RESEARCH ARTICLE

Evaluation of Human Papillomavirus Infections in Prostatic Disease: a Cross-Sectional Study in Iran

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Abstract

Background: The role of inflammation in prostate diseases is suggested by the presence of inflammatory cells within the prostate in benign prostatic hyperplasia (BPH) and prostate cancer (PCa) patients. In addition, bacterial and viral infection may lead to chronic and recurrent inflammation of the prostate. The human papillomaviruses (HPVs) are a family of sexually transmitted viruses which have been implicated in the aetiology of cervical cancer and several other malignancies. This study evaluated the frequency of HPV infection in individuals with prostatic disease in Iran. Materials and Methods: The study included formalin fixed paraffin- embedded tissue samples of 196 primary prostate cases, including 29 PCa and 167 BPH samples. HPV DNA was purified and amplified through MY09/MY11 and GP5+/GP6+ primers with nested PCR. All patients were interviewed using a questionnaire to collect demographic information. Results: Nested PCR showed that HPV DNA was found in 17.2 percent of PCa samples and 4.8 percent of BPH samples (not significant). Conclusions: Our data do not support a significant role of HPV infection in prostatic disease in Iranian patients, but demographic data indicated a probable association between presence of HPV DNA and risk of inflammation in prostate tissue which might lead to prostate carcinoma. Further studies are required to elucidate any roles of HPV infection in prostatic disease.

Keywords: Human papillomavirus - prostate carcinoma - benign prostatic hyperplasia - Iran

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Introduction

Papillomavirus are a group of genetically related organisms, which infect epithelium and infuse proliferation variation in infected cells, which can lead tissues in both benign and malignant tumors (Maghrabi et al., 2007). More than 100 different types of HPV recognized to date, that infect epithelial cells and bring about the formation of benign hyper proliferative lesions more commonly identified as warts, for example types 6 and 11 infect the genital system and beget genital warts, to these so- called low- risk HPV types, since high- risk HPV types 16 and 18 are linked to cancer and have been shown to passes oncogenic potential (Dell and Gaston, 2001; Yahyapour et al., 2012).

Prostate diseases, benign prostate hyperplasia and prostate cancer, are chronic diseases that need a long period for development from small lesion to became clinical manifestation. In these both prostatic diseases, there were an imbalance between prostate cell growth and apoptosis (Hamid et al., 2011). Up to 30 percent of men may affect by symptomatic BPH in early 70 s (Webber, 2006). Acute and chronic urinary retention are depends on most common and significant complications of long-term BPH (Stamatiou, 2009), also prostate cancer is fifth common cancer and the second most common cancer in men (Silvestre et al., 2009).

Although it is confirmed that Androgens effect on progress of BPH and PCa but recent data investigate that the action of Androgens alone cannot explain the hyperplastic development of the prostate gland. Definitely several mitogenic growth factors have been implicated in the aetiology of enlargement, despite several significant studies efforts, the aetiology and pathophysiology remained unclear. Different important sources exist for the initial event, including direct infection with viruses, fungi, mycobacteria and parasites (Prakash et al., 2002; Hamid et al., 2011). Viral infection may lead to chronic and recurrent inflammation of the prostate and initiate or promote carcinogenesis (Martinez-Fierro et al., 2010).

The impact of inflammation in prostate diseases is expressed by the attendance of inflammatory cells within the prostate tissues in BPH and PCa patients. Several histopathologic results confirmed that inflammation is

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much more common in the transition zone and peripheral zone of the prostate gland, which is a prediction for BPH and PCa (Hamid et al., 2011). A relationship between HPV infection and prostate carcinoma has been suggested and some studies demonstrated and confirmed that HPV infection play a main role in the progression and tumorigenesis (Dell and Gaston, 2001; Maghrabi et al., 2007).

Carcinogenesis is a multistep complex involving several genetic and epigenetic events in protooncogenes, tumor suppressor genes and antimetastasis genes (Anwar et al., 1992). The frequency of ras mutation has increased in prostate carcinoma patients with higher Gleason score. Also the clinical correlation of the Gleason score with other prognostic factors including metastasis is well established. Similarly, the HPV infection rate has also increased significantly with the increasing Gleason score (Anwar et al., 1992). Al- Maghrabi has demonstrated that the rate of HPV infection has increased in patients with stage promotion of the tumor and with higher Gleason score (Maghrabi et al., 2007). Downregulation of KAI1 metastasis suppressor protein is associated with dismal prognosis in a variety of cancers and Mutation of p53 was proposed to be involved whit KAI1-downregulation. In cervical cancer, p53 is inactivated by HPV oncoprotein E6 with the stage of inactivation depending on the HPV type (Schindl et al., 2001).

This study evaluating the frequency of HPV infection in patients with prostatic diseases in capital of Iran on a cross- sectional study.

Materials and Methods

Study population

The study included formalin- fixed and paraffin embedded tissue samples of 196 primary prostate cases, including 29 prostate adenocarcinoma and 167 BPH samples. Specimens were obtained from patients of Mostafa Khomeini Hospital of Tehran city. All patients were interviewed by inspectors using a questionnaire to collect demographic information and they had negative histories of exposure to either chemotherapy or radiotherapy prior to surgery. BPH and PCa confirmed by pathologist through standard criteria. Demographic and medical information including age, habitant, Gleason score and metastasis collected from patients medical records.

DNA extraction

5- 10 slides (depends on type of sampling: transurethral resection of the prostate or total prostatectomy) of about 10 μm wide were deparaffinized in xylene and absolute ethanol, then DNA was extracted using High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany). Value index and purity of extracted DNA was studied by Nanodrop (Thermo Scientific, NanoDrop 1000, Wilmington, USA) and then ß- Globin was used as a control to DNA viability with PCO3/ PCO4 primers.

Nested PCR

HPV DNA was amplified through MY09/MY11 and GP5+/GP6+ primers by Nested PCR. The MY09/MY11 primer set (MY09 5'– CGT CCA/C AA/GA/G GGA A/ TAC TGA TC –3' and MY11 5'– GCA/C CAG GGA/T CTA TAA C/TAA TGG –3'). Which amplify the L1 gene of HPV was capable of amplifying a wide spectrum of HPV types to produce a PCR product of 450 bp. The PCR reaction for MY09/MY11 primers was performed using the following steps; 5 min at 94˚C; then 40 cycles at 94˚C for 1 min, 55˚C for 1 min and 72˚C for 1 min. Finally reactions were carried out to a final extension for 5 min at 72˚C. Also nested PCR completed by amplified GP5+/ GP6+ primers. The GP5+/GP6+ primers set (GP5+ 5'– TTT GTT ACT GTG GTA GAT ACT AC –3' and GP6+ 5' – AAA AAT AAA CTG TAA ATC ATA TTC –3') is a non-degenerate primer set that detects a wide range of HPV types using a lower annealing temperature during PCR and produces a PCR product of approximately 150 bp. Second round of PCR reactions were as follows; 4 min at 94˚C; then 38 cycles at 94˚C for 1 min, 40˚C for 2 min and 72˚C for 2 min. finally reactions were carried out to a final extension for 4 min at 94˚C. Each batch of samples included negative controls without a DNA template; and one positive control containing HPV- 18 was extracted from HeLa cell line and HPV- 16 and HPV- 18 approved by WHO.

PCR products were separated by electrophoresis through 1% and 2% Agarose gel respectively and then HPV positive samples were detected.

Statistical analysis

All statistical analysis was performed with SPSS 14 software. The significance level was set at $P<0.05$.

Results

Of 196 confirmed prostatic patients enrolled to our study, which is divided in 29 (14.8%) PCa and 167 (85.20%) BPH patients. The mean age in PCa patients was

Table 1. Demographic Data and Medical Records of PCa and BPH Patients Entry into Study

Variable	Benign prostatic hyperplasia		Prostate carcinoma	
	HPV-positive (n=8)	Negative (n=159)	HPV-positive (n=5)	Negative (n=24)
Mean age (SD)	70.08±4.20	68.84±6.06	64.20±4.37	67.23±5.07
Marital status				
Married	7 (87.5%)	145 (91.19%)	5 (100%)	22 (91.66%)
Not married	1 (12.5%)	14 (8.80%)	-	2 (8.33%)
Location				
Urban	7 (87.5%)	125 (78.61%)	5 (100%)	20 (83.33)
Rural	1 (12.5%)	34 (21.38%)	-	4 (16.66)
Surgery				
TUR-P*	6 (75%)	46 (28.93)	2 (40%)	17 (70.83)
TP**	2 (25%)	113 (71.06)	3 (60%)	7 (29.16%)
Gleason score				
Low	-	-	-	14 (58.33%)
Moderate	-	-	2 (40%)	6 (25%)
High	-	-	3 (60%)	4 (16.66%)
Distant metastasis				
Present	-	-	2 (40%)	4 (16.66 %)
Absent	-	-	3 (60%)	20 (83.33%)

*TURP: Transurethral resection of the prostate. **TP: Total prostatectomy

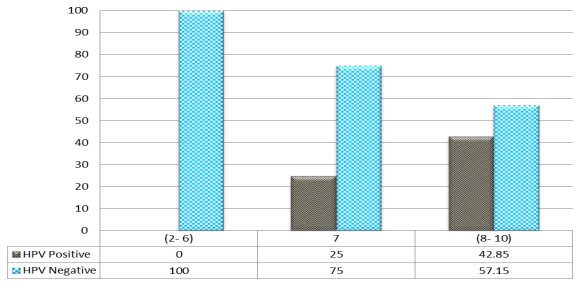

Figure 1. Frequency of HPV Infection in PCa Patients Based on Gleason Score (%)

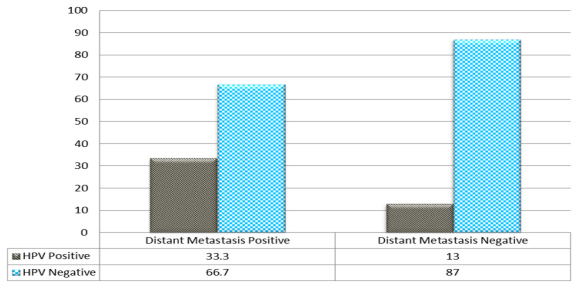

Figure 2. Frequency of HPV Infection in PCa Patients Based on the Presence of Distant Metastasis (%)

66.1±6.11 years (range 50-88 years) and in BPH patients 69.3±6.30 years (range 49-90 years). The mean Gleason score of PCa cases was 6.1±1.04 (range 2-9). Distant metastasis was seen in 6 (20.96%) of 29 PCa patients.

Value index and purity of DNA extraction processes and DNA viability were confirmed for all samples. Presence of HPV- DNA corroborated in 5 (17.2%) of 29 PCa patients and 8 (4.79%) of 167 BPH patients by nested PCR method. There was no obvious difference between PCa and BPH following the presence of HPV infection. Also, the mean Gleason score of HPV infected carcinoma cases was 7.9±1.10 (range 7-9) and distant metastasis was seen in 3 (60%) of 5 PCa patients infected with HPV. Also the Table 1 has shown much demographic and medical information about patients.

Discussion

This study screened the role of HPV infection in prostate enlargements. Although no statistically significant difference between HPV infection and prostate carcinoma or benign prostatic hyperplasia was found, but medical records has indicated some reasons about association between HPV infection and accession of prostate carcinoma.

The oncogenic types of HPV are recognized as the major cause of intraepithelial neoplasia of the anogenital tract (Korodi et al., 2005; Aghakhani et al., 2011). An association of prostate cancer with sexual history, particularly sexual transmitted diseases like HPV infection, has been reported in several studies (Adami et al., 2003; Korodi et al., 2005; Martinez-Fierro et al., 2010; Chen et al., 2011). Most investigations reported a high frequency of HPV infection in BPH and PCa samples (McNicol and Dodd, 1991; Silvestre et al., 2009; Aghakhani et al., 2011), other investigations found a possible association between HPV and prostate carcinogenesis (Silvestre et al., 2009). On the other hand, wide ranges of studies are not clearly understood any association between HPV infection and prostate carcinogenesis and refuse the causal role of HPV infection in progression of BPH (Dodd et al., 1993; Noda et al., 1998; Leiros et al., 2005; Gazzaz and Mosli, 2009; Aghakhani et al., 2011; Chen et al., 2011; Smelov et al., 2011).

We designed a cross sectional study to clarify the status of HPV infection in prostatic diseases in Iranian population, therefore there is great variation between the range of PCa samples and BPH samples. This study expressed higher rate of HPV infection in PCa patients than BPH patients. Thus there is a possible role for HPV infection to association in prostate carcinogenesis. We found rare incidence of HPV infection in BPH samples and statistical analysis confirmed no relation between HPV infection and tumorigenesis, therefore our results do not support the role of HPV infection in benign prostatic hyperplasia.

The contributions of immune and inflammatory responses to the development of cancer have been well recognized in different malignancies. Viral infections may lead to chronic or recurrent inflammation of prostate and initiate or promote carcinogenesis. It is well known that the first line of defense against viral infection is the interferon response, as E6 and E7 HPV proteins can inhibit the signaling capacity of interferon pathway. This may be associated with disease aggressiveness (Grivennikov et al., 2010; Martinez-Fierro et al., 2010). HPVs are oncogenic viruses and present oncogenic activity through spoiling mucosal immune resistance and destroying tumor suppressor genes (Yahyapour et al., 2012).

In a study by Hrbacek et al. (2003), it has been shown that positive association between HPV infection with higher grade of Gleason score (Hrbacek et al., 2011), also the frequency of the HPV infection increased in patients with advanced stages of the prostate carcinoma and with the higher Gleason score (Anwar et al., 1992). Several studies investigated positive role between HPV infections with metastasis, these results clearly explain the importance of HPV infections in the tumor aggressiveness (Goldenberg et al., 2008; Marklund et al., 2010).

However, it was expressed in a study that no association between HPV infection (HPV-16 and HPV-18) temperament and Gleason score, grade of disease or a combined measure of disease aggressiveness (Karin et al., 2003). Also investigators reported no association between HPV infections and tumor aggressiveness represented by Gleason score and development and progression of the disease (Tu et al., 1994; Martinez-Fierro et al., 2010). Hence there isn't obvious conclusion about the role of HPV infections in tumor aggressiveness and development of carcinogenesis.

According to (Figure 1) Gleason score in HPV infected patients is significantly higher than non-infected patients, also the incidence of distant metastasis is most common in HPV infected patients (Figure 2 and Table 1). Our results confirmed previous investigations about the positive role of HPV infection in development of carcinogenesis and tumor aggressiveness. Demographic data including age, marital status, habitant and type of surgery showed there was no important difference between HPV infected and

non- infected PCa and BPH samples.

In conclusion, our results do not support the significant role of HPV infection in prostatic disease in Iranian population, but medical records expressed a supportive role for HPV infection in progression of prostate cancer and risk of inflammation in prostate tissues and may lead to carcinogenesis and metastasis.

Successful administration of the HPV vaccination and education of healthy sexual behavior would be appropriate schedules in order to prevention of HPV infection and reduce the risk of prostate cancer in adolescents.

Further studies in comprehensive populations would help improvement our understanding of the role of HPV infection in the etiology of prostatic diseases.

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