$). *M. hominis* and *T. vaginalis* were found in 9/68 (13.2%), and 1 patient, respectively. HSV was recovered from the urethra in 7/68 males (10.3%) – 3 with HSV-1, 2 with HSV-2, and 2 were seronegative for HSV. None of these males had genital lesions. Although a single etiologic agent was identified in 45/68 infected men (66.2%), co-infection was common: 2 organisms in 15 (22%) and 3 organisms in 8 (11.8%).

Conclusion: *C. trachomatis* and *U. urealyticum* were the most common infecting and co-infecting pathogens in this cohort of men with NGU. Unrecognized genital HSV infections are common in males attending our STD clinic, and symptomatic shedding of HSV occurs without genital lesions. Still, the microbial etiology in this group remains unclear in many patients despite careful microbiologic evaluation.

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Non-gonococcal urethritis is among the most widespread conditions for which care is provided in the western world and is the most common diagnosis in men attending clinics for sexually transmitted diseases [1,2–5]. In 1972, gonorrhea was surpassed by NGU as the more common diagnosis made at private physician offices. Since then, the divergence between the two has progressively increased [6]. The morbidity and complications associated with NGU are known to be approximately equal in severity to those of gonococcal disease. However, in contrast to gonorrhea, the infectious agents causing NGU with the exception of *Chlamydia trachomatis* are non-reportable. Therefore, the sexual partners of NGU patients are mostly untreated. This may account for the rising incidence and relative incidence of NGU with respect to gonococcal disease [7].

NGU is manifest clinically by mucoid or purulent urethral discharge accompanied by dysuria and/or itching at the urethral meatus. In the majority of clinics its diagnosis is one of exclusion due to the failure to detect "typical" intracellular diplococci on a stained smear of urethral exudate. Reassessment the morning before the patient voids may be necessary. This is "satisfactory" to the extent that tetracycline is the regimen of choice for most NGU cases of undetermined etiology.

C. trachomatis has been recognized as the cause in 40–50% of NGU cases and is considered to be the most common of all sexually transmitted pathogens. Other causes of many *C. trachomatis*-negative cases of NGU include: *Ureaplasma urealyticum*, *Mycoplasma hominis*, and *Trichomonas vaginalis*. Genital herpes is largely unrecognized, both by physicians and patients. Among individuals who are HSV-2 seropositive, only a minority has recognized symptomatic disease. A very small proportion, estimated at 20% of patients with herpes infection, is correctly diagnosed as having genital herpes. In an ongoing study, we have microbiologically and clinically evaluated the etiology of NGU and their co-infecting pathogens in males attending an STD clinic in northern Israel.

Materials and Methods

The Bnai Zion Medical Center STD clinic provides care to approximately 350 patients per year from the metropolitan Haifa area and northern Israel. For a total of 19 months spanning September 1996 to July 1998 a cohort of 281 male patients attending this clinic was prospectively enrolled. These patients presented with urethritis manifested by urethral discharge, dysuria, or itching at the urethral meatus. Patients who had taken antibiotics within the previous 2 weeks or patients who had urinated within the previous 2 hours were not eligible for the study. After informed consent was obtained, a questionnaire addressing

NGU = non-gonococcal urethritis
STD = sexually transmitted disease
HSV = herpes simplex virus

Table 1. Techniques used in pathogen identification

Pathogen	Source of specimen	Diagnostic test
Neisseria gonorrhea	Urethra	Stained smear Selective culture (Hylabs, Rehovot, Israel)
Ureaplasma urealyticum	Urethra	Selective culture (Mycofast, France)
Chlamydia trachomatis	First catch urine	PCR (Amplicor, Roche Diagnostic Systems)
Trichomonas vaginalis	Urethra	Saline wet mount
Mycoplasma hominis	Urethra	Selective culture (Mycofast, France)
Herpes simplex virus	Urethra	Antigen detection ELISA (Dako, IDEIA, UK) HSV-1 & 2 IgG ELISA (Gull Laboratories, USA)

sexual behavior and symptoms suggesting an STD was administered. Among the 281 males, 43 were excluded either due to fitting the exclusion criteria previously stated or reluctance to consent. The cross-sectional study observed 238 males with a median age of 34 years. A presumptive diagnosis of NGU in men included the presence of four or more polymorphonuclear cells per oil immersion field of a gram-stained urethral smear or pyuria noted in urine analysis, and the absence of observed gram-negative diplococci. Our aim was to detect a new STD during this period. STDs included new episodes of gonorrhea, *C. trachomatis*, *U. urealyticum*, *M. hominis*, *T. vaginalis*, and herpes simplex infection.

Specimen collection and storage [Table 1]

All patients underwent a physical examination and collection of genitourinary specimens. Four urethral swab and discharge specimens were obtained after a prostate massage by inserting a narrow shafted Dacron-tipped swab 2–3 cm into the urethra. The swabs were placed into a transport vial (Copan, Italy). The specimens were transported at room temperature and were immediately processed. The first swab was inoculated on Thayer Martin Media (Hylabs, Rehovot, Israel) for gonococcal culturing. Gram-negative diplococci were confirmed as *N. gonorrhea* by glucose utilization profiles (Api-NH, bioMérieux, France). The second swab was used for detection of *U. urealyticum* and *M. hominis* (Mycofast International Mycoplasma, France). The third swab was used for direct detection of *T. vaginalis* by wet mount (Hylabs, Rehovot). The fourth swab was used for HSV antigen detection (Dako, IDEIA, UK).

In addition, 30–50 ml of first-catch urine was collected from each patient. A 500 ml aliquot was stored at 4°C for up to 7 days from the time of collection until it was processed for polymerase chain reaction testing for *C. trachomatis* (COBAS AMPLICOR, Roche, USA) [Table 1].

HSV-specific antibodies

We examined blood samples from all patients positive for HSV antigen for the presence of specific antibodies against HSV-1 and HSV-2 using an enzyme immunoassay (EIA-gG; Gull, USA). This examination confirmed the presence of specific viral glycoprotein, gG, which distinguishes between HSV-1 and HSV-2 species.

Table 2. Pathogens recovered in male patients with NGU.

Pathogen	No. of patients with NGU in which pathogen was found			
	Overall	Single (45 cases)	Double (15 cases)	Triple (8 cases)
Chlamydia trachomatis	35 (51.5%)	23	8	4
Ureaplasma urealyticum	31 (45.6%)	17	7	7
Mycoplasma hominis	9 (13.2%)	4	3	2
Trichomonas vaginalis	1 (1.5%)	1	0	0
HSV	7 (10.3%)	0	5	2
HBV	2 (2.9%)	0	1	1
Gardenella vaginalis	2 (2.9%)	0	2	0
Candida albicans	2 (2.9%)	0	2	0
Treponema pallidum	2 (2.9%)	0	2	0
Human immunodeficiency virus	0	0	0	0

Statistical analysis

Univariate analysis for testing the associations between categorical groups was performed using chi-square or Fisher's exact test, when appropriate. A P value less than 0.05 was considered statistically significant. Results were expressed as numbers and percentages of patients.

Results

A total of 238 urethral, first-voiding urine, and serum specimens were available for laboratory evaluation. The median age and range of patients was 34.0 $\pm$ 10 years. The patients included 179 Israeli Jews (75%), 38 Israeli Arabs (16%), and 21 foreigners (9%). Ninety patients (38%) had had two or less sexual partners in the previous 6 months. All but two patients were heterosexual; two males were bisexual. Of the total number of patients, 147 (62%) reported that they seldom use condoms.

Among the 238 male patients with symptoms of urethritis, an etiology was found for 71 (29.8%). In three of these patients (4.2%) gram-negative intracellular diplococci were observed on urethral smears and/or *N. gonorrhea* was isolated on selective cultures, whereas in 68 of the 71 patients (95.8%), the typical diplococci were neither found on a urethral smear nor isolated in culture. Although a single agent was identified in 45 of the infected patients with NGU (66.2%), co-infection with other sexually transmitted organisms, such as HSV, *Treponema pallidum*, and hepatitis B virus was common [Table 2]. Two infectious organisms were isolated in 15 of the 68 patients (22%), and three microorganisms were isolated in 8 (11.8%). *C. trachomatis* and *U. urealyticum* were the most common infecting and co-infecting pathogens ($P < 0.0001$). *C. trachomatis* was detected in 35/68 (51.5%), *U. urealyticum* in 31/68 (45.6%), *M. hominis* in 9/68 (13.2%) and *T. vaginalis* in 1/68 (1.5%). Using antigen detection, HSV was found in 7/68 patients (10.3%); none of them had a history of genital lesions. Three of these males were seropositive for HSV-1, two for HSV-2, and two were seronegative for both HSV-1 and HSV-2.

Discussion

In this cohort of males with NGU the most common infecting and co-infecting pathogens were *C. trachomatis* and *U. urealyticum*. This

finding is consistent with current reports [4]. Recent findings have proposed that the proportion of NGU cases caused by *C. trachomatis* and *U. urealyticum* is actually lower than reported levels [8–10]. This could be attributed to the use of new and more sensitive isolation techniques such as nucleic acid amplification and to better control programs. In other words, as technology allows us to more accurately isolate the pathogen in question, the literature will grow ever closer to the true relative proportions of different microbes. In our cohort, the proportions of *C. trachomatis* and *U. urealyticum* remain relatively high. Since we employ the PCR for detection of *C. trachomatis*, this discrepancy is most likely due to the lack of screening and control programs in Israel.

When examined separately, *C. trachomatis* is known to be the most important etiologic agent causing NGU [11], and is responsible for 30–50% of NGU cases [12–15]. In 25–60% of heterosexual men with NGU, *C. trachomatis* was recovered from the urethra [16]. *C. trachomatis* was the most prevalent pathogen associated with urethritis in this cohort of men, both as a single agent and in association with others in multiple infection [5]. *C. trachomatis* is recognized as the most common bacterial cause of STDs. This is due both to the asymptomatic natural history in both men and women and to its persistence in genitourinary infections [5].

Our findings indicate that the next most common pathogen involved in NGU is *U. urealyticum*, which reflects the existing literature [14]. This attests that 20–50% of men with NGU are infected with *U. urealyticum*. Findings by Bowie et al. [17] suggest that antibiotic treatment may play an important role in the pathogenesis. This is only one of numerous explanations for the presence and pathogenesis of *U. urealyticum* in NGU patients. However, this information has been challenging to construe because urethral colonization with *U. urealyticum* has been found to be directly proportional to the patient's degree of sexual promiscuity [18]. Furthermore, other investigators have found that the presence of *U. urealyticum* in patients with NGU equals that in men without any clinical disease [17]. In contrast, one study found an increased presence of *U. urealyticum* in men with urethritis, which was even more evident in men with *Chlamydia*-negative NGU compared to men with *Chlamydia*-positive NGU [19]. In contrast, *M. hominis* and *T. vaginalis* were relatively uncommon. Among others, these pathogens have been examined for their role in NGU and appear to account for the minority of cases of NGU, especially with regard to single-pathogen etiology [20]. Furthermore, relative frequencies vary among different observers. This may be a function of geography and/or isolation techniques.

As mentioned above, NGU is the most common clinical diagnosis for men seeking care at STD clinics. In 1972, gonorrhea was surpassed by NGU as the more common diagnosis made at private physician offices. Although the complications of NGU are rare in men, infection of the female partner may have devastating long-term consequences, such as chronic pelvic pain, pelvic inflammatory disease, tubal infertility, and ectopic pregnancy. Consequently, it is of prime importance that an etiologic agent be detected early to allow for appropriate and immediate therapy, which will reduce complications. However, our findings as well as the current standards indicate that in the majority of NGU cases no pathogen was detectable. The current literature suggests that in 20–30% of men with NGU, no organism was isolated despite exhaustive efforts to grow them [21,22]. Some research groups report the presence of rare pathogens such as *Haemophilus equigenitalis* and *Mycoplasma* species [22,23]. However, direct relationships remain unclear. There is a clear discrepancy between the frequency of our cases with no isolated organism and the literature. We found a significantly greater proportion of patients of this type. This discordance may be accounted for by geographic differences. Within the category of geography there are nutritional and antibiotic differences that may influence the host susceptibility to certain organisms. Additional geographic effects may also be seen at the level of the microbial location, whereas different species of bacteria exist at different proportions across the globe. Additionally, these discrepancies may be attributed to differences in the diagnostic tests and techniques used, as well as to variability among manufacturers. Furthermore, our exclusion criteria disqualified patients who had been taking antibiotics within 2 weeks of the study. This cut-off may not have been sufficient with respect to time. All of these factors may account for the decreased proportion of our NGU cases for which a pathogen was isolated.

Furthermore, while early and accurate detection for the purpose of proper and immediate antimicrobial treatment receives most of the research attention, education is paramount in the battle against STDs. Efforts should be made to educate both the patient and the sexual partners, both previous and future, with regard to condom use and other forms of prophylaxis such as abstinence. Moreover, specific health personnel should be employed to take on this easy yet overlooked task.

Since unrecognized genital HSV infections are common in males attending our STD clinic, genital HSV infection should be considered in the evaluation of males with NGU. As we previously reported [24], symptomatic shedding of HSV can occur without the presence of genital ulcers or a previous history of genital lesion. Antibody assays performed on serum can now correctly distinguish past HSV-1 from past HSV-2 infection. Individuals who are carriers of infection or who acquired HSV-2 in the past can be identified by these assays. Two of the seven HSV NGU patients were seronegative for both HSV-1 and HSV-2, which may be due to the reduced sensitivity of serology during the acute phase of infection.

Our data suggest that the microbial etiology of NGU still remains unclear in many cases despite careful microbiologic evaluation. The importance of clarifying the etiology of NGU is obvious since it may contribute to new approaches of antibiotic therapy, reducing morbidity and improving quality of life. For the treatment of NGU, we suggest a combination of third-generation cephalosporins and doxycycline. This medical treatment has not been changed by the findings reported here. However, due to the low percentage of cases in which a pathogen was isolated, we are more inclined to reserve antibiotic therapy for those cases with positive cultures. Furthermore, since NGU is among the most

PCR = polymerase chain reaction

prevalent conditions for which care is provided in the western world and the most common clinical diagnosis in men seeking care at STD clinics, the economic impact of this condition is astounding [3,4,25]. Etiologic clarification can more accurately align antibiotic treatment, which in turn will reduce primary spending as well as reduce re-infectivity and complications, which secondarily reduces spending.

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