

## RESEARCH ARTICLE

# Human Papilloma Virus Detection by INNOLiPA HPV in Prostate Tissue from Men of Northeast Mexico

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### Abstract

**Background:** Prostatic adenocarcinoma by Prostate cancer (PCa) is the most prevalent cancer and the second cause of cancer-related death among men in the Western world. Human papilloma virus (HPV) may be considered as a preventable risk factor. In this study, we assessed the frequencies of HPV infection in prostatic adenocarcinoma and benign prostatic hyperplasia (BPH) cases in Northeast Mexico. **Materials and Methods:** A total of 87 paraffin-embedded blocks (from 25 and 62 patients with definite diagnoses of BPH and adenocarcinoma, respectively) were selected and subjected to INNOLiPA HPV Genotyping to detect 28 high- and low-risk HPV types. The rates of infection were compared in the two studied groups. **Results:** INNOLiPA HPV demonstrated great sensitivity for HPV detection on paraffin-embedded tissue. Global prevalence was 14.9% (13/87). HPV infection was positive in 19.4% (12/62) of patients with adenocarcinoma and 4.0% (1/25) of patients with BPH. HPV-11, which is considered to be low risk, was more prevalent. Interestingly, one patient with BPH and six with prostate cancer showed examples considered to be high risk (HPV-18, -51, -52, and -66). **Conclusion:** A higher rate of HPV infection among Mexican patients with prostatic carcinoma than among those with BPH was observed. HPV infections may thus contribute to the risk of prostate cancer. Further studies are required to elucidate any roles of HPV infection in prostate disease in Mexico and the effect of prevention and treatment of HPV infection on prostatic adenocarcinoma.

**Keywords:** : Prostatic carcinoma- benign prostatic hyperplasia- human papilloma virus- INNOLiPA HPV- Mexico

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### Introduction

Prostate cancer (PCa) is the second leading cause of cancer-associated mortality in the United States and the first in Mexico among men. PCa is the most commonly diagnosed cancer in males (Siegel et al, 2012).

The role of inflammation in prostate diseases is suggested by the presence of inflammatory cells within the prostate in benign prostatic hyperplasia (BPH) and PCa patients, and has been associated with prostate cancer carcinogenesis and progression. Infections, especially sexually transmitted ones and those that reach the urogenital tract, are possible causes of intraprostatic inflammation. Infectious organisms are essential for the maintenance of local inflammatory processes, which can cause cellular changes responsible for genetic and epigenetic changes that lead to cell transformation (Sfanos et al., 2013). In this context, special emphasis is given to human papillomavirus (HPV).

Human papillomaviruses (HPVs) are small epithelium

tropic viruses belonging to the Papillomaviridae family and consist of 8 kb double-stranded circular DNA surrounded by a nonenveloped capsid. More than 150 different HPV types have been described, forty  $\alpha$ -HPV types have been shown to infect the anogenital tract of which 13 are classified as high-risk or oncogenic according to the World Health Organization (Dell and Gaston, 2001). These sexually transmitted viruses are classified either as “low-risk,” or nononcogenic HPV-type (e.g., HPV-6 and -11), or as “high-risk,” or oncogenic (e.g., HPV-16 and HPV- 18) (Dell and Gaston, 2001). HPV codes for the oncoproteins E6 and E7 that are able to immortalize prostate tissue cells, in vitro through the inactivation of the tumor suppressor genes, P53 and PRb (Choo et al., 1999).

A relationship between HPV infection and prostatic carcinoma has been suggested, and some studies have demonstrated and confirmed that HPV infection plays a main role in progression and tumorigenesis (Dell and Gaston, 2001). A recent meta-analysis study showed a significantly increased risk of prostate cancer with the

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positivity of overall HPV detected in prostate tissues (Yang et al, 2015).

The prevalence of HPV DNA in tumor tissue of patients with prostate cancer has been poorly investigated in Mexico (Martinez Fierro, 2010). Therefore, the present study examined the prevalence of high- and low-risk HPV, using a highly sensitive assay, in BPH and prostatic adenocarcinoma blocks in Northeastern Mexico.

## Material and Methods

### Patients

Biopsies in formalin-fixed paraffin-embedded tissues were collected for 62 patients who were diagnosed with primary prostate cancer and 25 patients with BPH in a single institution in the Northeastern area of Mexico (seven states) from January 2014 to December 2015.

BPH and PCa were confirmed by a pathologist through standard criteria. Tumors were staged using standard criteria by Gleason score and TNM staging.

All patients had negative histories of exposure to either chemotherapy or radiotherapy prior to surgery, and they had no other diagnosed cancers. Demographic and medical information including age, habitat, Gleason score, and metastasis was collected from patients' medical records.

The use of formalin-fixed and paraffin-embedded prostate cancer tissues and the related clinical information were approved by the Research and Ethical Committee of Unidad Médica de Alta Especialidad (UMAE) 25.

### DNA extraction

To extract DNA, tumor fragments were incubated in 200  $\mu$ L TE solution (1 mM EDTA, Tris 10 mM, pH 7.4) with 20 mg/mL of proteinase K at 65°C overnight or until complete fragment digestion. Subsequently, the proteinase K was inactivated at 95°C for 15 min, and the total product was used for phenol-chloroform DNA extraction. The value index and purity of extracted DNA was studied by NanoDrop (Jenway, NanoDrop, OSA, United Kingdom).

### Detection-Genotypification HPV

The INNO-LiPA HPV genotyping test (Innogenetics NV, Ghent, Belgium) directs the amplification of a 65-bp region of the HPV L1 gene and allows the identification of 28 anogenital HPV genotypes, with the inclusion of a human DNA internal control, HLA-DPB1 (270 bp), and two HPV controls.

Results from specimens that failed to amplify the controls, indicating invalid samples, were excluded from the study.

INNO-LiPA HPV permits simultaneous detection of multiple genotypes in a single sample.

## Results

The mean age for the BPH group and the prostatic adenocarcinoma group was  $61 \pm 11.9$  and  $64 \pm 6.4$  years respectively ( $P = 0.73$ ). The 25 patients with BPH had undergone transurethral prostatic resection of the prostate,

and the 62 with prostatic adenocarcinoma had undergone open prostatectomy. The 35% was Gleason 2-6, 43% Moderate-Gleason 7, and 22% High- Gleason 8-10.

The overall prevalence of HPV infection in all studied samples was 14.9% (13/87). The frequency of HPV infection in patients with BPH and prostatic adenocarcinoma was 4% and 19.4% respectively.

The analysis of HPV genotype was performed. Of 13 samples HPV positive, HPV-11 considered as low risk, was more prevalent (46.1%). One patient with BPH present HPV-18 considers as high risk. Interestingly, in six patients with prostate cancer were detected HPV-high risk (types 51, 52, and 66). Two samples (15.4%) showed multiple infections (two types of HPV).

Sensitivity of INNO-LiPA HPV. The results obtained using the INNO-LiPA HPV kit were compared with PCR using MY09 and MY11 primers. Of the 87 samples analyzed, three were true positive, 74 true negative, 10 false negative, and none false positive. Validation analysis revealed a sensitivity of 23% and 100% specificity of PCR (MY09/MY11) with respect to the INNO-LiPA kit.

## Discussion

HPV prevalences in patients with prostate cancer demonstrate large variations worldwide, ranging between 2% and 65% (Anwar et al., 1992; Stricker et al, 1998). The prevalence of HPV infection in Mexican patients with prostate cancer (19.4%) is according to the global prevalence (18.9%) reported in a recent meta-analysis study (Yang et al., 2015).

A recent study using Real Time PCR showed that HPV DNA was found in 20.0% of 200 patients with PCa (Atashafrooz and Rokhbakhsh-Zamin., 2016).

Our results are in accordance with previous reports that indicated an association between presence of HPV-DNA and risk of inflammation in prostate tissue, which might lead to prostatic carcinoma.

The mechanism of HPV infections and prostate cancer development is far from clear. It has been proposed that exposure to environmental factors such as infectious agents and dietary carcinogens, and hormonal imbalances lead to injury of the prostate gland and to the development of chronic inflammation and regenerative 'risk factor' lesions, referred to as proliferative inflammatory atrophy (Ghasemian et al., 2013).

McNicol and Dodd (1990), were the first to demonstrate HPV genomic sequences in prostate tissues. Subsequent studies, such as, provided further evidence to support a relationship between HPV and prostate cancer (Anwar et al., 1992; Martinez-Fierro et al., 2010, Atashafrooz and Rokhbakhsh-Zamin., 2016). The oncogenic types of HPVs are recognized as the major cause of intraepithelial neoplasia of the anogenital tract (Aghakhani et al., 2011).

In our study, we found different types of oncogenic high-risk HPV (types 18, 51, 52, and 66) without the presence of a predominant HPV type, suggesting that the HPV association with PCa is not due to a specific type of HPV, as implied in previous reports, particularly because HPV16 was not detected in this study (Serth et al.,

1999). However, further studies are needed to determine the prevalence and association of type-specific HPV with prostate cancer.

On the other hand, several studies have found that HPV DNA is not preferentially associated with either BPH or prostate cancer (Strickler et al., 1998; Aghakhani et al., 2011).

These discrepancies may be explained by the following. (a) Geographic differences. A previous study suggested the link between HPV infections and prostate cancer, although the risk estimates varied by study region. A high prevalence of HPV has been reported in Africa (68.3%) with respect to North America, Europe, Asia, Latin America, and Oceania (15.2% to 20.2%). (b) Differences in sexual behavior. An association of prostate cancer with sexual history, particularly sexually transmitted diseases like HPV infection, has been reported (Adami et al., 2003; Martinez-Fierro et al., 2010). (c) Gleason score. In a study by Hrbacek et al. (2011), a positive association has been shown between HPV infection and a higher Gleason score. The frequency of HPV infection also increased in patients with advanced stages of prostatic carcinoma and with a higher Gleason score (Anwar et al., 1992). Several studies have investigated a positive role between HPV infections and metastasis, and their results clearly explain the importance of HPV infections in tumor aggressiveness (Marklund et al., 2010). (d) Technical difficulties in HPV DNA detection. There are several possible reasons for false-positive or false-negative results, most notably PCR contamination, degradation, or cross-contamination of DNA, and tumor samples that are not representative (Adami et al., 2003).

Sensitive and accurate detection of HPV genotypes in such archival tissues could be affected, as DNA is often degraded as a result of long and poor storage conditions. Such damage to DNA includes chemical modification, cross-linking, and fragmentation, all of which can reduce the efficiency of PCR amplification (Medeiros et al., 2007).

We demonstrated a high sensitivity of the INNOLiPA test with respect to PCR using MY09/MY11. LiPA has a greater sensitivity for detection, given that the amplicon target (65 bp) is smaller than MY09 and MY11 (450 bp). Utilization of assays targeting longer amplicons could result in decreased sensitivity on paraffin-embedded tissue.

In conclusion, the higher rate of HPV infection among Mexican patients with prostatic carcinoma than among those with BPH indicates a probable role of HPV in the pathogenesis of prostatic carcinoma. Further studies are required to elucidate any role of HPV infection in prostate disease and the effect of prevention and treatment of HPV infection on prostatic adenocarcinoma.

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