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THE RELATIONSHIP BETWEEN HUMAN PAPILLOMAVIRUS TYPE 16 AND 18 WITH PROSTATE ADENOCARCINOMA

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ABSTRACT

Background: Prostate cancer is the most common cancer in men. Several factors in the incidence of this disease have been reviewed; recently, the role of high-risk types of human papillomavirus (HPV) in creating this condition is evaluated. Therefore, we decided to survy the role of this virus in the incidence of prostate cancer.

Materials and methods: The 40 tissue blocks with prostate cancer as case group and 40 blocks with benign prostatic hyperplasia (BPH) as the control group were studied in order to assess the presence of HPV type 16 and 18. Virus extraction and purification of DNA was done using PCR method. The findings analyzed by software SPSS 16 and Fisher exact statistical test. P <0.05 was considered significant.

Findings: Of 40 prostate cancer blocks, 4 cases (10%) includes 3 cases (7.5%) of HPV-18 and a case (2.5%) of HPV-16 were found. Of 40 BPH blocks, 1 case (2.5%) was positive for HPV-18. No HPV-16 was found in BPH samples. No significant relationship was between samples with prostate cancer and BPH.

Conclusions: The results obtained in this research study did not show the role of HPV type 16 and 18 in creation of prostate cancer, and therefore more review is necessary about this.

Keywords: Prostate cancer, benign prostatic hyperplasia, Human papilloma virus

INTRODUCTION

Adenoarcinoma of the prostate is the most commonform of cancer in men, accounting for 29% of cancer inthe United States in 2012. Cancer of the prostate is typically a disease of men older than age 50 years, approximately 70% in men between the ages of 70 and 80 years (1). The frequency with wich incidental carcinoma is found at post mortem examination varies between 15% to 70 %(2). According to available statistics, the prevalence rate of prostate cancer in Iran is 3.43 %(3) and its incidenceis 5.1 per 100,000 person-years(4). Our knowledge about the causes of prostate cancer is still not perfect. A variety of factors, including age, race, family history, environmental influences and hormone levels have a suspect role. An increased incidence of the disease with immigration from the region with low incidence to an area with high incidence is the represent of environmental agent's role. There are many environmental effective factors, but none has been proven as the cause (1). Case-control studies have shown that having multiple sexual partners in a lifetime, or the start of sexual activity early in life, significantly increases the risk of prostate cancer (5). This communication, would suggest a sexually transmitted infection (STI) that may have role in some cases of prostate cancer (6). Studies on sexually transmitted diseases in a decade ago or more, shows a significant association between prostate cancer and sexually transmitted diseases caused by HPV-16, HPV-18, HSV-2 (7). HPV is an icosahedral nonenveloped, 8000-base-pair, double stranded DNA virus with a diameter of 55 nm. More than 125 types of HPV have been identified and numbered. Some of the HPVs are known as high risk, such as the type 16, 18, 31, 33, and 45 which are involved in the creation of cervical cancer(8). High risk types of HPV have two oncoprotein E6 and E7which causes to disable two tumor suppressor protein,called P_{53} and Rb(1). A lot of studies have been done about the relationship between HPV with prostate cancer. In1991 Nicol and Dodd by PCR method found HPV types 16 and 18 in prostate cancer and BPH, but no significant relationship was found between HPV and cancer (9).Also Aghakhani. et al in the year 2010 by PCR method did not found the relationship between high risk HPV and prostate cancer(10). On the other hand, Serth in 1999 by PCR method showed the presence of HPV type 16 in 21% of cases of prostate cancer indicating a significant relationship between the virus and prostate cancer (11).

Also Carrozi in 2004 (12) and Martinez in 2010 (13) reported significant relation between HPV and prostate cancer. According to the existing contradictions about the role of HPV in the creation of prostate cancer and using Gardasil vaccine for the prevention of HPV type 6 , 11 , 16 and 18(14), we decided to survy the role of HPV virus type 16 and 18 in carcinoma of the prostate in a case-control study. If HPV would have causative role, this vaccine will reduce cancer prevalence. Since there are several methods to determine the virus, we choosed polymerase chain reaction (PCR) on the tissue blocks due to its high accuracy and power to detection.

MATERIALS AND METHODS

This case-control study was done as cross-sectional on paraffinized blocks of prostate samples sent to the pathology laboratory of Kashan Shahid beheshti hospital during the years 2001 until 2013. Tissue blocks of prostate cancer (case group) and benign prostatic hyperplasia (control group) were selected from pathology archive. According to previous studies and in order to compare the ratio of the frequency of HPV virus in two groups, using the formula compare two ratio and considering P1 and P2 as 53.8 and 20 percents(using Carrozi study) (12) and ensuring 95% and 80% of power, the sample size obtained 33 in each group. Considering to sample missing, 40 blocks were studied in each group.

$$n' = \left[\frac{(z_{1-\alpha/2}\sqrt{2\bar{p}(1-\bar{p})} + z_{1-\beta}\sqrt{p_1(1-p_1)+p_2(1-p_2)})}{p_1 - p_2} \right]^2$$

Laboratory methods.In this study we examined the HPV type 16 and 18. Three to five micron sections transfered to 1.5 mL tubes.Then the tissue DNA virus was extracted with three-stage including deparaffinizing, extraction and purification. These steps were done by using the catalog Guide of 5-PRIME CO. The steps are as follow:

Sample deparaffinization

1- Placing 5-10 mg finely minced tissue into a 1.5 ml tube. Adding 300 µl xylene and incubation for 5 min with constant gentle mixing at room temperature.
2- Centrifuging at 13000-16000 xg for 1-3 min to pellet the tissue and then Discarding the xylene.
3- Steps 1 and 2 will be Repeat for twice.
4- Adding 300 µl 100% ethanol and incubation for 5 min with constant mixing at room temperature.
5- Centrifuging at 13000-16000 xg for 1-3 min to pellet the tissue, then Discarding the ethanol.
6- Repeating steps 4 and 5.

Cell lysis

1- To add 300 µl cell lysis solution and homogenize using 30-50 strokes with a microcentrifuge tube pestle.
2- Adding 1.5 µl proteinase k solution to the lysate, mixing by inverting 25 times, and incubation at 55ºC for 3 h to overnight. If tissue is not completely digested after overnight incubation, should add an additional 1.5 µl proteinase k solution and continue incubation at 55ºC for 3 h to overnight, inverting tube periodically during the incubation if possible.
3- To continue the purification by beginning with step 1 of RNase treatment.

RNase treatment

1- Adding 1.5 µl RNase A solution to the cell lysate.
2- Mixing the sample by inverting the tube 25 times and incubation at 37ºC for 15-60 min.

Protein precipitation

1- Cooling sample to room temperature by placing on ice for 1 min.
2- Adding 100 µl protein precipitation solution to the cell lysate.
3- Vortexing vigorously at high speed for 20 s to mix the protein precipitation solution uniformly with the cell lysate.
4- Centrifuging at 13000-16000 xg for 3 min. precipitated proteins will form a tight pellet. repeating step 3 followed by incubation on ice for 5 min, then repeating step 4 if the protein pellet is not tight.

DNA precipitation

1- Pouring the supernatant containing the DNA into A clean 1.5 ml microcentrifuge tube containing 300 µl 100% isopropanol. If the DNA yield is expected to be low, should add a DNA carrier such as glycogen(adding 0.5 µl Glycogen solution per 300 µl isopropanol).
2- Mixing by inverting gently 50 times.
3- Centrifuging at 13000-16000 xg for 5 min.
4- Pouring off supernatant and draining tube on clean absorbent paper then adding 300 µl 70% ethanol and inverting tube to wash the DNA pellet.
5- Centrifuging at 13000-16000 xg for 1 min, then Carefully pouring off the ethanol.
6- Inverting and draining the tube on clean absorbent paper and allowing to air dry for 5-10 min.

DNA hydration

1- Adding 20 µl DNA hydration solution.
2- Rehydration of DNA by incubating sample for 1 h at 65ºC and/or overnight at room temperature with Taping tube periodically to aid in dispersing the DNA.

At the end of this stage, extracted and purified DNA was ready and kept in 4ºC(15). In the next step to determine the amount of impurities caused by the presence of protein, absorption was measured with the nanodrop device, once the wavelength of 260 nm (the length of a wave in which DNA has a maximum absorption) and other time the wavelength of 280 nm (which is the maximum protein absorption), then the ratio of absorption at 260 nm to 280 nm was calculated. Samples with the ratio more than 1.8 were used. At the end, PCR was used to replicate and detection virus genome. Thermocycler of the Eppendorf CO and PCR kit of Russian DNA- technology CO was used. In this kit, FLASH PCR method has been used, based on the fluorescence (Fig.1-4).

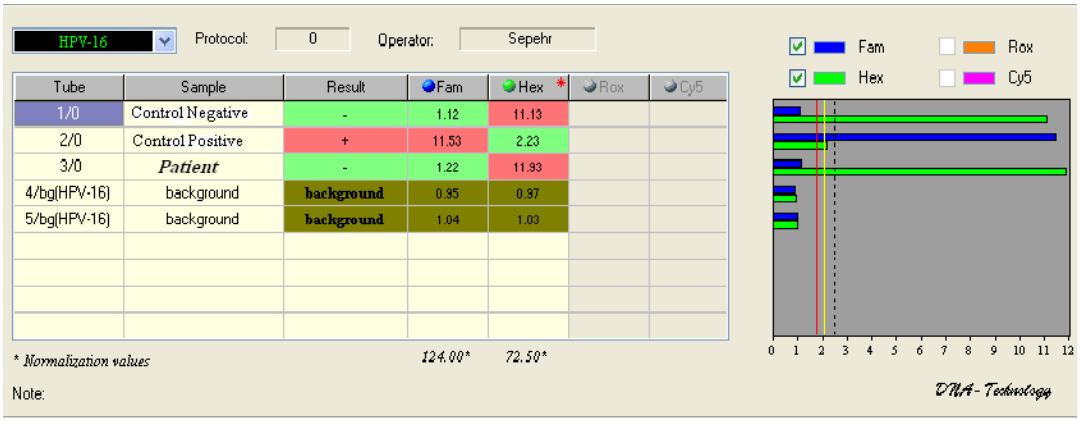

Fig.1. Negative sample for HPV-16

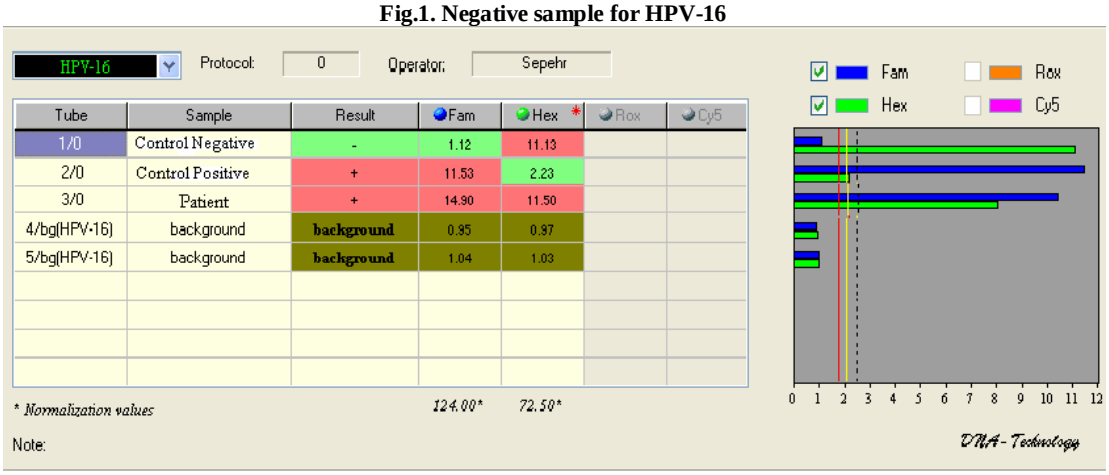

Fig.2. Positive sample for HPV-16

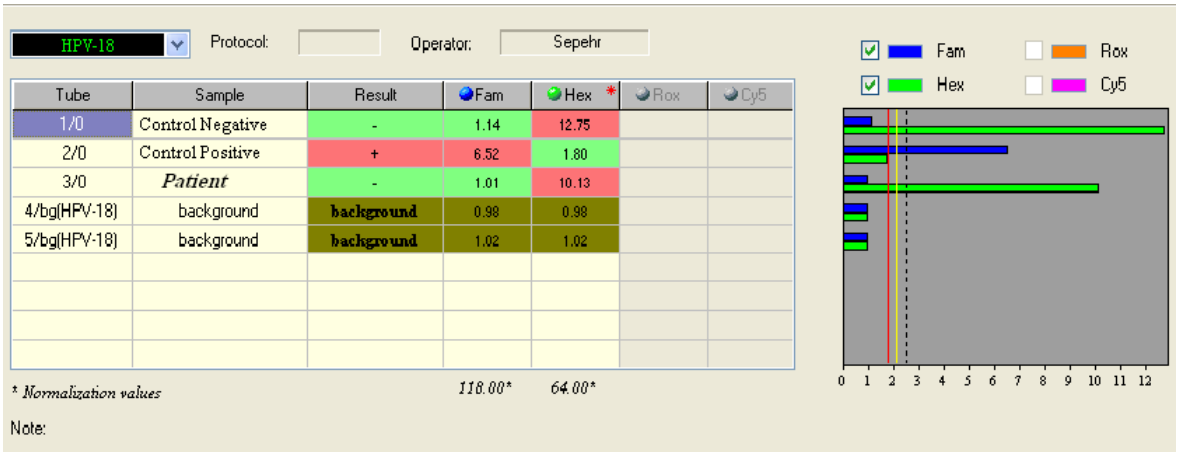

Fig.3. Negative sample for HPV-18

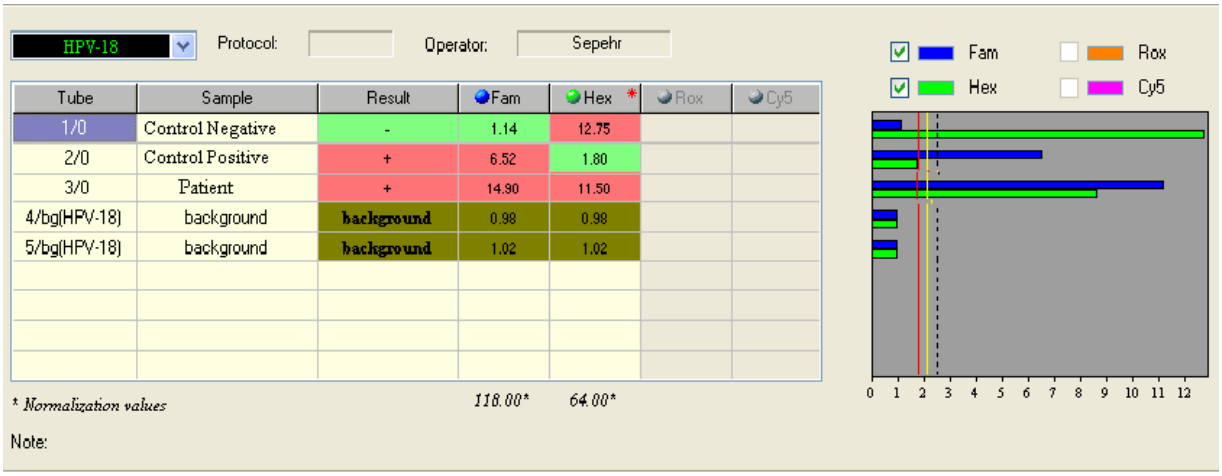

Fig.4. Positive sample for HPV-18

STATISTICAL METHODS

After the initial information gathering, the findings were analyzed with the software SPSS16 and fisher exact test. P< 0.05 was considered significant. The results expressed as the absolute number and percentage.

RESULTS

In this study, the average age of the prostate cancer group was 73 with range of 58 to 91 years and the average age of the BPH group was 70.25 with range of 55 to 90 years (P-value = 0.13)(Table 1). Of 40 blocks of prostate cancer, 4 blocks (10%) were positive, including 3 cases (7.5%) HPV-18 and a case (2.5%) HPV-16. Of the 40 blocks of BPH was a case (2.5%) positive for HPV-18. No HPV-16 found in blocks of BPH (Table 2).

Table 1- Age of prostate cancer and benign prostatic hyperplasia patients

	BPH	cancer
average age	70.25	73
SD	7.75	8.44
NO	40	40

Table 2- HPV type16 and 18 frequency in prostate cancer and benign prostatichyperplasia

BPH	cancer	
0	**(2.5%)**1	**Positive cases of HPV-16**
(2.5%)1	**(7.5%)**3	**Positive cases of HPV-18**
(2.5%)1	**(10%)**4	**Total positive cases**
(97.5%)39	**(90%)**36	**Total negative cases**
(100%)40	**(100%)**40	**Total sum**

Of 40 prostate cancer samples, 3 cases (7.5%) and of 40 BPH samples, 1 case (2.5%) were positive for HPV-18. There was no significant relationship between cancer and BPH about HPV-18 (P = 0.03) (Table 2). One case (2.5%) of 40 prostate cancer was positive for HPV-16. Of 40 BPH samples, no case was positive for HPV-16. No significant relationship was between cancer and BPH about HPV-16 (P ~1)(Table 2). Totally, of 40 cases of prostate cancer, 4 cases (10%) and of 40 cases of BPH, 1 case (2.5%) were positive for HPV. No significant relationship was between cancer and BPH about HPV(P = 0.18)(Table 2).

DISCUSSION

The results obtained in our study did not show causing role of HPV in cancer of prostate. This result is matched with the results of Effert(16), Tu(17), Gherdovich(18), Noda(19), Chen(20), Lin Y(21), Groom(22), Ghasemian(23), Whitaker(24), Yow ma(25) and Aghakhani(10). In contrast, Sarkar(26), Leiros(27), Mokhtari(28), Neha Singh(29) and Bae Jong-Myon(30) reported a significant relationship between HPV and prostate cancer. Regarding to discrepancy in the results, more studies is necessary. Some comments is necessary: confoundings such as high fat diet, heredity, environmental and geographic factors and etc may have a role in prostate cancer. So these confoundings may cause discrepancy. For example, in a person with prostate cancer who has undergone prostate HPV infection and a positive family history of prostate cancer, whether it can be said that prostate cancer in this person who simultaneously have two risk factors, have been associated with the presence of the virus or with a positive family history? Definitely, finding the causative agent and the relationship between cause and effect in this case is difficult, especially because in some studies tissue blocks have been chosen as sample, it is not possible to access patients. With regarding to our use of tissue blocks, this issue is also applies about our study. Therefore, the interpretation of the results of the studies in this field should be done considering these items. Such confoundings justify discrepancy between the findings of various studies. Instudies that the correlation between HPV and cancer has been significant, is not determined whether the confounding factors have effects on their study or not. On the other hand, in different

studies, different methods has been used for HPV detection, in most of them PCR is used for it's accuracy, also in some studies measuring the level of antibodies against virus has been used, the other used method has been immunehistochemistery (IHC). One of the causes of discrepancy in results of the studies, may be due to the difference in methods.

Another noteworthy point is that sometimes, with statistical perspective, in a study may not exist significant differences between the case and control groups about the presence of a factor, but this is not a meaning of that this factor is not important clinically, especially in cases cause death, in which a slight positive number has also great importance. Considering that prostate cancer is one of the most common and deadly cancers in men, it is necessary to wide research on this disease and it's risk factors, to reduce them.

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