

The Role of *Ureaplasma urealyticum* in male non-gonococcal urethritis in Tabriz

E. Ebrahimi¹ Msc. In Microbiology sahand30@yahoo.com

Mohsen Amjadi² M.D. Associate Prof. of Urology amjadizm@yahoo.com

¹ Department of Medical Microbiology, ² Department of Urology, Imam Khomeini hospital, Faculty of Medicine; Tabriz University of Medical Science, Tabriz, I.R. Iran

Objective: The aim of the current study was to assess the incidence of *U. urealyticum* and *Mycoplasma hominis* in men with nongonococcal urethritis in Tabriz.

Materials and Methods: Two hundred men with confirmed urethritis and 50 healthy men of similar age as control group were investigated for *Ureaplasma urealyticum* at the medical microbiology laboratory of Sina and Emam hospitals of Tabriz University between 1st October 2003 and 30th April 2004.

Results: Fifty six (28%) of the 200 patients had gonococcal urethritis whilst 144 (72%) had non-gonococcal urethritis. Twenty nine (20.14%) of specimens showed urea positive organisms in broth cultures which in incubation on solid medium, 27 (18.75%) of them were identified as *U. urealyticum*. *Mycoplasma hominis* was detected in only five cases (3.47%). In wet preparation three (2.08%) *Trichomonas vaginalis* were observed. The difference in the occurrence of *U. urealyticum* between the patients and the control group was highly statistically significant ($p < 0.001$). The age range of peak incidence among the patients investigated was 26-35 years. There was evidence of urethritis in all the 27 *U. urealyticum* positive cases as shown by the presence of increase in the number of polymorphonuclear leucocytes in the specimens collected.

Conclusion: Isolation of 27(18.75%) *U. urealyticum* among studied population showed that this organism has comparatively high prevalence in patients with non-gonococcal urethritis.

Key words: *Ureaplasma urealyticum*, non- gonococcal urethritis, *Mycoplasma hominis*

Introduction

Mycoplasmas are the smallest identified free-living organisms that comprise a large group of microorganisms widespread in nature and are unique among prokaryotes in that they lack a cell wall. This feature is largely responsible for their biologic properties, including lack of a Gram stain reaction and non-susceptibility to commonly prescribed antimicrobial agents, including beta-lactams¹. They can be isolated as commensals or pathogens from plants, insects, animals and humans, some of them are considered normal flora of the respiratory or genitourinary tract. Among 17 species isolated from human seven species of mycoplasmas can be isolated from genitourinary tract but only three species of *M. hominis*, *U. urealyticum* and recently discovered *Mycoplasma genitalium* have been implicated in human disease.² They are referred to as “sexual mycoplasmas”, as they cause the infection via sexual contacts.

In humans, both mycoplasma and ureaplasma species may be transmitted by direct contact between hosts (ie, venereally through genital-to-genital or oral-to-genital contact), vertically from mother to offspring (either at birth or in utero), or by nosocomial acquisition through transplanted tissues³. The existence of *U. urealyticum* (formerly known as T-mycoplasma) in human was first recognized by Shepard in 1956, who described in details the morphologic and cultural characteristics of this microorganisms.⁴ Since then, *U. urealyticum* is incriminated in various diseases like nongonococcal urethritis (NGU)^{5, 6}, prostatitis⁷, epididymitis⁸, bacterial vaginosis⁹, acute pyelonephritis in males¹⁰.and etc. There is also evidence that they may play a role in infertility¹¹. The aim of the current study was to assess the incidence of *U. urealyticum* and *M. hominis* in men with nongonococcal urethritis in Tabriz.

Material and methods

The study group consisted of 200 male outpatients aged 19-49 years, with clinical symptoms of the genitourinary tract or urethral discomfort and 50 healthy men of similar age as control group, who were referred to the medical microbiology laboratory from the department of Urology in Emam and Sina hospitals of Tabriz Medical University. Between 1st October 2003 and 30th April 2004 from 183 of patients and all of control group, urethral discharge, and from 17 patients, fresh urine samples in addition to urethral discharge were collected (these patients had signs of urethritis but not enough discharges). Urethral swabs were preferred over urine samples for detection of genital mycoplasmal infections because the organisms are cell associated and calcium alginate swabs, not wooden cotton-tipped swabs, were used because the latter may inhibit growth of mycoplasma and ureaplasma organisms. Stuart's transport medium was used wherever necessary. The urine samples were centrifuged at 3000 rpm for 10 minutes and urine sediment was used for examination. Processing of the samples was done as follows: Wet preparation method for polymorphonuclear cells and *T. vaginalis*, Gram's staining for *Neisseria gonorrhoeae* and candida and Giemsa's staining for inclusion bodies of *Chlamydia trachomatis* were carried out. The samples were cultured on Thayer-Martin medium, McConkey's agar, Sabouraud's agar, PPLO broth supplemented with arginine for (*M. hominis*) at pH 7 and PPLO broth supplemented with urea (for *U. urealyticum*) at pH 6-6.5. After inoculation of PPLO broth medium, culture were mixed immediately and filtered through a 0.45µm pore size filter into another 2ml PPLO broth with urea for *U. urealyticum*. A-8 agar medium (commercially available media, Remel, A - 8 Agar, Selective REF: R20204) was used to study the colonies of *U. urealyticum*. The liquid media were examined after 48 hours. For *U. urealyticum*, as soon as the color change in the PPLO broth was noticed a sub-culturing into another broth and on A8 agar was done.^{12, 13} Simultaneously, the presence of urea splitting organisms like proteus and klebsiella was ruled out by examining McConkey's medium. Microbiology techniques such as Diens, Cresyl-fast violet and Geimsa staining, MnCl₂ (0.8%) coupled with urea (1%) were used to identify mycoplasmas and ureaplasmas.³ Cultural and serological tests studies for chlamydia were not done. Both *M hominis* and *U urealyticum* can be detected in culture within 2-5 days.

Results

Samples (urethral discharge and urine sediments) showing 4 or more polymorphonuclear cells (PMN) per high power field (hpf) were included in the present study. Patients with urethral cultures and Gram stain negative for gonococci were accepted as NGU. In 56 (28%) samples, N. gonorrhoeae was isolated. Out of

144 (72%) cases of NGU, 29 (20.14%) of them were urea positive which in incubation on a solid medium, 27(18.75%) grew completely and established colonies (figure) and then identified as an *U. urealyticum*. In five (3.47%) cases *M. hominis* and in three (2.08%) cases *T. vaginalis* were also detected. In control group, only in three samples (6%) *U. urealyticum* was isolated. The difference in the occurrence of *U. urealyticum* between the patients and the control group was highly statistically significant ($p < 0.001$). The age range of peak incidence among the patients investigated was 26-35 years (Table).

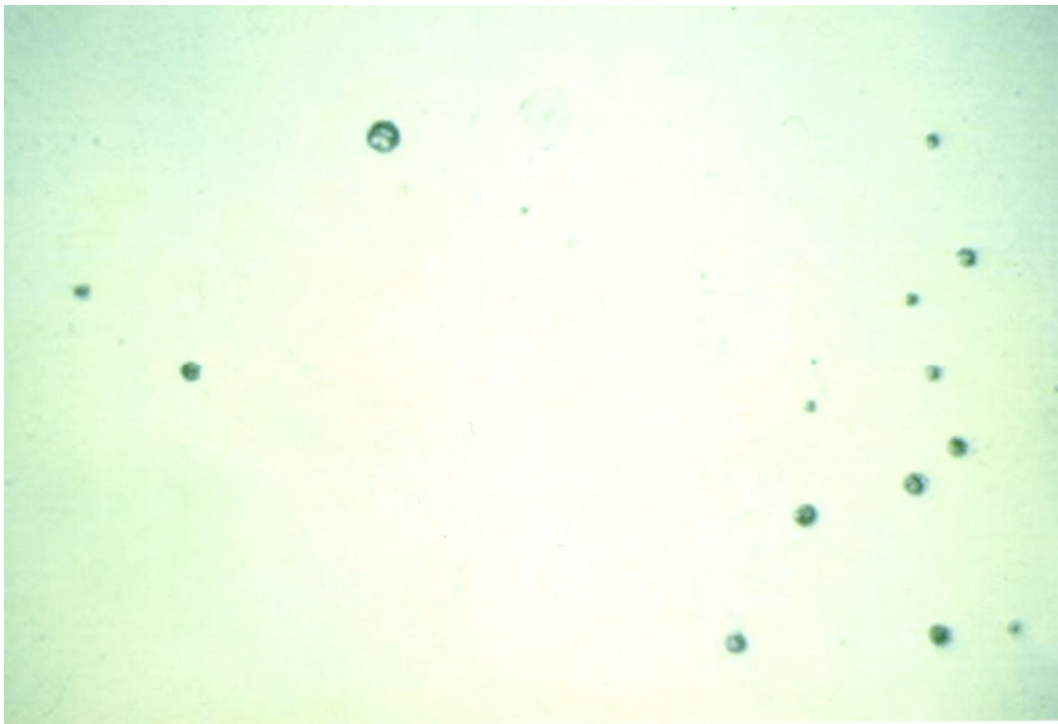


Figure: Colonies of *U. urealyticum* on A-8 agar medium stained with Diens's method.

Table : Incidence of *U. urealyticum* and *M. hominis* regarding age of patients

Age	<i>U. urealyticum</i>		<i>M. hominis</i>	
	n	%	n	%
19 (n = 8)	1	3.70	0	0
20-24 (n =21)	4	14.82	0	0
25-29 (n =27)	6	22.22	1	20
30-34 (n =36)	8	29.63	0	0
35-39 (n =47)	3	11.11	3	60
40-44 (n =44)	3	11.11	0	0
45-49 (n =17)	2	7.41	1	0
Total (n =200)	27	(100)	5	(100)

Discussion

Sexually transmitted diseases in men such as gonorrhoea and nongonococcal urethritis are endemic at high levels in most regions of the world, despite effective methods of diagnosis and treatment. NGU is a milder disease with a longer incubation period and longer duration of symptoms before treatment. One of the first sexually transmitted diseases to be associated with mycoplasmal infection is non-gonococcal urethritis in men and *U. urealyticum* is one of the most common and important aetiological factor of NGU.⁶ This has been confirmed by other researchers^(14, 15). Also Varela et al. have shown that there has been a growing incidence of *U. urealyticum*-induced urethritis in men recently¹⁶. NGU is about twice as common as gonorrhoea in Western countries, and on the basis of national statistics from Great Britain, its incidence has been thought to be increasing more rapidly¹⁷. *U. urealyticum* involvement in chronic NGU has been emphasized¹⁸. Horner et al. have demonstrated that acute urethritis is associated with *M. genitalium* and *C. trachomatis* infection, but not with *U. urealyticum*¹⁹. In a study by McKee et al. which involved 400 American soldiers with the symptoms of urethritis, *U. urealyticum* was isolated in 19%²⁰. Chandeying et al. performed microbiological examination of urethral swabs collected from 479 students in southern Thailand, detecting *U. urealyticum* in 10.9% and *M. hominis* in 1.3% of the patients²¹. The incidence of *U. urealyticum* in the present study was 18.75 and six per cent in the control group respectively which are comparable with those of earlier investigators^(21,6). This difference in the occurrence of *U. urealyticum* between the patients and the control group was highly statistically significant ($p < 0.001$). Most common cause of nongonococcal and nonchlamydial urethritis; characterized by urethral discharge, dysuria and meatal swelling can be associated with *U. urealyticum*. Yan ZH et al,²² in a similar study, have reported an incidence of 70.7 per cent, while Srugo et al²³ have reported 45.6.1% isolation of *U. urealyticum* in NGU. *U. urealyticum* is often present in non-gonococcal urethritis, accounting for 10%–20% of cases^(21, 24, 3, 8), and these results agree with our findings (19.2%). It is clear that most *Mycoplasma* spp. cannot be considered as important causes of nongonococcal urethritis because they are isolated so rarely from the genitourinary tract in either healthy or diseased states. Although *M. hominis* may be isolated from up to 30% of patients, our findings (3.47%) and many other studies have failed to implicate it as a cause of non-gonococcal urethritis^(21, 8, 24). However, the results of our study provide further evidence for an etiologic role of *U. urealyticum* in men with non-gonococcal urethritis. The majority of sexual mycoplasmas - positive results were obtained in men aged 25-29 years (25.93%) and 30-34 years (22.22%). According to literature data, young and sexually active men are most frequently affected²⁵. Similar results were obtained by Bakare et al⁶. Isolation of 27(18.75%) *U. urealyticum* among studied population showed that this organism is frequently found in the genitourinary tract in men with urethritis and has comparatively high prevalence in patients with non-gonococcal urethritis.

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