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ORIGINAL ARTICLE

Mycoplasmas in semen of chronic prostatitis patients

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Abstract

Objective. To evaluate the occurrence of mycoplasmas in the semen of chronic prostatitis patients. *Material and methods*. Genital mycoplasmas (Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum, Ureaplasma parvum) were sought in the semen of 121 chronic prostatitis patients [38 National Institutes of Health (NIH) category IIIa, 59 NIH category IIIb and 24 NIH category IV] and 40 controls. The commercially available kit Mycoplasma IST was applied to the semen samples of all 161 men, and polymerase chain reaction (PCR) to those of 60 randomly selected men. *Results*. Ureaplasmas were found in all study groups (at frequencies ranging from 12% to 25%) using the Mycoplasma IST test, but M. hominis was found only in one NIH category IIIb patient. Using PCR, most of the ureaplasmas appeared to be U. parvum, which was found in all prostatitis groups (18% of NIH category IIIa, 15% of NIH category IIIb and 25% of NIH category IV patients) but not in the controls. M. genitalium was found in 18% of the NIH category IIIa patients. All of the mycoplasmas occurred significantly more frequently in prostatitis patients than in controls and in NIH category IIIa patients than in controls. *Conclusion*. Mycoplasmas occur more frequently in the semen of prostatitis patients than in that of healthy controls, with U. parvum being the most frequently occurring species.

Key Words: *Mycoplasma genitalium, prostatitis, semen, Ureaplasma parvum*

Introduction

Prostatitis is a serious clinical problem, affecting half of all men at some time in their lives. However, its etiology is unknown in >90% of patients, and therefore treatment is frequently empirical and unsatisfactory. In the great majority of studies, only aerobic culture detection in prostate-specific specimens is used, although several fastidious or non-culturable microorganisms may also be important in the etiology of prostatitis [1].

Mycoplasmas are the smallest free-living organisms, which are widespread in nature. They are most unusual self-replicating bacteria, possessing very small genomes, lacking cell-wall components and displaying a genetic economy that requires a strict dependence on the host for nutrients and refuge. They are commensals or benign pathogens, causing mostly mild and chronic infections [2,3]. In recent years mycoplasmas have been associated with prostatitis syndromes. However, their actual role is still a matter of debate.

In this study we detected mycoplasmas in the semen of chronic prostatitis patients using two different methods.

Material and methods

Study group

The study was carried out at Tartu University Clinicum. The study group comprised 161 men who participated in a prospective study of the etiopathogenesis of chronic prostatitis: 38 men with inflammatory chronic prostatitis/chronic pelvic pain syndrome [National Institutes of Health (NIH) category IIIa], 59 with non-inflammatory chronic prostatitis/chronic pelvic pain syndrome (NIH category IIIb), 24 with asymptomatic inflammatory prostatitis (NIH category IV) and 40 controls. The

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study was approved by the Ethics Review Committee on Human Research of the University of Tartu. The study design and selection of patients have been described elsewhere [4,5].

Samples

Semen samples were collected by the patients following washing of the glans penis with soap and water and urinating. The samples were obtained by masturbation and ejaculated into a sterile collection tube in a private room near the laboratories. After ejaculation, the semen was incubated at 37°C for 25–45 min for liquefaction.

Semen samples (0.1 ml) were inoculated onto the bioMérieux transport medium R1 provided with the commercially available Mycoplasma IST kit (bioMérieux, Marcy L'etoile, France), immediately transported to the microbiology laboratory and processed within 1 h. Another portion of the semen was frozen (−20°C) for polymerase chain reaction (PCR) analysis.

Microbiological analyses

The Mycoplasma IST kit was applied to the semen samples of all 161 men to investigate Mycoplasma hominis and Ureaplasma urealyticum according to the manufacturer's instructions, as described previously [6].

PCR was additionally used in the semen samples of 60 randomly selected men to investigate Mycoplasma genitalium, Ureaplasma parvum and U. urealyticum. DNA was extracted from 200 µl of semen using the High Pure PCR Template Preparation Kit (Roche Biochemicals), and 10 µl of extracted DNA was used for PCR.

Primers MgPa1 and MgPa3 were used for specific M. genitalium genome amplification; they amplify a 281-bp segment of the 140-kDa adhesion protein gene [7]. The cycling parameters were as follows: 95°C for 2 min; 40 cycles at 95°C for 30 s, 65°C for 30 s and 72°C for 25 s; and 72°C for 5 min. Primers UMS-125 and UMA-226 were used for specific U. parvum genome amplification; they amplify a 403-bp segment of the multiple-banded antigen gene [8]. Cycling parameters were as follows: 95°C for 2 min 30 s; 40 cycles at 95°C for 40 s, 60°C for 50 s and 72°C for 40 s; and 72°C for 5 min. PCRs were carried out using the thermal cycler Mastercycler (Eppendorf). Recombinant Taq DNA Polymerase (Fermentas) was used.

Primers P6 and U8 were used for specific U. urealyticum genome amplification; they amplify a 1300-bp segment of the 16S rRNA gene [9]. Cycling parameters were as follows: 95°C for 3 min 30 s; 40 cycles at 95°C for 1 min, 56°C for 1 min 20 s and 72°C for 2 min; and 72°C for 5 min. PCRs were carried out using the thermal cycler RoboCycler Gradient 40 (Stratagene). Recombinant Taq DNA Polymerase (Fermentas) was used.

The PCR products were separated by electrophoresis in a 2% agarose gel and visualized under UV light with ethidium bromide.

Statistical methods

Fisher's exact test and logistic regression analysis were used to compare the occurrence of mycoplasmas between the different study groups. Cohen's kappa coefficient (κ) for diagnostic agreement was used to compare the two methods. $p \leq 0.05$ was considered significant.

Results

Using the Mycoplasma IST test, we found ureaplasmas in all groups studied (Table I). M. hominis was found out a low count ($<10^4$/ml) in only one NIH category IIIb patient.

Using PCR, most of the ureaplasmas found using the IST test were re-identified as U. parvum, which appeared to be the most frequent species. It was found in all the prostatitis groups but not in the controls. One patient in NIH category IV had both ureaplasma species. M. genitalium occurred only in NIH category IIIa patients. All differences between the groups were slightly above the significance level (Table I).

Further analysis involved only the PCR-confirmed mycoplasmas. All of the mycoplasmas as well as U. parvum occurred significantly more frequently in the prostatitis patients than in the controls (Figure 1). All of the mycoplasmas were also found more frequently in the NIH category IIIa patients in comparison with the controls (4/11 vs 1/25; $p = 0.023$).

We found substantial agreement between the two methods used for the genus Ureaplasma: in 48/60 men both tests were negative and in 7/60 both tests were positive ($\kappa = 0.69$, $p = 0.0007$). However, in four men, the IST test gave a positive result but PCR was negative, and in one man, the IST test was negative but PCR gave a positive result.

Discussion

A quarter of chronic prostatitis patients harbored PCR-confirmed mycoplasmas in their semen. This proportion was even higher in the case of inflammatory chronic prostatitis/chronic pelvic pain syndrome (NIH category IIIa) patients, one-third of whom

Table I. Occurrence of mycoplasmas in semen.

Test method	Microorganism	Positive specimens; *n* (%)			
		NIH IIIa	NIH IIIb	NIH IV	Controls
Mycoplasma IST test	Ureaplasma spp.	8/38 (21)	10/59 (17)	6/24 (25)	5/40 (12)
	M. hominis	0/38 (0)	1/59 (2)	0/24 (0)	0/40 (0)
PCR	U. urealyticum	0/11 (0)	1/20 (5)	1/4 (25)	1/25 (4)
	U. parvum	2/11 (18)*	3/20 (15)***	1/4 (25)	0/25 (0)*,***
	M. genitalium	2/11 (18)**	0/20 (0)	0/20 (0)	0/25 (0)**

*,**$p = 0.087$; ***$p = 0.080$; $p > 0.1$ for all other comparisons (Fisher's exact test).

were colonized by mycoplasmas. Mycoplasmas were present in only 1/25 healthy controls. The most frequent species in prostatitis patients was U. parvum, which was never found in the controls. M. genitalium occurred only in NIH category IIIa patients.

Previous investigators have found numerous mycoplasma species in humans. For certain species such as Ureaplasma spp., M. hominis and M. genitalium, the genital tract is thought to be the main site of colonization [2,3]. We found ureaplasmas in nearly 20% of prostatitis patients and in 12% of controls using the Mycoplasma IST test. U. urealyticum has been shown to be the most widespread species in the genital tract of both sexes, its reported prevalence in human semen varying from 10% to 40% [10]. U. urealyticum has been related to non-gonococcal urethritis and prostatitis but it also quite frequently colonizes asymptomatic men [3,11]. However, in most previous studies, no distinction has been made between U. urealyticum and U. parvum. U. parvum (formerly U. urealyticum biovar 1) was only recently separated from U. urealyticum [12], and it has been shown in some studies that most ureaplasmas in semen may actually be U. parvum [13]. A similar tendency could also be seen in our study, where U. parvum occurred more frequently among PCR-confirmed mycoplasmas than U. urealyticum and, interestingly, it was present only in prostatitis patients.

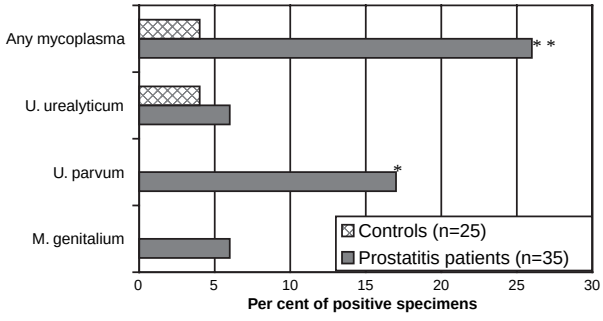

Figure 1. Statistical significance of occurrence of mycoplasmas in relation to prostatitis. *$p = 0.032$ (Fisher's exact test); **$p = 0.026$ (Fisher's exact test), $p = 0.029$ (logistic regression analysis).

M. genitalium has been found to be a probable cause of non-gonococcal urethritis [3,14], and is also associated with prostatitis [1], although some contradictory opinions can be found as well [15]. M. genitalium can affect fertility as it was shown to adhere to the spermatozoa, which became immotile when many M. genitalium were attached [16]. In our study this species was associated with NIH category IIIa prostatitis patients. In contrast, M. hominis, which is generally associated with female rather than male genital tract infections [3], was found in a low concentration in one patient only.

Although we found substantial agreement between the two methods used to detect the genus Ureaplasma, the IST test does not enable one to differentiate between U. urealyticum and U. parvum. As most of the ureaplasmas in our study appeared to be U. parvum, and this species was present only in prostatitis patients, there is an argument for using the PCR method instead of the Mycoplasma IST test. In addition, the IST test gave a false-positive result in four cases. A similar situation has been discussed by Stellrecht et al. [17], who found some culture-positive but PCR-negative semen specimens, although they cultured mycoplasmas on A7 agar instead of using the Mycoplasma IST test. In our study, the false-positive results may have been caused by other urease-positive microorganisms, as the IST test detects ureaplasmas by means of this enzyme. It was possible to confirm this hypothesis in three out of four cases by means of routine microbiological analysis (data not shown).

We conclude that mycoplasmas occur more frequently in the semen of prostatitis patients than in that of healthy controls, with U. parvum being the most frequently occurring species.

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