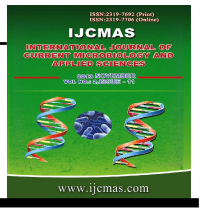
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 11 (2013) pp. 105-113**



Original Research Article

Trichomonas vaginalis infection as a risk factor for prostate cancer

Qasim Sharhan Al-Mayah[1], Mohammad A. K. Al-Saadi[2*] and Rebah Najah Jabbar[3]

[1]Al-Nahrain University/College of Medicine/Medical Research Unit, Iraq
[2]University of Babylon/College of Medicine/Department of Microbiology, Iraq
[3]Al-Nahrain University/Biotechnology Research Center, Iraq
*****Corresponding author*

K e y w o r d s

Trichomonas vaginalis; prostate cancer; chronic prostatitis; ant-trichomonas IgG.

A B S T R A C T

Infection plays a role in the pathogenesis of many human malignancies. Prostate cancer (PCa) is an important health problem in men worldwide. *Trichomonas vaginalis* infection is the most common non-viral sexually transmitted infection which can cause chronic prostatitis in infected men. This study aimed to investigate the assumption of *T. vaginalis* infection as a risk factor for PCa. Serum samples from 50 confirmed PCa patients and 40 healthy control were analyzed by means of enzyme-linked immunosorbent assay for the presence of ant-trichomonas IgG antibodies. In addition, DNA was extracted from 23 paraffin-embedded prostate tissue samples referred to the PCa patients. The extracted DNA was used for detection of hypothesized *T. vaginalis* nucleic acid by polymerase chain reaction technique using specific primers designed for this purpose. There was positive non-significant association between ant-trichomonas IgG antibodies and PCa (p=0.047, Odds ratio=3.895, 95% CI=1.016-14.929). No evidence for *T. vaginalis* nucleic acid with the host genome was observed. *T. vaginalis* may increase the risk of PCa when there is prolonged infection within the prostate.

Introduction

Prostate cancer (PCa) is now considered as one of the most important medical problems facing the male population. It is the most common noncutaneous neoplasm accounting for one quarter of all such cancers, and the second leading cause of death among men in the United States and many Western industrialized countries (Siegel *et al.*, 2011). As early as the 1950s Ravich and Ravich postulated that sexual transmission of a carcinogenic agent might explain observed differences in prostate cancer rates between men of varying religious backgrounds, owing to differences in circumcision practices among individuals with differing religious beliefs (Ravich and Ravich, 1951). *Trichomonas vaginalis* infection is one of the most prevalent non-viral sexually transmitted disease in the world (van der Pol, 2007). This protozoan has been proposed as a possible cause of PCa,

primarily because of its ability to infect and elicit inflammation within the prostate, and because of its common occurrence (Chen *et al*., 2013). Using PCR technique, Lee *et al*. (2012) detected *T. vaginalis* in 21.2% of Korean patients complaining of lower urinary tract symptoms, 71.4% of them have chronic prostatitis. Sutcliffe *et al*. (2006) have previously hypothesized that this parasite is frequently asymptomatic or non-specific presentation that may allow it to persist undetected and untreated in the male urethra and thus possibly ascend to the prostate with greater frequency than other more symptomatic sexually transmitted infectious agents that are now readily detected and treated (e.g., *N. gonorrhoeae*). Accordingly, these researchers conducted a nested case-control study of trichomonosis and PCa risk in the Health Professionals Follow up Study (HPFS), using serology to ascertain a history of trichomonosis. In that study, they observed a positive association between *T. vaginalis* serostatus and overall PCa risk (OR =1.43, 95% CI: 1.00-2.03), and a suggestion of a more pronounced association for high-grade disease (OR=1.76, 95% CI: 0.97-3.18). These results encouraged them to conduct two additional investigations of trichomonosis and PCa risk, one in the Prostate Cancer PreventionTrial (PCPT)(Sutcliffe *et al*., 2009), and the other in the Physician's Health Study (PHS)(Stark *et al*., 2009). While results in the PCPT were null, results in the PHS were more consistent with their original findings.

This study aimed to investigate the association of *T. vaginalis* through the detection with the PCa incidence, and the probability of the presence of the protozoan nucleic acid within the DNA of the prostate cells from PCa patients.

Materials and Methods

Study Design

This cross section study included 50 men with histopathologically confirmed PCa during the period from September 2011 to July 2012 from the Hospital of Radiation and Nuclear Medicine, and Al-Kadhumyia Teaching Hospitals in Baghdad-Iraq. The control group consists of 40 age-matched, unrelated men from Al-Kadhumyia Teaching Hospital and College of Medicine/ Al-Nahrain University. The mean ages of patients and control were 69.44±1.44 years and 66.48±1.299 years respectively. Informed consent from patients as well as control was taken which included age, smoking, drinking, residence, and family history of cancer.

Samples

Blood samples

Five mL of freshly venous blood was collected from each participant in plane tube which underwent centrifuagation where the serum was obtained and preserved at -20°C until be used.

Tissue samples

A total of 23 paraffinized prostate tissue samples that referred to patients with confirmed PCa were obtained from each patient.

Preparation of tissue sections

Tissue sections from paraffinized prostate tissue samples were prepared according to Greere *et al*. [9]. Five to six sections were cut from each block (each with 5 μM

thickness), and placed in a sterile 1.5 mL microcentrifuge.

Deparaffinizing sections

Method described by Greere *et al*. was used to remove paraffin from prostate tissues.

Estimation of serum level of prostate specific antigen (PSA) in control group

As some individuals with PCa have no obvious clinical signs, we conducted PSA test to confirm that control group have no PCa. The test was done using commercial kit (ACON laboratories, Inc./USA) according to the manufacturer's instructions.

DNA extraction from tissue samples

DNA was extracted from tissue samples using ready kit (gSYNC^{TM} DNA Mini Kit Tissue Protocol/ Geneaid/ Korea) according to manufacturer's instructions.

ELISA for detection IgG antibodies against *Trichomonas vaginalis*

A ready kit (Trichomono-IgG/Medical Biological Union/Russia) based on detection of α-actinin protein, was used to estimate the titer of specific IgG antibodies against *T. vaginalis* in patients and control groups. The test was done according to the manufacturer's instructions.

Primers and PCR protocols

It is possible that the process of formalin fixing and paraffin embedding lead to fragmentation of DNA (Greere *et al*., 1994).To ensure that our samples satisfy the criteria of PCR and produce the expected length of PCR products, we conducted a preliminary assessment to the DNA using a primer pair for human β-globin gene (HBB)(GenBank accession number Gu324922.1). The forward and reverse sequences of the primers are : 5'-TAGCTGTTTGCAGCCTCCC-3' and 5'-CCCTGGCCCACAAGTATCAC-3' respectively, with expected PCR product length of 311 base pairs. Cycling parameter was as follow: initial denaturation at 95°C for 4 min, followed by 35 amplification cycle of 94°C for 30sec, 59°C for 30 sec, and 72°C for 1 min. The final extension was at 72°C for 5 min.

Extracted DNA from tissue samples was used in PCR for amplification of supposed genes belong to *T. vaginals* incorporated within genome of prostate cells in patients with PCa. We used two pairs of primers to increase the probability of dectection. The first pair was for β-tubulin 3 gene (genBank accession number L05470.1), a gene which encodes for the amino acids sequence of beta-tubulin protein, a major component of *T. vaginalis* cytoskeleton. The forward and reverse primers were: 5'-TCCAAAGGTTTCCGATACAGT-3' and 5'-GTTGTGCCGGACATAATCATG-3' respectively, with expected product length of 195 bp. The other pair targets the conserved regions of the 18S ribosomal gene (GenBank accession number U17510.1). The forward and reverse primers were: 5'-TGAATCAACACGGGGAAAC-3' and 5'-ACCCTCTAAGGCTCGCAGT-3' respectively, with expected product length of 323 bp. Cycling conditions for both primers were as follows: 95 °C for 5 min followed by 35 amplification cycles of 94 °C for 15 sec, 55 °C (57 °C fo the second pair) for 30 sec and 72 °C for 30 sec. The final extension was at 72 °C for 7 min.

Agrose gel electrophoresis:

Gel was prepared by dissolving 2 gm of agarose (Biobasic/Canada) in 100 mL of 1x Tris Borate EDTA (TBE) (Biobasic/Canada), then put in an microwave for 5 min. After about 10 min, the gel was poured in the tray of the electrophoresis apparatus. When the gel solidifies it was transferred into the tank and enough amount of TBE was poured to just cover the gel. The comb was removed and a 10-µL aliquot of PCR product was mixed with 2 µL loading dye and loaded into the wells. Power supply was adjusted into 100 volt and run for 45 min. The gel then was stained with ethidium bromide (Biobasic/Canada)(0.5 µg/mL) for 20 min and examined using U. V. transilluminator with camera. The amplified products was determined by comparison with a commercial 1000 bp ladder (Kapa Biosystem/ United States).

Statistical analysis

The Statistical Package for the Social sciences (SPSS) 14.0version was used for statistical analysis. Chi-square test was used to estimate the correlation between serostatus and cancer. Risk association between the serostatus and PCa susceptibility was estimated by odds ratio and 95% confidence intervals using multivariate logistic regression.

Result and Discussion

Seropositivity for *T.vaginalis*

There was a positive significant (*P*=0.047 0.1625) association between IgG anti *T. vaginalis* antibodies serpositivity and PCa. Out of 50 confirmed PC patients, 12 (24%) cases gave sero-positive results for *T. vaginalis* and 3 cases gave equivalent results compared to only3 (7.5%) positive cases and 3 equivalent among 40 controls (Figure 1).

In logistic regression analysis, men who gave seropositive result to T. *vaginalis* have a 3.895 fold risk of PCa (OR= 3.895, 95% CI=1.016-14.929).

Of note, the prevalence of seropositivity was higher among younger patients than older ones. Out of 17 PC patients of 45-65 years age class, there was 8 (47.1%) subjects gave positive results compared to only4 (12.1%) patients of ≥ 66 years gave positive results with significant difference (table 1).

On the other hand, there are no significant differences in the seropositivity between rural and urban residences. Out of 26 patients of rural residency, there were 7 patients (26.9%) had positive result compared to 4 patients (16.7%) having seropositive result among 24 urban residence (table 1).

***T. vaginalis* nucleic acid detection**

Figure 2 shows the results of PCR for *β-globin* gene amplification in 23 tissue samples from confirmed PC patients. Only 19 samples had PCR products of 311bp, which indicates that these samples still have integrated DNAs ≥ 311bp. So these samples were candidate to further investigation for detection of supposed *T. vaginalis* nucleic acid within their DNAs. However, none of these gave positive result for the primers of BTUB3 or 18S rRNA gene

Figure.1 Seropositivity of PCa patients and control to *T. vaginalis*

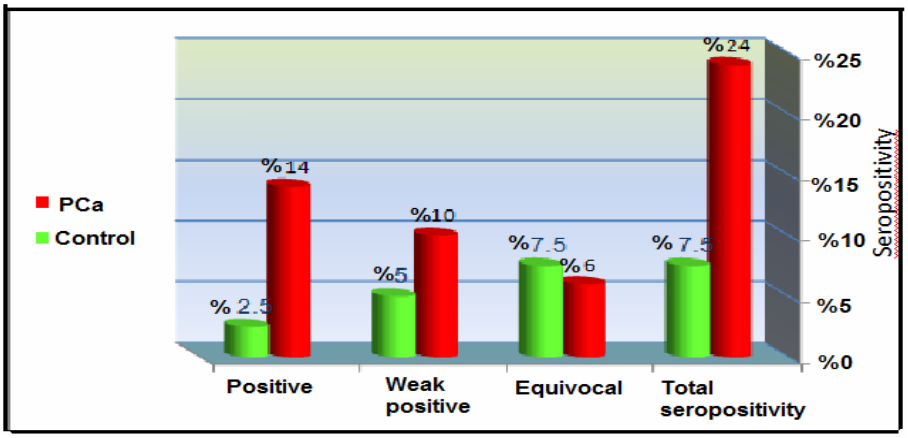
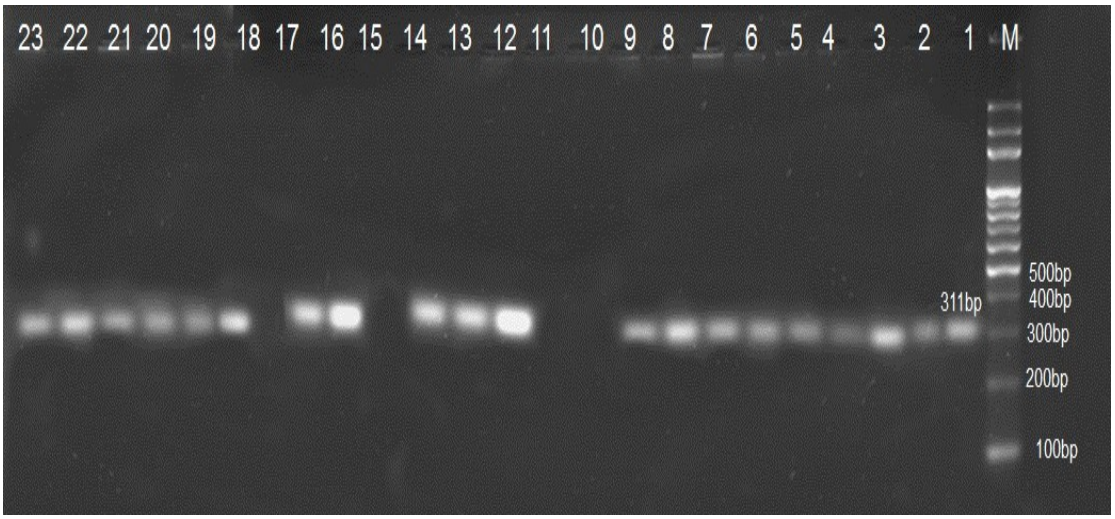


Table.1 Seropositivity to *T. vaginalis* in different age classes and in rural and urban residence

Risk Factor	Serostatus		χ^2	*p*-value
	Positive No (%)	Negative No(%)		
Age (Years)				
45-65 (n=17)	8 (47.1%)	9 (52.9%)	7.509	0.006
≥ 66 (n=33)	4 (12.1%)	29 (87.79%)		
Residence				
Rural (n=26)	8 (30.77%)	18 (69.23%)	1.361	0.243
Urban (n=24)	4(16.7%)	20(83.3%)		

Figure.2 gel electrophoresis for detection of BHH gene in DNA extracted from PCa tissue. Expected PCR pproduct is 311 bp. M=marker, Lane 1-9,12-14, 16,17, 19-23 positive amplification, lane 10-11, 15,18 no PCR product.



Multiple risk factors, among which is the chronic inflammation, are known to cooperate to induce PCa Trichomonads have been observed to infect and elicit a inflammatory and immune response within the prostate (Chen et al., 2013). The α-actinin protein is one of the most immunogenic protein of *T. vaginalis.* This protein is constantly observed on the surface of epithelial cells lysed by the parasite and thus it exposed to immunologic recognition (Bricheux *et al*., 1998) [10]. Moreover, it is not found among other microorganisms and share little amino acid sequence identity with human homolog (Sutcliffe *et al*., 2012). It is because this property, antibodies formed against this protein became a target for the detection of *T. vaginalis* infection. However, when these antibodies are of IgG type, it generally cannot be explained as current infection. Rather, it indicates current or past infection. The significant association between seropositivity to *T. vaginalis* and PCa in our study reflected the role of this protozoan in the initiation of PCa. The ability of *T. vaginalis* to induce inflammation and direct lysis of epithelial cells, and subsequent renewal in the prostate is well documented. But it is hard to postulate that every microorganism , or even non-living factor, with these properties can induce PCa. *Chlamydia trichomatis*, for example, can induce inflammation in prostate but, to our knowledge, all literatures not only refuted the possible role of these bacteria in PCa but also some of them even reported negative association (Dennis et al., 2009). Although the precise mechanism by which *T. vaginalis* can be involved in the initiation of PCa is not yet known, many hypotheses have been postulated.

One hypothesis suggested that the adhesion of *T. vaginalis* to the epithelial cells of the prostate upregulates the expression of anti-apoptotic genes, such as defender against cell death and cyclogenase (Sutcliffe *et al*., 2006). Wang *et al*. (2010) showed that COX-2 expression is induced in primary human vaginal epithelial cells upon interaction with either *T. vaginalis* or purified trichomonad adhesion AP56. Cox-2 overexpression in PCa has been documented and was associated with both cancer initiation and progression by affecting cell proliferation, mitosis, cell adhesion, apoptosis and immunosurveillance, and/or angiogenesis (Sutcliffe *et al*., 2012).

The other hypothesis is the alteration of spermine and putrescine polyamines involved in the cellular growth, differentiation and death (Wallace *et al*., 2003). Trichomonads cannot synthesize spermine, and instead, they secrete large amount of putrescine during growth, evidenced in genital secretions from patients with trichomonosis (Garcia *et al*., 2005). Therefore they must import exogenous spermine through antiporter system coupled to the secretion of two molecules of putrescine for each molecule of spermine (Yarlett *et al*., 2000), with subsequent decreased levels of spermine in prostate cells. Interestingly, spermine has been shown to have free radical scavenger properties (Gugliucci, 2004), let alone its activity of inhibition carcinoma growth in vitro and in vivo (Smith *et al.*, 1995) and reduced level of spermine has been found in malignant prostate tissue compared to normal or hyperplastic tissue (van der Graaf *et al*., 2000).

The relatively high incidence of seropositivity among younger age class of PCa patients may be referred to the sexual activity of this class. Men are infected with *T. vaginalis* from infected women

who are considered as the source of infection (despite the ability of infected man to transfer the infection to healthy women by sexual contact). So, it is reasonable to assume that the more frequent sexual contact, the more chance for male to get infection from infected female. Although there is no information about the age of patients' spouses neither their situation regarding infection with *T. vaginalis*, we can deduce their age over 40 years old. The incidence of *T. vaginalis* in this age group of women in Iraq ranged from 15.6%-35% (Mahdi *et al*., 2001; Kadhum, 2012), a percentage which is high enough to be regarded as a source for husbands infection especially when we take into account that *T. vaginalis* can be recovered from 30%-60% of male partners of infected women (Madico *et al*., 1998).

Many previous reports have shown higher prevalence of *T. vaginalis* in rural area compared with urban (Mahdi *et al*., 2001; Kadhum, 2012; Ryder *et al*., 2012). In this study, there was slight non-significant difference between the two residencies which may be due to small sample size. However, the increased rate of infection in rural areas may be referred to low hygienic measures and reduced health care seeking among infected population. It is worth mention that if trichomonosis is left without treatment, the infection can persist for at least 3 months (Zhang *et al*., 1995). During this relatively long period, *T. vaginalis* transmits among dyads and can cause chronic infections to the infected organs, with probable risk of PCa in men and cervical cancer in women (Zhang *et al*., 1995; Pustan *et al*., 2010).

Horizontal gene transfer (HGT) is a relatively new issue in parasites and has been received less attention than that in case of bacteria. Parasite can be donors, recipient, or conduit for/of HGT (Wijayawardena *et al.*, 2013). Apicomplexa, *Theileria* infection in bovine leukocytes induces transformation of host cells and infected leukocytes can be kept indefinitely in culture (Durrani *et al*., 2012). Despite the fact that *T. vaginalis* is extracellular protozoan compared with intracellular *Theileria*, exchange of genomic segments with the host cell is not impossible, however, this study did not give solid evidence for that. From the results of this study we can conclude that *T. vaginalis* could be a player in PCa onset when there is prolonged, mild, untreated inflammation with this parasite.

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