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Lithogenic Potential of *Ureaplasma* in Chronic Prostatitis

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Ureaplasma urealyticum · Chronic prostatitis · Prostate · Calcifications

Abstract
Introduction: The role of *Ureaplasma* spp. (UPs) in the pathogenesis of chronic prostatitis is debated. The lithogenic potential of UPs could be a risk factor for the development of chronic prostatitis. ***Methods:*** A total of 143 patients with identification of UPs were retrospectively selected from a database including patients with prostatitis-like symptoms who were studied according to the same protocol including clinical, microbiological and microscopic evaluation, and transrectal prostate ultrasound. A control group of patients with negative UPs was considered including 393 with chronic bacterial prostatitis (CBP), 42 patients with *Chlamydia trachomatis* (CT), and 781 patients with chronic pelvic pain syndrome. UPs and *Mycoplasma hominis* (MH) were identified using a semiquantitative assay. ***Results:*** Calcifications were observed more frequently in patients with UPs (64%) than in patients with CBP without UPs (39%), CT infection (37%), and chronic pelvic pain syndrome (29%) ($p < 0.0001$). UPs were isolated in VB1 alone in 35 patients (urethral UPs), in expressed prostatic secretion (EPS) or post-massage urine (VB3) or sperm in 77 patients (prostatic UPs) and associated with other pathogens in 31 patients (associated UPs). Calcifications were more frequent in prostatic UPs (71%) and associated UPs (73%) than in urethral UPs (34%). Mean NIH-CPSI scores were not significantly different between groups, although mean WBC counts of sperm of patients with urethral UPs were significantly lower than in patients with prostatic UPs ($p = 0.000$) and associated UPs ($p = 0.002$). ***Conclusions:*** UPs identification in the urogenital fluids is related to higher rates of prostate calcifications. The ability of UPs to promote the formation of calcifications could be related to the chronicization of prostate infection. In particular, the presence of UPs in VB3/EPS/sperm is associated with higher rates of calcifications and high WBC sperm counts, suggesting a partial or full causative role of UPs in the pathogenesis of this disease.

Introduction

The possible causative role of *Ureaplasma urealyticum* in chronic prostatitis was initially hypothesized by studies based on quantitative cultures, demonstrating that *U. urealyticum* was the sole pathogen isolated by means of the

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4-glass location test in patients with chronic prostatitis symptoms. Brunner et al. [1] isolated *U. urealyticum* in expressed prostatic secretion (EPS) or urine voided after prostatic massage (VB3) in 13.7% of a large series of patients with chronic prostatitis, in association with low counts – or in the absence – of *U. urealyticum* in first voided (VB1) or midstream urine (VB2). Sugata et al. [2] detected *U. urealyticum* in EPS or VB3 in about 40% of patients with chronic prostatitis in association with elevated WBC counts. The same group later showed that *U. urealyticum* was the only isolated organism by 4-glass testing in 8% of patients with chronic prostatitis [3]. Treatment of *U. urealyticum* infection with tetracycline, macrolides, or ofloxacin achieved resolution of symptoms and decreased WBC counts in EPS and VB3 [4]. More recently, *U. urealyticum* was shown in 5% of 1,400 patients with symptoms of chronic prostatitis being associated with WBC counts >10/field in EPS or VB3 in 45% of cases [5]. Xiao et al. [6], who used specific PCR to investigate microorganisms in EPS of both chronic prostatitis patients and normal young male adults, found *U. urealyticum* in 22.4% of patients with inflammatory EPS and in 17.0% of patients with noninflammatory EPS, whereas none of the controls was positive for *U. urealyticum*. The number of microorganisms was associated with disease severity and correlated with WBC counts and lecithin levels. In addition, *U. urealyticum* was found more frequently in semen of infertile men (9%) compared to healthy controls (1%), being associated with lower rates of normal and motile sperm cells and lower semen volumes [7]. Conversely, a recent study by Park and Lee [8] concluded that the presence of *Ureaplasmas* (UPs) in urine was not related to the presence of chronic prostatitis and that *U. urealyticum* increases WBC counts in urine but not in EPS. On the other hand, in a previous study [9], *U. urealyticum* was not identified in the perineal prostate biopsy tissues from patients with idiopathic chronic prostatitis. The causative role of other mycoplasms (*Ureaplasma parvum*, *Mycoplasma hominis* [MH], *Mycoplasma genitalium*) in chronic prostatitis is controversial [10, 11].

UPs are also characterized by their capacity to split urea promoting calcification of calcium phosphate. Formation of urinary calcifications in the presence of UP infection of the urine has been well demonstrated [12, 13], whereas information about the possible lithogenic effect of the infection in prostatic fluid and tissue is lacking. The aim of this study was to assess the presence of prostate calcifications and the clinical characteristics in patients with chronic prostatitis symptoms and documented presence of *Ureaplasma* spp. (UPs) by means of the 4-glass localization test or in sperm cultures.

Materials and Methods

A database including the clinical records of >2,500 patients with chronic prostatitis (categories II and III, NIH criteria) was retrospectively reviewed to select patients with identification of UPs in 4-glass localization test or sperm culture. As control group, patients with negative UPs were considered, including patients with chronic bacterial prostatitis (CBP, category II), patients with identification of *Chlamydia trachomatis* (CT), and symptomatic patients with negative cultures (category IIIa and IIIb). All patients were studied with the same protocol that included medical history, objective examination, administration of the NIH-CPSI questionnaire [14], microbiological and microscopic evaluation (4-glass localization test and sperm culture), and transrectal ultrasound of the prostate. MH and UPs were identified using the semiquantitative assay Mycoplasma IST 2 kit (BioMerieux). This commercial system is a liquid method based on the ability of UPs and MH to grow in a culture medium and to metabolize urea and arginine. UPs and MH were identified and enumerated according to a set threshold of color change. CT was identified by PCR. Presence and pattern of calcifications at prostate ultrasound were evaluated by subjective evaluation of the same urologist using an Aloka ProSound 3,500 Plus system equipped with endocavitary biplane convex/linear probe at frequency range of 5–7.5 MHz.

Images of calcifications in the transverse and sagittal planes were evaluated. We defined as positive for calcifications the presence of multiple hyperechoic areas plus 3 mm in the largest diameter. Hyperechoic foci were classified by the presence of shadowing or not and according to distribution into the prostate gland as periurethral, central, peripheral, or diffuse (periurethral + central + peripheral).

Results

UPs were detected in 143 patients. They were isolated in first void urine (VB1) alone in 35 patients (urethral UPs); in EPS or post-massage urine (VB3) or seminal fluid (SF) in 77 cases (in association with UPs in VB1 in 41 patients and with negative VB1 in 36 patients) (prostatic UPs); UPs was demonstrated in association with other pathogens in 31 patients (associated UPs). MH was demonstrated alone in 3 and in association with UPs in 4 cases (in VB1 in 1 case, in VB3 in 4 cases, and in VB1 plus VB3/SF in 2 cases). In other 7 cases, MH was associated with other pathogens. No growth of microorganisms was observed in midstream urine (VB2). As control group, 393 patients with CBP (category II), 43 patients with identification of CT (15 in VB1 and 27 in VB3 and/or EPS and/or seminal fluid), and 781 symptomatic patients with negative cultures (category IIIa and IIIb) were considered.

In Table 1, rate and pattern of calcifications assessed with transrectal prostate ultrasound in patients with and without identification of UPs are shown. Calcifications were observed more frequently in patients with prostatic UPs (71%)

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Table 1. Calcifications in patients with prostatitis symptoms with and without UPs

	UPs positive			UPs negative			Total	Sig
	urethral	prostatic	associated with other pathogens	CBP	CT	CPPS IIIa-b		
N	35	77	31	394	43	781	1,363	
Total	12 34%	55 71%	25 73%	154 39%	16 37%	228 29%	490 36%	0.000
Periurethral	4 11%	22 28%	13 38%	67 17%	5 12%	45 57%	156 11%	0.000
Central	12 34%	48 62%	23 68%	131 33%	11 20%	205 26%	431 32%	0.000
Peripherical	5 14%	11 14%	8 23%	36 9%	0	24 3%	84 6%	0.000
Diffuse	4 11%	6 8%	5 15%	27 6%	0	13 2%	55 4%	0.000

UP, *Ureaplasma*; CBP, chronic bacterial prostatitis; CT, *Chlamydia trachomatis*; CPPS, chronic pelvic pain syndrome; UPs, *Ureaplasma* spp.

and in patients with UPs associated with other pathogens (73%) than in patients with urethral UPs (34%), patients with CBP without UPs (39%), patients with CT (37%) and patients with negative cultures (29%) ($p < 0.0001$).

When hyperechoic areas without shadowing were excluded, the difference within groups was confirmed. Similarly, differences were confirmed when prostatic calcifications were subdivided by zone distribution.

In Table 2, mean age value, mean NIH-CPSI score values, rate of history of urinary tract infection or sexually transmitted disease, length of follow-up, and WBC counts (VB1, VB3, and SF) are presented. No difference was observed between the different groups except for the value of WBC in SF. Mean WBC counts of sperm in patients with the presence of urethral UPs (5.0 ± 3.7) were significantly lower than in patients with prostatic UPs (12.5 ± 7.7), and associated UPs (10.4 ± 7.2) ($p < 0.0001$). No differences were observed for other ultrasound findings, PSA values, uroflowmetry findings, ejaculatory symptoms, and symptom scores.

Discussion

The role of mycoplasmas in chronic prostatitis has often been debated, as their isolation in the urine of patients with chronic prostatitis has been explained in various ways. Particularly, intracellular incorporation of pathogens could act as a trigger for chronic inflammation or recurrent epithelial damage, aggravating chronic genitourinary pain, or inducing chronic prostatitis. UP species are the most prevalent genital mycoplasmas isolated from the urogenital tract and are divided into 2 biovars, *U. parvum* and *U. urealyticum*[15]. In patients with chronic prostatitis, *U. urealyticum* has been considered a component of the normal opportunistic genital flora or a causal agent of a concomitant urethritis or a full or incomplete causative agent of chronic prostatitis. In our series, the presence of UPs in EPS/VB3 or sperm was associated with symptom score values and WBC counts similar to those observed in patients where the presence of UPs was associated with infection with other known pathogens, suggesting that the former may have a role in provoking prostatitis-like symptoms. In addition, patients with UPs in EPS/VB3 or sperm showed higher WBC counts in sperm when compared to patients where UPs had only been identified in VB1, thus, confirming that the localization of UPs in the prostate, but not in the urethra, causes prostate inflammation. In assessing the potential effect of *U. urealyticum* in provoking or promoting the appearance of chronic prostatitis, its peculiar biological properties must be taken into account. In fact, *U. urealyticum* has the ability to form biofilms [16] and has a specific urease activity. In the urine, urease splits urea into am-

Table 2. Demographic and clinical characteristics of patients with urethral (VB1) or prostatic (VB3/EPS/sperm) UPs and patients with UPs associated with other pathogens

	Urethral UPs	Prostatic UPs	UPs associated with other pathogens	Total UPs	Sig
N	35	77	31	142	
Age, years	44±12	45±13	45±14	45±13	0.960
CPSI pain	8.6±3.7	10.4±4.4	9.2±4.6	9.7±4.3	0.131
CPSI micturition	4.4±2.8	4.5±2.7	3.4±2.3	4.3±2.7	0.153
CPSI QoL	7.2±3.2	7.6±2.7	7.1±3.7	7.4±3.1	0.678
CPSI total	20.3±7.5	22.7±7.9	19.8±9.0	21.4±8.1	0.172
WBC in VB1	2.5±3.8	3.3±4.4	3.2±3.1	3.1±4.0	0.554
WBC in EPS	11.2±21.5	6.8±2.7	8.0±5.6	8.4±12.2	0.764
WBC in VB3	6.4±8.7	8.5±8.4	7.3±6.2	7.8±8.1	0.433
WBC sperm	5.0±3.7	12.5±7.7	10.9±5.8	10.4±7.2	0.000
WBC ≥10 in VB3	5 14%	26 34%	9 29%	40 28%	0.103
UTI history	13 37%	29 37%	17 54%	58 41%	0.222
STD history	15 43%	41/77 53%	18 58%	74 52%	0.433
UTI+STD	7 20%	19 25%	14 45%	40 28%	0.137
Follow-up duration	20±7	22±7	19±9	21±8	0.257

UPs, *Ureaplasma* spp.; UTI, urinary tract infection; STD, sexually transmitted disease. Urethral < prostatic $p = 0.000$. Urethral < associated $p = 0.002$.

monium and carbamate, which in turn, in the presence of water, forms bicarbonate, resulting in an increase in the pH value of urine. In addition, ammonium can directly damage epithelia for its toxic action. This proved to be crucial in promoting the formation of struvite urinary stones. In fact, *U. urealyticum* was cultured in 16% of infection stones and in 30% of voided urine from patients with infection stones [12, 13]. Experimental studies have shown that inoculation of *U. urealyticum* into rat bladders resulted in the formation of struvite stones and that *U. urealyticum* is capable of causing damage to the urothelial mucous coat, thus, promoting retention of struvite crystals [17, 18]. *U. urealyticum* can also act similarly in ducts and prostate tissue causing the formation of extensive calcifications, as anecdotally described in the Skene's gland [19]. Prostate calcifications are a substrate that promotes the formation of biofilms in which the growth of other chronic prostatitis pathogens can be promoted [20].

Urea is present in seminal fluid in concentrations around 45 mg/dL which originated from local production or blood circulation [21]. They should be sufficient to allow the growth of UPs and to form the substrate for the formation of calcifications. In fact, the growth of UPs depends on the presence of urea whose hydrolysis plays a primary role in the energy metabolism of UP cells by promoting ATP synthesis [22]. On the other hand, it is possible that the concentrations of urea in the seminal fluid of patients with prostatitis may be higher by urethroprostate reflux of urine, which has a urea content about 20 times higher than seminal fluid. Already 50 years ago, it had been hypothesized that patients with prostatitis

may have a reflux of urine in the prostate ducts due to failure of the external sphincter to relax during micturition [23]. Kirby et al. [24] using a suspension of carbon particles demonstrated that reflux of urine into the prostatic ducts during micturition can occur. A more recent experimental study in the rat confirmed that urine reflux into the prostate can induce prostatic inflammation [25]. Finally, the ejaculate obtained by electrocution in spinal cord injured patients has higher concentrations of urea than the ejaculate obtained by masturbation, suggesting urinary contamination due to the procedure itself [26]. We previously demonstrated that CPSI scores for domains of micturition were higher in patients with calcifications than in patients without calcifications, although no difference was observed at uroflowmetry [27, 28]. It cannot, therefore, be ruled out that the formation of calcifications could be facilitated by the presence of alterations of micturition that could be associated with urine reflux in the prostate ducts.

In our series, the presence of prostate calcifications was observed in 73% of patients with UPs in VB3/EPS/sperm in comparison with lower rates observed in patients without UPs or with UPs only in VB1. The presence of UPs in prostatic fluids seems to be associated with a high lithogenic potential that is compatible with the biological characteristics of this microorganism. The formation of prostate calcifications could be a predisposing factor to the chronicization of infection by UPs or other pathogens. Quantitative computerized assessment of the extent of calcifications may be useful for better defining the role of UPs in their formation. In a small series of 4 cases, the presence of UPs was associated with the presence of the most extensive calcifications [29]. A strength of our study was the search for sexually transmitted pathogens in all samples of the 4-glass test, which allowed to identify in a significant number of cases the presence of sexually transmitted infections that could remain unknown. In addition, our protocol provided for the systematic evaluation of the seminal fluid that was obtained as the fifth adjunctive sample of the 4-glass test after the collection of VB3. The addition of the semen examination increased the number of patients in which the presence of UPs was identified. One possible limitation of the study was the use of a semiquantitative assay for MH and UPs. In fact, molecular methods, such as PCR assays, are more sensitive and rapid for diagnostic purposes than culture [30]. Furthermore, these assays do not rely on the viability of the bacterium for detection and when using a multiplex PCR assay allow detection of >1 target in a single reaction. Some studies compared the diagnostic effectiveness of semiquantitative methods with that of molecular assays for the detection of mycoplasmas. A commercial kit showed a 96% overall agreement between the 2 methods [31], and a second one showed sensitivity and specificity of a semiquantitative assay of 77.3 and 80.0%, respectively, with positive and negative predictive values of 97.1 and 28.6% [32]. Finally, cultures cannot distinguish between the species *U. parvum* and *U. urealyticum*, and analysis with additional molecular methods is needed for speciation. On the other hand, PCR assays are expensive and rely on specialized skills of the laboratory personnel.

Another undoubted limitation of our study is the subjective evaluation of the presence of calcifications; a diagnosis with more objective criteria is certainly desirable as it allows a numerical evaluation of the extent of calcifications. To our advantage is the fact that all ultrasounds were performed by the same operator that eliminated interobserver variability. On the other hand, most studies of the prevalence of prostate calcifications were performed based on subjective assessment of sonographic images by classifying patients in patients with or without calcification. Only a few studies adopted classifications to divide patients with smaller multiple calcifications from those with larger and coarser calcifications [33].

In conclusion, the presence of UPs in EPS and sperm is associated with prostatitis-like symptoms and increased WBC counts in the sperm, thus, suggesting a causative role for this species in the pathogenesis of this disease. UPs identification in the seminal fluids is related to higher rates of prostate calcification than in infection from other pathogens. The ability of UPs to promote the formation of calcifications could be related to the chronicization of prostatic infection and should be fully elucidated by specific studies designed to quantitative evaluation of prostatic calcifications.

Statement of Ethics

This study was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. This paper is exempt from Ethical Committee approval because ethical formal consent is not required for observational noninterventional studies (DL no. 211 June 24, 2003).

Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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Author Contributions

Conception or design of the work: Vittorio Magri and Alberto Trinchieri. Acquisition of data: Vittorio Magri. Drafting the paper: Alberto Trinchieri. Paper critically revising: Gianpaolo Perletti, Konstantinos Stamatiou, and Emanuele Montanari.

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