DOI: 10.4081/aiua.2022.1.62

ORIGINAL PAPER

Propionibacterium acnes in urine and semen samples from men with urinary infection

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Summary ***Objective: Propionibacterium acnes has been implicated in the pathogenesis of prostate disease as acute and chronic prostatic inflammation, benign prostatic hyperplasia and prostate cancer although it should still be clarified if Propionibacterium acnes (P. acnes) is a commensal or accidental prostate pathogen. Aiming to evaluate the pathogenic potential for genitourinary tract of Propionibacterium acnes, we investigated the frequency of P. acnes genome in urine or semen samples from men with recurrent symptoms of urinary infection and negative testing for the most common urinary tract pathogens and sexually transmitted infections (STI) agents as Chlamydia trachomatis, Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma parvum and Ureaplasma urealyticum.***
Materials and methods: The DNA extracted from urine and semen samples was analyzed for evaluating the P. acnes genome presence by real-time polymerase chain reaction (PCR). Infections were treated with vancomycin and cephalosporins antibiotics and then the search for the P.acnes genome by real-time PCR was repeated.
Results: The P. acnes qualitative real-time PCR revealed the genome in 73 out of 159 samples examined (108 urine and 51 semen). After antibiotic therapy, P. acnes was never detected.
Conclusions: These results suggested that P. acnes genome determination should be performed in cases of chronic inflammation in the urinary tract to identify an unknown potential pathogen of genitourinary tract.

KEY WORDS: Propionibacterium acnes; Prostate hyperplasia; Prostate cancer; Urinary tract infections.

Submitted 18 October 2021; Accepted 3 December 2021

INTRODUCTION

Propionibacterium acnes (*P. acnes*) is a gram-positive, non-motile, non-spore forming, anaerobic bacillus. It is ubiquitous and part of the normal flora of the skin. Despite it is considered part of our commensal microbiota there are a number of reports correlating *P. acnes* with several diseases. Indeed *P. acnes* contributes at the pathogenesis of acne vulgaris and was found to be implicated in a wide-range of post-operative infectious conditions, such as endocarditis, endophthalmitis and intravascular nervous system infections. *P. acnes* is also frequently detected in prostate tissue of patients diagnosed with *benign prostate hyperplasia* (BPH) and cancer (1). Chronic infection and inflammation have been linked to cancer of several organs suggesting that also prostatic inflammation could contribute to the etiology of prostate cancer as well as BPH. Kakegawa et al. showed in a series of repeat prostate biopsies that patients with high serum PSA and initial biopsy negative for cancer progressed more frequently to prostate cancer in subsequent biopsies if the initial biopsy was positive for the presence of *P. acnes* (2, 3).
In the present paper we evaluated the potential pathogenic role of *P. acnes* in the genitourinary tract. For this purpose, we evaluated by real time PCR the presence of *P. acnes* DNA in urine or seminal fluid of patients with recurrent symptoms of urinary infection but negative testing for the most common urinary tract pathogens and *sexually transmitted infections* (STI) agents as *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma parvum* and *Ureaplasma urealyticum*.

MATERIALS AND METHODS

Patients

Male patients with recurrent symptoms of urinary tract infection were considered in the present study. Patients sought medical attention of the urology specialist due to voiding symptoms (weak stream, straining and hesitancy) or storage problems (urgency, frequency and dysuria) and pain poorly localized in the lower back, hypogastrium, pelvis or genitalia. Digital prostate palpation showed tender, swollen and warm prostate or also nodularity. Patients with diabetes, spinal cord injury and catheter use were excluded.
Patients were subjected to urinalysis and urine culture and to semen analysis in case of history of couple infertility. Samples were screened for predominant pathogens involved in *urinary tract infections* (UTI) as *Escherichia coli*, *Klebsiella spp.*, *Proteus spp.*, and *Enterococcus spp.* and for *Sexually Transmitted Infection* (STI) agents, by molecular methods. Patients with negative testing for the most common urinary tract pathogens and *sexually transmitted infections* (STI) agents were investigated for the presence of DNA *P. acnes*.

Specimen Collection, Urinalysis, Urine and Semen Culture

Urinary and semen specimens were obtained from patient with recurrent symptoms of urinary infection. All the urinary samples were subjected to urinalysis and urine cul-

No conflict of interest declared.

ture. Midstream urine was collected in a clean vessel and analyzed within 2 h of collection. Urinalysis included physical, chemical and microscopic examinations. Chemical urinalysis was performed by Automated Urinalysis System U500 (*InterMedical Diagnostics*) detecting blood, protein, glucose, leukocyte esterase and nitrite. Microscopic examination was performed to identify cells, bacteria, casts and crystals. Physical and chemical findings were: hazy or cloudy appearance, blood presence, glucose > 1000 mg/dl, protein more than trace quantities, leukocyte esterase or nitrite positivity. Microscopic findings were: detection of *red blood cells* (RBC) and/or *white blood cells* (WBC) ≥ 4 for *high power field* (HPF) and/or bacteria (data not shown). Urine and semen samples for microbiology analysis were collected in sterile vessel. Selective and differential solid media (*Integral System Enterobatteri, Liofilchem, Italy*) were used for cultivable microorganisms. *Lactobacillus species*, coagulase-negative *Staphylococcus* and *Streptococcus* were considered normal flora or contaminants and thus urine or semen culture was considered negative. Growth and identification of *Enterobacteriaceae*, *group B Streptococcus*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Enterococcus spp.*, *Klebsiella spp.*, *Proteus spp.*, *Candida spp* at > 10000 CFU/ml and 5000 CFU/ml for urine and semen respectively were considered positive culture.

DNA extraction and Sexually Transmitted Infection (STI) agents detection

The urine and semen samples were screened for *Chlamydia trachomatis* and *Genital Mycoplasmas* with real time PCR. Briefly, the DNA extraction was made using Prime DNA/RNA Rapid Extraction kit (*Astra Biotech, Berlin, Germany*) and the analysis was made by real-time PCR using the kit *Mycoplasma genitalium/Mycoplasma hominis* Multiplex PCR kit, Ureaplasma urealyticum/Ureaplasma parvum Multiplex kit and *Chamydia trachomatis* PCR kit (*Astra Biotech, Berlin, Germany*) according to the manufacturer's instructions.

Propionobacterium acnes identification

The DNA extracted from urine and semen samples was analyzed for *P. acnes* genome presence using the *P. acnes* recA gene (*Primer Design Genesig, UK*) according to the manufacturer's instructions.

The kit is designed with the broadest possible detection profile to ensure that all clinically relevant strains and subtypes are detected. The analysis was made by real-time PCR and all amplification reactions of the genomic materials were performed with a AriaDX Real-time PCR System (*Agilent Technologies*).

Antibiotic therapy

The treatment of *P. acnes* infections can be made using antibiotics such as cephalosporine, vancomycin, penicillin, tetracyclines, rifampicin and erythromycin.

In our study all the patients with *P. acnes* infections were treated, initially, with cephalosporins for three months.

After cephalosporins therapy if the urine or semen samples were still positive for *P. acnes* an alternative antibiotic regimen with vancomycin for three months was proposed.

Results

DNA P. acnes detection from urine and semen sample

Out of the 159 samples examined (108 urine and 51 semen samples) the genome of *P. acnes* was identified in 56 urine and 17 semen samples (Tables 1, 2). *P. acnes* positive patients were treated with cephalosporins therapy. After cephalosporins therapy, *P. acnes* real time was repeated in 56 urine and 17 semen samples and *P. acnes* genome was not detected in 51 urine and in 16 semen sample whereas 6 samples (5 urine and 1 semen) were still positive for *P. acnes*. These patients were treated with vancomycin, an alternative antibiotic therapy, and then were re-evaluated for presence of *P. acnes* genome. At the end of the vancomycin therapy, *P. acnes* real time testing was negative per *P. acnes* genome.

Table 1.
Total samples examined.

Tot. n. samples	**159**
Urine	108
Semen	51

Table 2.
Presence of P.acnes genome in urine and semen samples.

Samples in which the P. acnes genome was identified	
Urine	56
Semen	17

Discussion

UTI is the most common urological infection with annual incidence increasing with age in men (4, 5). The presence of localized genitourinary symptoms and signs of urinary tract inflammations and a urine culture with an identified urinary pathogen are suggestive of UTI and antibiotic therapy is necessary for the treatment of the infection. Any microorganism, ascending the urethra or by reflux of urine into prostatic duct, can infect prostate gland and to cause chronic inflammation in connection with *benign prostatic hyperplasia* (BPH), chronic prostatitis (CP) and prostate cancer (6). In addition to bacterial infections, other factors such as hormone imbalances, dietary carcinogens and environmental factors promote prostate chronic inflammation and lead to injury of the prostate (7).

Several studies showed that the *P. acnes* identification was associated with acute and chronic inflammation and, moreover, a high prevalence rates of this bacterium in prostate tissue samples from men with prostate cancer has been demonstrated (8, 9). *P. acnes* is a common skin anaerobic organism that is capable to resist to phagocytosis through complex cell wall structure, to persist intracellularly within macrophages and to produce exocellular enzymes that can damage the host tissue and induce pro inflammatory cytokines (10). In this study we analyzed, with molecular biology techniques, the urine and semen sample of patients with recurrent symptoms of urological

infections, that were negative for more common genitourinary pathogen and STI agents (11, 12). Patients with samples positive for *P. acnes* were treated with specific antibiotic therapy and showed a positive response in terms of improved clinical condition.
A limitation of the present study is the lack of comparative date between urine and semen samples from the same patient, although it is conceivable that the pathogen could be detectable indifferently in both samples. A further objective of our work will be to assess the correlation between *P. acnes* infection and couple infertility. In fact, our preliminary experience has shown that three patients with a history of couple infertility were able to obtain the pregnancy after antibiotic therapy to eradicate the *P. acnes* infection. This finding should be confirmed by larger controlled series.

Conclusions

Our results show that real time PCR genome screening for *P. acnes* could be a useful diagnostic tool for UTIs. *P. acnes* infection could start a pathogenic cascade causing, in the long term, an inflammatory process of the prostate. Indeed, an amount of evidence suggests that the invasion of prostate epithelial cells by *P. acnes* contributes at the prostate gland diseases (13).

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