Detection of *Trichomonas vaginalis* in benign hyperplastic prostate tissue

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Introduction

Prostate cancer and benign prostatic hyperplasia (BPH) represents the most common urologic disease among the elderly males resulting in more than 2 million visits per year; 8% of total urologic visits and 1% of family physician visits (1,2). Its prevalence among men at risk is believed to be between 5% and 8% (3,4). BPH and PCa are considered chronic diseases, with early initiation and slow progression. The pathogenesis of BPH is not yet completely understood however, the role of chronic inflammation is emerging as an important factor in BPH development and progression. *Trichomonas vaginalis* is a common nonviral sexually transmitted infection, with an estimated 174 million annual infections globally. Recently, the studies have found that *T vaginalis* is associated with asymptomatic infections in 50%-75% of infected men and a number of observations support an association between *Trichomonas vaginalis* and prostatitis.

In this study we investigated the possibility of asymptomatic persistence of *T. vaginalis* in the prostate gland using benign hyperplastic prostate tissue as prostate condition other than clinical prostatitis in Mubarak Al-Kabir Teaching Hospital, Kuwait.

Main Objectives of the Study:

1. To investigate the possibility of asymptomatic persistence of *T. vaginalis* in the prostate gland using benign hyperplastic prostate tissue.

2. To detect antibodies to *T. vaginalis* in patients suspected of BPH as measure of *T. vaginalis* infection

Materials and methods:

Number of Patients: 75 of > 50 years of age

Patient selection: Suspected BPH cases reporting at the Surgical Clinic, Mubarak Al-Kabir Teaching Hospital, Kuwait for further evaluation during the period 2013-2014. All enrolled patients were given a code number to preserve the patient confidentiality.

Patient Samples:

The following specimens were collected from all the suspected BPH to confirm the diagnosis:

i, Prostate tissue: 5-6 small biopsy specimen were taken from the prostate for histopathology analysis.

ii, Blood specimen: 5 ml. blood for routine blood analysis; CBC, Blood sugar and blood chemistry profile and detection of immunoglobulin (IgG, IgG subtypes, IgM, and IgA) antibodies to *T. vaginalis*.

Test Protocols/Methodologies:

Immunohistochemistry of fixed prostate tissues:

1. *Trichomonas vaginalis parasite antigen*

The fresh biopsies were fixed in formalin, processed and the embedded in paraffin wax and a 4-5µm sections were cut. Staining was done after deparaffinization and rehydration by graded alcohols. Endogenous peroxidase activity was blocked using 0.3% hydrogen peroxide in Tris-buffered saline. For antigen retrieval the sections were treated with 10 mmol/l citrate buffer (pH 6.1) in microwave for 5 min and then incubated with the mouse primary monoclonal anti-*Trichomonas vaginalis* antibody. EnVision Flex Dual Link horseradish peroxidase/DAB visualisation system (Dako) was used and counterstained with haematoxylin. Positive control slides with *Trichomonas vaginalis* parasies were used for each run of samples.

2. *P16 antigen Test:*

The slides were processed as above till antigen retrieval and then incubated with the mouse primary anti-human p16 antibody (clone G175-405 BD Biosciences Pharmingen San Diego CA). The staining and the counterstaining was done above. Human uterus adenocarcinoma tissue were used as the positive control (5).

3. Polymerase Chain Reaction (PCR) for detection of *T. vaginalis* in Prostate tissue:

The DNA was extracted from the fresh tissue using QIAamp DNA Mini kit (Qiagen). A set of primers targeting a conserved region of the beta-tubulin genes of *T. vaginalis* (*btub1*, *-2*) were used.

The sequence used:

For BTUB 9: 5′ CAT TGA TAA CGA AGC TCT TTA CGA T 3′ (positions 850 to 874);

For BTUB 2: 5′ GCA TGT TGT GCC GGA CAT AAC CAT 3′ (positions 961 to 938).

The DNA was amplified by PCR using an automated thermocycler (Perkin-Elmer Cetus, Norwalk, Conn.) using the standard final PCR reaction mixture (50 µl). Cycling times were optimized. Negative controls, including uninoculated transport media, were used through-out the specimen preparation and PCR process. PCR products were separated and visualize on a 2% agarose gel containing ethidium bromide. (6).

4. Detection of anti-*T. vaginalis* antibodies by ELISA:

Indirect ELISA antitrichomonas IgG, IgM, and IgA antibody responses were determined in serum of men infected with *T. vaginalis* by ELISA following manufacturer's instructions coating plates over night at 4°C with 100 µl (1 µg/ml) of *T. vaginalis* antigen (*Trichomonas vaginalis*, strain C-1: NIH). Each specimen was added in duplicate in dilutions ranging from 1:200 to 1:800 for IgG, IgG subclasses, IgM, and IgA.

The plates were incubated for 1 h at 37°C. Antihuman IgG/IgM/IgA conjugated with horseradish peroxidase (Sigma, St. Louis, MO, USA) was used at optimum dilutions (1:40,000, 1:3,000, and 1:10,000, respectively) in PBS–Tween 20–BSA.

Ethical Clearance:

Ethical clearance was taken from the Committee for the Protection of Human Subjects in Research-HSC & KIMS, Kuwait University, Kuwait.

Results:

We detected *T. vaginalis* DNA by PCR in BPH tissue from 18 of 75 (24.0%) patients. Four patients were later confirmed having prostate cancer (CaP) on histopathology (Table 1) and were later removed from the statistical analysis.

T. vaginalis DNA was detected by PCR in prostate tissue from 16 of 75 (22.5%) patients with BPH. P16 antigen was detected in 16/71 (22.5%) of BPH tissue samples, of which all were positive by PCR (Table 2). A total of only 10 (14.1%) BPH tissues were positive by immunocytochemistry (ICC), of which 7 were also positive by PCR however, three *T. vaginalis* DNA-negative prostate tissues were also positive by ICC (Table 2).

T. vaginalis-specific antibodies with predominantly IgG4 antibodies were detected in 23 (32.4%) cases.

Table 1: *Trichomonas vaginalis* detection status and age of BPH patients.

Diagnosis	*T. vaginalis* positive		*T. vaginalis* negative	
	N (%)	Mean age ± SD	N (%)	Mean age ± SD
BPH and iCaP	18/75 (24.0)	61.7 ± 4.2	57/75 (76.0)	60.2 ± 7.1
BPH only	16/71 (22.5)	62.7 ± 5.3	55/71 (77.5)	61.8 ± 9.3

BPH: benign prostatic hyperplasia, iCaP: incidental prostate cancer

Table 2 shows a comparative analysis of the three diagnostic tests (PCR, P16 antigen assay, immunocytochemistry assay) using PCR as the standard test. P16 antigen assay shows perfect strength of agreement with the PCR however, strength of agreement with ICC assay is moderate.

Table 2: Comparative performance of PCR, P16 antigen test and Immunocytochemistry assay to detect *T. vaginalis* in prostate tissues from BPH patients.

		PCR		TOTAL
		+	-	
P_{16}	+	16	0	16
	-	0	55	55
	TOTAL	16	55	71
ICC*	+	7	3	10
	-	9	52	61
	TOTAL	16	55	71

***** *ICC: Immunocytochemistry*

Statistical evaluation of P16 antigen test & ICC Assay:

P_{16} antigen test:

McNemar's test: not applicable
Kappa (agreement)= 1.000
SE of kappa = 0.000
95% confidence interval: From 1.000 to 1.000
The strength of agreement is perfect.

Immunocytochemistry assay (ICC):

McNemar's test: p-value = 0.1459 (not sig. different to sensitivity)
Kappa= 0.442 (Moderate)
SE of kappa = 0.132
95% confidence interval: From 0.182 to 0.701
The strength of agreement is considered to be 'moderate'

Conclusion:

Our preliminary study suggests a direct evidence of *T. vaginalis* in BPH tissues with no clinical signs of prostatitis. Both PCR and P16 antigen assay to detect *T. vaginalis* in the prostate tissue from BPH patients showed perfect agreement however, the ICC assay showed moderate agreement and was also positive in 3 PCR-negative tissues.

The unexpected relative high detection rate of *Trichomonas vaginalis* in BPH tissue may suggest a possible implication of this organism in the pathogenesis of BPH however, more studies are needed to further strengthen this hypothesis.
We hypothesize that chronic T. vaginalis infection of prostate tissue may lead to BPH in elderly people.

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