Original Article

The Prevalence of *Ureaplasma Urealyticum* and *Mycoplasma Genitalium* in Patients with Prostate Cancer in Shohada Hospital in Tehran, Iran

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Abstract

Background: Prostate cancer (PCa) is an important health problem in the aging male population in the world. It is the third most common cancer in the world. Despite of its importance, relatively little is known about its etiology. Sexually transmitted infections (STI) and urogenital pathogens such as Mycoplasma and Ureaplasma, have been proposed as a risk factor for prostate cancer development. This study aimed at detecting the prevalence of *Ureaplasma urealyticum* (*U. urealyticum*) and *Mycoplasma genitalium* (*M. genitalium*) in PCa and the controls group with benign prostate hyperplasia (BPH) in Shohada hospital.

Materials and Methods: A total of 124 paraffin-embedded prostate tissues (62 PCa patients and 62 controls with BPH) were included in this study. The subjects'specimens were investigated by the polymerase chain reaction method for the presence of *U. urealyticum* and *M. genitalium* DNA.

Results: *U. urealyticum* was detected by standard PCR in 1.61% of the 62 PCa patients and there was no DNA *U. urealyticum* in the 62 controls with BPH. No *M. genitalium* was detected by standard PCR in the prostates of 124 paraffin-embedded prostate tissues.

Conclusion: According to our results, there is no association between *M. genitalium* and *U.urealyticum* with PCa. We recommend further studies using a large sample to determine role of *Ureaplasma* and *Mycoplasma* in PCa because understanding the role of infectious agents on PCa might be useful for developing new therapeutic approaches and prevention of PCa.

Keywords: *Ureaplasma urealyticum*, *Mycoplasma genitalium*, Prostate Cancer, benign prostate hyperplasia, paraffin-embedded prostate tissues

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Introduction

Nowadays prostate cancer (PCa) is one of the most important health challenge in the aging male population and the third most common cancer in the world[1,2]. There is a direct relation between incidence and age in this disease (about 65 years or more)[3]. The higher rates of PCa are seen in Western countries,

North America and in some African regions. The lowest incidence is seen in Asian countries. However, with aging populations and using prostate specific antigen (PSA) screening, the incidence of prostate cancer in the Asian countries has risen in the past decade[4]. The incidence of PCa is much lower in Iran than developed countries. In the recent studies, the incidence of PCa in Iran was approximately 3.5%[5,6]. Although the rate of PCa is very common in the world, relatively little is known about its causes[7]. It has been well established worldwide that at least 15-20% of all cancers are associated with infections, as etiologic agent, such as the association between *Helicobacter pylori* infection with gastric cancer[8-11]. Recent studies proposed that chronic infections may develop PCa. Infections such as urinary tract infections (UTIs), sexually transmitted infections (STIs) caused by *mycoplasam* and *Ureaplasma* were shown to be associated with PCa as well as the genitourinary tract can be colonized with *Ureaplasma urealyticum (U. urealyticum)* and *Mycoplasam genitalium (M. genitalium)*[12-16].

Mycoplasma and *Ureaplasma* are small, without cell wall and with sterols in membrane bacteria[17]. Today, it has been demonstrated that chronic infection with *Mycoplasma* can induces malignant transformation of human cells[18-22].

In the present study, we examined the prevalence of *M. genitalium* and *U. urealyticum* in PCa and the control group with benign prostate hyperplasia (BPH) using standard Polymerase Chain Reaction method (PCR) in a population of men in Shohada hospital, Tehran, Iran.

Methods

This was a descriptive survey of 62 PCa and 62 non PCa pathological specimens collected from patients aged between 50-89, referred to Shohada hospital, Tehran-Iran from 2007-2012. Demographic data, including the histological type of cancer, gleason score (GS), prostatic intraepithelial neoplasia (PIN) and PSA were recorded. Then formalin-fixed and paraffin-embedded tissue sections of open prostatectomy, core needle biopsy and trans urethral resection of the prostate (TURP) samples were examined from patients with PCa and from patients with BPH. A single pathologist experienced in urogenital pathology performed microscopic evaluation of the microscopic slides to determine the cancerous and non-cancerous tissue differentiation. The best paraffin-embedded block containing cancerous tissue of the patients with prostate cancer was selected for examination. Samples were transported to Department of Microbiology, Medical School, Shahid Beheshti University of Medical Sciences for further analysis.

DNA was extracted from formalin-fixed and paraffin-embedded tissue blocks by G-spin TM Total DNA Extraction Kit (iNtRON Biotechnology Co., Korea). First, the paraffin blocks were sliced into thin pieces using a sterile razor blade and were placed in a 1.5 ml tube (not more than 25 mg). According to the manufacturer's instructions, xylene was used for the removal of paraffin and then the bacterial DNA was extracted from tissue. DNA concentration and purity was measured by spectrophotometry. Mean and standard error of the mean were calculated and analyzed for significance in different. The extracted DNA was stored at -20˚C.

The PCR assay for detection of *M. genitalium* and *U. urealyticum* performed by *mycoplasma genitalium* PCR Detection Kit (Pars tous Co., Iran) and *Ureaplasma urealyticum* PCR Detection Kit (Pars tous Co., Iran), respectively. Primers within the *M. genitalium* PCR Mix and *U. urealyticum* PCR Mix was specific for the conserved 16srRNA coding region in the *Mycoplasma* and *Ureaplasma* genome.

The PCR reaction mixture contained 14.6 µl of *M.genitalium* PCR mix and *U. urealyticum* PCR mix (for detection of *M. genitalium* and *U. urealyticum*, respectively), 0.4 µl of HS-Taq DNA polymerase and 5 µl of DNA template was used for adjusting to a final volume of 20 µl. For negative and positive controls used 5 µl of PCR grade water and 5 µl positive controls, respectively.

PCR mixtures were cycled under the following thermal conditions: Initial denaturation 94˚C for 10 min, 40 cycles in denaturation 94˚C for 30 sec, annealing 56˚C for 30 sec, extension 67˚C for 30 sec and final extension 72˚C for 5 min.

The PCR products were analyzed on 2% agarose gel electrophoresis and the gel was stained with ethidium bromide (0.5 µg/ml) and viewed by UV transilluminator. The presence of 255 base paires (bp)

and 370 bp fragments were positive for *M. genitalium* and *U. urealyticum* respectively.

Demographic data were analyzed using Statistical Package for Social Sciences (SPSS) software (version 16).

Results

The mean age of PCa patients was 67.8±7.9 years (range50-86 years) and in BPH patients 68±9.1 years (range 40-89 years). The characteristics of the study population and GS information (grade of PCa) are summarized in Table1.

The genomic DNA extraction results using G-spin TM Total DNA Extraction Kit was shown high quality and quantity of DNA collected from all samples (Normal range of purity of DNA=1.9±0.2 and normal range of A260= 0.1-1).

To detect *M. genitalium* and *U. urealitycum* in PCa patients and controls with BPH, we used PCR assay for each one separately. The results of PCR showed the *U. urealitycum* was positive in 1 (1.61%) of the 62 PCa patients (Figure 1) but the *U. urealitycum* was not detected in control samples. None of the 124 prostate samples were positive for the *M. genitalium* (Figure 2) and no significant differences were found between the presence of these bacteria and PCa [p value >0.001; OR=1; 95% CI (0.985-1.049)]. PSA value was 60 ng/ml in the patient who was *Ureaplasma* DNA positive. He was 50 years old and he had poorly differentiated tumor (Gleason 9).

Discussion

Since the early 1950s, when sexually transmitted infections (STIs) were first proposed as a possible PCa risk factor[23], numerous epidemiologic studies have been examined. There is compelling evidence that urogenital infections are associated with PCa[12, 13]. However, in several studies did not find an association between PCa and any specific bacterial infections[24,25]. Therefore, the evidence is not clear-cut and require more subtle investigation. In this study, we investigated the possible role of *M. genitalium* and *U. ueralyticum* as risk factors in the PCa using PCR method. *M. genitalium* and *U. urealyticum* has been identified as one of the main causes of non-gonococcal urethritis in the world[26,27]. Our study showed that the incidence of *M. genitalium* and *U. urealyticum* positively was 0.0 % and 1.6 % in the case group with

Table 1: The study population characteristics.

Characteristic	PCa	BPH
Total number of patients	62	62
Mean age (range, years)	67.8±7.9 (50-86)	68±9.1 (40-89)
< 60	10	11
60-69	23	22
70-79	28	24
>79	1	5
Source		
Needle biopsy	22 (35.5%)	4 (6.5%)
TURP	8 (12.9%)	40 (64.5%)
Prostatectomy	32 (51.6%)	18 (29%)
Mean PSA (ng/mL)	19.19±16.43	4.16±3.8
0-4 (ng/ml)	3	38
4.1-10 (ng/ml)	19	19
10.1-20 (ng/ml)	22	5
> 20 (ng/ml)	18	0
Median Gleason Score (range)	6.92±1.23 (5-10)	-
5	7	-
6-7	39	-
8-10	16	-
PIN	11 (17.7%)	-

PCa Prostate cancer, BPH Benign prostate hyperplasia, TURP Transurethral resection of the prostate, PSA Prostate specific antigen, PIN Prostatic intraepithelial neoplasia

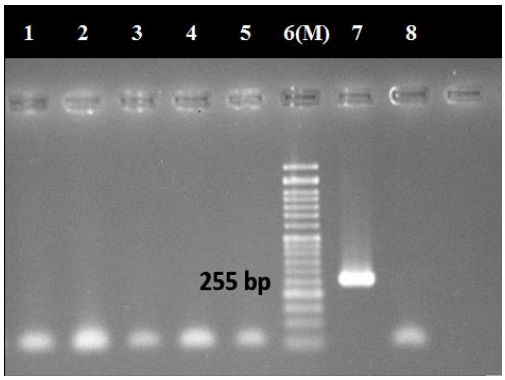

Figure 1. Results of standard PCR assay for detection of *M. genitalium* DNA (16S rRNA gene). (1-5): DNA from prostate tissue of patients, 6(M): 50 bp ladder, (7) Psitive control, (8) negative control, *M. genitalium* (255 bp fragment).

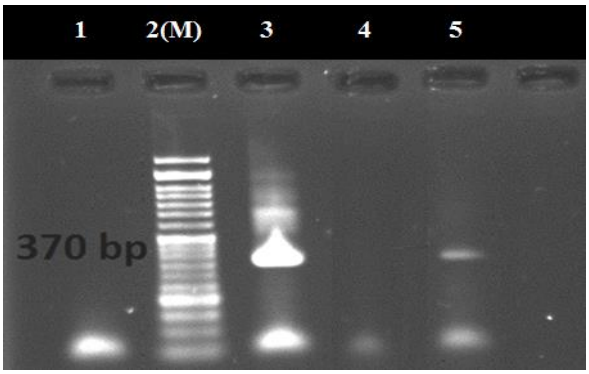

Figure 2. Results of standard PCR assay for detection of *U. urealyticum* DNA (16S rRNA gene). (1): negative control, 2(M): 50 bp ladder, (3): positive control, (4): *U. urealyticum* DNA negative from prostate tissue of patient, (5) *U. urealyticum* DNA positive from prostate tissue of patient.

***U. urealyticum* (370 pb fragment)**

***M. genitalium* (255 bp fragment).**

PCa, respectively. No *M. genitalium* and *U. urealyticum* were detected in the control group with BPH. Until now, as far as we know, there have been no studies in Iran about the incidence of bacterial infection in PCa, but in several studies have been examined detection of *M. genitalium* and *U. urealyticum* in men in other diseases. Salari and Karimi (2003) reported that the prevalence of *M. genitalium* and *U. urealyticum* in urethral swab samples from men with non-gonococcal urethritis in Tehran, Iran was 19.2 % and 7.2%, respectively[28]. Yeganeh et al. (2013) have been shown the prevalence of *M. genitalium* in Urine specimens was revealed to be 12% and 2% symptomatic and asymptomatic men, respectively[29]. The prevalence of *U. urealyticum* was 12% in semen specimens of infertile men and 3 % in healthy men[30].

Differences in the prevalence of Mycoplasma and Ureaplasma in our study with other studies in Iran could be related to the type of diseases and samples. In a similar study, Erturhan et al. reported that mycoplasma infections play a role in the etiopathogenesis of the PCa. The *Mycoplasma spp.* DNA was found to be positive in 35.4% patients in PCa and there was no mycoplasma DNA in the control group with BPH[15]. The racial, geographical and cultural differences could be the reason for this difference. Also the difference in results could be due to the type of sampling. In his study, all samples were collected using the TURP procedure. In this method, a piece of the prostate is removed through the urethra. Because numerous species of Mycoplasma can be present as normal flora in the urethra, so there is a possibility of contamination of the prostate samples during the sampling by TURP procedure. But more than 50% of the cancer subjects in our study was the prostatectomy specimens. Similar to our study, in other studies that prostate cancer tissues collected by prostatectomy have not detected the presence of Mycoplasma spp[11,31].

Barykova et al. detected *M. hominis* infection in 15.2% and 37.4% of PCa patients using PCR and real-time PCR, respectively. While, *M. genitalium* and *U. ueralyticum* were only detected in 5.6% and 0.8% of the patients using PCR, respectively. None of the three species were detected in control samples. Therefore, they have been suggested that *M.hominis* may be involved in PCa development[14]. In his study, *M. hominis* DNA was detected in transrectal prostate biopsy samples. Although most commonly known as a commensal organism in the human genitourinary tract, *Mycoplasma hominis* is also present in the human rectum[32]. Whereas, *M. hominis* have not detected in studies that samples were collected by prostatectomy[11,33].

Recently, three case-control study based on serology (such as ELISA and immunofluorescence), has investigated the relationship between mycoplasma and prostate cancer. As they has been shown that antibodies against *M. hyorhinis*, *M. hominis and U. urealyticum* is associated with an increased risk of prostate cancer[12,16,34].

According to our results, there is no association between *M. genitalium* and *U. urealyticum* with PCa using PCR. A possible explanation for this result is that the detection infectious agent in clinical tissues by using PCR techniques is difficult, because in these techniques only a small fraction of the tissue is examined. Knowing that inflammation, and probably also bacteria and viruses, are unevenly distributed in the prostate, the risk of missing focally infected areas is high. On the other hand, this difference may be due to the fact the tissue samples were formalin-fixed and paraffin-embedded and bacterial DNA may be damaged due to the long storage samples. Also, the sample size was small and the incidence of *Mycoplasm*a infections is much lower in Iran than other countries with different cultures. So, we recommend further experimental studies using a large sample to determine the role of *Ureaplasma* and *Mycoplasma* in PCa because understanding the role of infectious agents on PCa might be useful for developing new therapeutic approaches and prevention of PCa.

Conclusion

In this study, we conclude that the association between PCa and infections caused by Mycoplasma and Ureaplasma is not supported. As regards role of mycoplasma and Ureaplasma in the genesis of PCa is not conclusively proven yet, further studies are needed.

Acknowledgments

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