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Non – Detection of HPV DNA in Prostatic Cancer and Benign Prostatic Hyperplasia: a case- control study in Kerman

Elham Jafari, M.D.[1], Shariar Dabiri, M.D.[2], Abbas Mortazaeizadeh, M.D.[3], Ahmad Reza Sayadi, Ph.D.[4], Sahar Amir Poor Rostami, M.Sc.[5]

1- Assistant Professor, Pathology and Stem Cells Research Center, Kerman University of Medical Sciences, Kerman, Iran (Corresponding author; E-mail: ejfarda@yahoo.com)

2- Professor of Pathology, Physiology Research Center, Institute of Basic and Clinical Physiology Sciences, Kerman University of Medical Sciences, Kerman, Iran

3- Researcher, Pathology and Stem Cells Research Center, Kerman University of Medical Sciences, Kerman, Iran

4- Assistant Professor, Social Determinants of Health Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

5- Pharmaceutics Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran

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Abstract

Background: Different studies assessed the role of inflammation in malignancy of different organs. Prostatitis has been suggested as a cause of cancer and BPH. Till now, more than 100 types of human Papilloma virus (HPV) are recognized including low and high-risk groups for carcinogenesis. Among them HPV-16 and HPV-18 have shown further association with cancer. Regarding the presence of E6 and E7 in HPV and ability for modification of basal epithelial cells, related role in prostate cancer (as well as cervical and genital malignancies) is hypothesized. The aim of this study was to determine the association of HPV-16 and HPV-18 with prostate cancer and malignancy degree.

Methods: A total of 75 consecutive paraffin-embedded blocks including 50 samples with primary prostate cancer and 25 samples with benign prostate hyperplasia (BPH) as control were studied. Amplisense kit was used for replication at real-time polymerase chain reaction (PCR) to determine genotypes of HPV-16 and HPV-18. DNA purity was assessed by Nano Drop.

Results: The results of real-time PCR demonstrated that none of the samples of BPH and prostate cancer had amplification of HPV DNA.

Conclusion: The results revealed that HPV-16 and HPV-18 are not causes of prostate cancer.

Introduction

Prostate carcinoma is the fifth most common malignancy and the second most common cancer among men worldwide (1). In Iran, it is the second most common cancer among men after gastric cancer (2). Age is an important risk factor for prostate carcinoma. However in 25% of cases, it occurs in subjects under 65 years of age (3). Other contributing factors of prostate cancer include genetic factors such as Hereditary prostate cancer 1(HPC1) and Predisposing for cancer of prostate 1(PCAP), fat-enriched regimen, hormones especially the ratio of luteinized hormone and testosterone to di-hydro-testosterone, consumption of alpha-reductase drugs and inflammation (4).

Inflammation is an important factor in several types of malignancies especially prostate cancer (5). Chronic prostate inflammation, especially due to sexually transmitted disease

(STD) and viral ascending urinary tract infections by human Papilloma virus (HPV), Herpes simplex virus (HSV), and Cytomegal virus (CMV), might have role in prostate cancer development.

Human Papilloma virus includes two main types; low-risk subtypes such as HPV-6 and HPV-11 accompanied with genital wart and high-risk subtypes such as HPV-16 and HPV-18 accompanied mainly with cervical dysplasia or cancer and with lower rate with vulva, penis, anus, and prostate (6). HPV genome has three separate regions; long control region (LCR), early region including E1 to E7 evolved in carcinogenesis and replication; and latent region encoding capsid structural proteins (L1 and L2). HPV would result in infection and basal cell involvement via ulcers or epithelial abrasions (7). Association of HPV-16/18 and prostate cancer has been assessed by Nested PCR, SOUTHERN BLOT, and D-PCR in some studies (8). Since HPV has several carcinogenic factors such as E6 and E7 and higher HPV infection is seen in those with higher malignancy grade (9), it may be evolved in the development of prostate cancer. This cross-sectional study was performed to assess the association of HPV genotypes 16 and 18 with prostate cancer.

Materials and methods

This cross-sectional study was performed on 75 consecutive paraffin-embedded blocks including 50 samples of primary prostate cancer and 25 samples of benign prostate hyperplasia (BPH) as control. All samples had been taken from subjects referred to health care centers in Kerman, Southeastern Iran since 2011 to 2015. All samples had been assessed in a single pathology center. After diagnosis confirmation and Gleason grading by a single pathologist, samples were enrolled. The nucleic acid was extracted by DNA-Sorb-C kit (manufactured in Russia, distributed by Arian-Gen Co.) and for real-time PCR, HPV16-18 kit (manufactured by ROCH Co. Germany) was used.

For DNA extraction, as instructed by DNA-Sorb-C kit 10, cuts of 5 to 10 micron from samples were transferred into a 1.5 ml microtube and for deparaffinization, the samples were put in Xylen and Xylen-diluted with alcohol 100%, 95%, 70%, and 50% with 1:1 ratio, respectively for three minutes. Then, they were washed with PBS and after adding Reagent buffer solution (400 microliter) and Lysis reagent solution (17 microliter), they were placed in shaker-incubator with 60 centigrade degree for 120 minutes. Then, the microtube was centrifuged for five minutes in 12000rpm. The supernatant was transferred into a new microtube and 25 lambdas from universal sorbent were added and 10 minutes shaking and 5 minutes centrifuging in 10000rpm were done.

The supernatant was removed and washed in two stages; 500 lambdas of washing solution-1 were added and it was centrifuged at 5000rpm for 1 minute and the supernatant was removed. Again, 500 lambdas of Wash Buffer-2 were added and it was centrifuged at 10000rpm for 1 minute and the supernatant was removed. Then, 50 lambdas of TE-Buffer solution were added and shaker-incubator was used with 65 centigrade degree for 10 minutes and finally it was centrifuged at 12000rpm for one minute. DNA-containing supernatant with NANO DROP-certified purity was stored at -20 centigrade degree. Absorption rate was calculated at wavelengths of 260 and 280 nanometers to determine the purity degree with optimal level of 1.8. Also, DNA amount was determined according to absorption rate at 260 nanometers.

Amplisense kit was used for replication at polymerase chain reaction (PCR) to determine genotypes of HPV-16 and HPV-18. For this, real-time PCR was done on extracted DNA in specific promoters-containing media (in Master-Mix Kit), initial substrates for synthesis of DNA or nucleotides, temperature and buffer-thermal DNA-polymerase with temperature schedule demonstrated in table 1. Positive control in kit was used and without-DNA mixture was used as negative control. 7 lambdas from PCR-mix-1-FEP/FRT HPV16/18 and 8 lambdas from PCR-buffer-FRT and polymerase (TaqF) were used and then, they were put in three separate 0.2 milliliter microtubes with volume of 10 lambdas from the above-mentioned solution and 10 lambdas from sample, negative control, and positive controls and reaction was performed by Thermocycler BIONEER (Excicycler). Internal control was with beta-globin gene.

Table 1. Temperature, time and number of cycles in real-time PCR steps

Step	Temperature, ˚C	Time	Fluorescence detection	Repeats
	95	15 min	-	1
2	95	20 s	-	45
	60	1 min	FAM, JOE/HEX, ROX	

Data analysis was performed on75 samples including 50 samples with primary prostate cancer and 25 samples with benign prostate hyperplasia. Data analysis was performed by SPSS (version 24.0) software. Chi-Square test was used and P value less than 0.05was considered as statistically significant.

Results

In this study, 50 samples with primary prostate cancer (66.7%) with mean age of 66.4 ±12.2 years and 25 samples with benign prostate hyperplasia (33.3%) with mean age of 67.9 ± 11.6 years were assessed. All malignant tumors were classified according to Gleason Score (International Society of urologic pathology consensus conference on Gleason Grading of Prostate Carcinoma, 2014)into the five groups; 18 subjects with score 6 (Group 1), 12 patients with Gleason Score=4+3 (Group 2), 5 cases with Gleason Score=3+4 (Group 3), 8 patients with Gleason Score=8 (Group 4), and 7 subjects with Gleason Score=9 (Group 5) as shown in tables 2-4.

Since this study was performed by existing data, further information was not available. The success rate for maximal amplification rate of DNA according to light absorption in wavelength of 260 nanometers was certified (near to 1.8). However none of the samples had amplification of HPV DNA (figure1).

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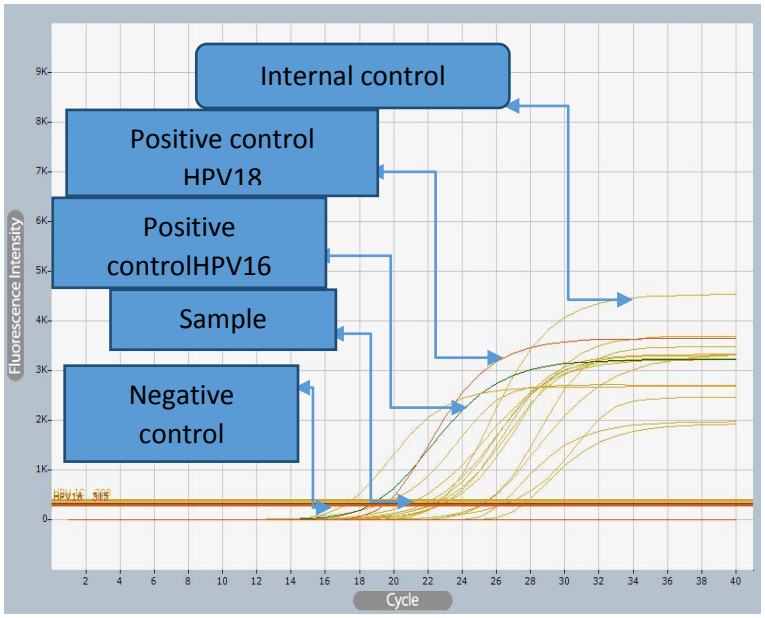

Figure1. Real-Time PCR grahs

Table 2. Relative frequency of the case group (based on the age group and Gleason grouping) and control group based on the age group

Age Group (year)	Case group based on Gleason Grouping						
	1	2	3	4	5	BPH group	Total
40-49		1 (2%)		1 (2%)		2 (8%)	4 (5.3%)
50-59	8 (%16)	5 (%10)	1(%2)	3 (%6)	2 (%4)	7 (%28)	26 (%54.7)
60-69	6 (%12)	3 (%6)	1(%2)	3 (%6)	4 (%8)	8 (%32)	25 (%33.7)
70-79	3 (%6)	2 (%4)	1(%2)		1(%2)	5 (%20)	12 (%16)
80-89	1 (%2)	1(%2)	2 (%4)	1 (%2)		3 (%12)	8 (%10.7)
Total	18 (%36)	12 (%24)	5 (%10)	8 (%16)	7 (%14)	25 (%100)	75 (%100)

Table 3. The frequency distribution of PIN in the case group (based on Gleason grouping) and control group

PIN*	Pca Gleason grouping					BPH
	1	2	3	4	5	
Low grade		3 (%6)	1 (%2)	2 (%4)	1 (%2)	5 (%20)
High grade	1 (%2)	4 (%8)	7 (%14)	1 (%2)	5 (%10)	3 (%12)

*Prostatic intraepithelial Neoplasia

Table 4. Frequency distribution of the case group (based on Gleason grouping) and control group according to the sampling method

Sampling method	PCa Gleason grouping						BPH
	1	2	3	4	5	total	
TURP*	11 (%22)	7 (%14)	2 (%4)	3 (%6)	5 (%10)	28 (%56)	19 (%76)
Core Needle Biopsy	7 (%14)	5 (%10)	3 (%6)	5 (%10)	2 (%4)	22 (%44)	6 (%24)
Total	18 (%36)	12 (%24)	5 (%10)	8 (%16)	7 (%14)	50 (%100)	25(%100)

*Trans-urethral resection of Prostate

Discussion

In this study, we had no positive case for HPV DNA types of 16 and 18 in 75 patients. Our results were similar to those reported by previous researchers such as Aghakhani et al (10) and also Ghasemian et al (11) in Iran. Effert et al (1992) have also assessed 30 paraffin-embedded samples of prostate adenocarcinoma for HPV types of 16 and 18 with Southern blot PCR differential and reported no positive case (12). Bergh et al (2007) assessed 201 samples with prostate cancer and BPH cases transformed to malignant tumors and also some match controlled cases for HPV and reported no positive case (13).

Our results were not in congruence with some other studies; for example, in Nehu Singh et al study in India (2014), Anwar and colleagues study in India (1992) and also in Leiros et al study(2005), high concordance rate of prostate cancer with HPV (40% to 42%) have been reported (14-16).

These different results might be due to application of various methods for detection of HPV DNA. Also, there are multiple etiologies for false positive results such as PCR-contamination or Cross- contamination of samples. It should be considered that HPV is related to high-risk sexual behaviors such as STDs history, multiple sexual partners, first sexual intercourse at early age, and repeated high-risk sexual activities. Hence, cases without such high-risk behaviors might demonstrate different results. HPV distribution is related to geographical region, age, sexual history, immunity status, and genetic factors (17).

We assessed only 75 cases and in comparison with other studies such as Singh et al (14), Effert et al (12) and Anwar et al (15) studies, the obtained results might be due to some limitations for larger sample size. Further studies with larger sample size are required to confirm or reject the results of the present study. Primer selection and DNA quality are two main factors leading to some errors during PCR. However, selection of primers in our study was similar to previous studies such as Ghasemian et al (11) and it was relatively near to 100%.

Also, it should be mentioned that in all studies the contamination of stratified squamous cells by HPV and related morphological alterations and HPV-related factors have been studied extensively and frequently. But, there is no study about other epithelial cells other than squamous types and performing such studies would be beneficial.

Our suggestions are carrying-out studies about function and effect of HPV on epithelial cells other than squamous types, association between PIN and high and low-risk HPV, association between other types of HPV such as HPV-30 and HPV-31 with prostate disease and association of HPV genotypes with prostate malignancy grade.

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