Molecular detection of Human Papillomavirus genotype-16&-18 in tissues from patients with prostate cancer and benign prostatic hyperplasia

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Summary:
Background: High oncogenic-risk genotypes of human Papillomavirus (HPV) infect a wide range of human cells, including prostate tissue that give rise to benign prostatic hyperplasia and prostatic adenocarcinomas.

Objectives: This study aimed to detect DNA of HPV genotype-16 &18 using in situ hybridization technique in prostatic tissues from benign prostatic hyperplasia and prostatic adenocarcinomas, and elucidate the association between these HPV genotypes and prostatic carcinogenesis.

Patients and methods: Forty-eight (48) formalin-fixed, paraffin embedded prostatic tissue blocks were obtained from different grades and (20) benign prostate hyperplastic tissue blocks as well as (10) apparently normal prostate tissue as well as (10) apparently normal prostate tissues. Detection and genotyping of HPV was done by highly sensitive in situ hybridization technique.

Results: The signals of in situ hybridization reactions of both HPV-16 and HPV-18 in prostate cancer cases in the present study was 25% (7 / 28) whereas in BPH, HPV-16 was detected in 45 % (9 /20) and HPV-18 was presented in 35 % (7 / 20). Neither HPV-16 nor HPV-18 was detected in the apparently healthy control group. The percentages of HPV 16 and HPV18 were increasing with advancing of grade of prostate cancer.

Conclusion: Our results indicate that the oncogenic HPV-16 might contribute to the development of subset of prostate tumors. In addition, HPV16&18 might have a crucial role in progression of the prostate cancer and benign prostatic hyperplasia.

Key word: HPV-16; HPV-18; prostate cancer, benign prostatic hyperplasia, in situ hybridization.

Introduction:
Most common neoplasms of the male genital tract involve the prostate gland (1). Prostate cancer is the fifth common cancer in the world and the second in cancer mortality exceeded only by lung cancer (2,3). Viral factors are the most important class of infectious agents associated with human cancers (4). It was estimated that 17-20% of all worldwide incidence of cancers are attributable to a viral etiology (5).

Human papilloma virus is sexually transmitted in adults. Human papilloma viruses (HPVs) are regarded as specific epitheliotrophic DNA viruses (6). HPVs can persistently infect prostate epithelium in non immunocomprised hosts (7).

To date, more than 100 types of HPVs have been reported, which are classified into low –oncogenic risk and high- oncogenic risk types according to their associations with malignant tumors (8).

High oncogenic risk HPV types may integrate into the host cell chromosome; here they interrupt the integration of E2 gene that regulates the transcription& expression of HPV-E6 & E7 oncoproteins. The E6 and E7 genes represent transforming genes and their products are responsible for the alteration of growth patterns of the infected cells as well as acting, at least in part, by interfering with host cell control of transcription and the cell cycle(9). These oncoproteins inactivate the cellular tumor suppressor gene products of p53 and Rb, respectively (10,11).

It is clear that continued expression of these viral oncoproteins is necessary for histopathologic progression and the malignant phenotype of anHPV-associated tumors (12).

The involvement of oncogenic (HPVs) in the pathogenesis of prostate cancers is a subject of great controversy (13). However, molecular detection of HPV DNA was documented in 2.4%(through 53%) and up to 100% in prostate cancer and in 32% - 93%
of benign prostatic hyperplasia (1,14, 15,13) So this study aims to assess the in situ hybridization expression of HPV-16 and HPV-18 in BPH & prostate cancer and to elucidate the correlation of these two high-risk oncogenic HPV-genotypes with development of BPH & prostatic carcinogenesis.

Materials and methods:

Patients and tissue samples: Fifty-eight (58) formalin-fixed, paraffin embedded tissues were collected from prostate biopsies that were related to (28) prostatic carcinoma, (20) benign prostate hyperplasia and (10) apparently normal prostate. They were collected from records of pathological archives of Teaching Laboratories of Medical City Hospital and Forensic Medicine Institute / Baghdad during the period of November 2009 to April 2010. The age of these individuals ranged between 55-95 years.

The diagnosis of these tissue blocks were based on their accompanied records. A consultant pathologist reexamined all these cases to confirm the diagnosis following trimming process of these tissue blocks.

Methods: Detection of HPV by ISH kit (Maxim biotech Inc, USA) was performed on 4µm paraffin embedded tissue sections using a biotinylated long DNA probe for HPV 16and HPV 18(cat. No. IH-60058 and IH-60059, respectively). One section was treated then with proteinase K solution.

One drop of the biotinylated long cDNA probe for HPV 16 and HPV 18 was placed on each specified slides. Hybridization solutions was placed on the tissue section and placed in the oven at 95°C for 8-10 minutes to denature the double stranded DNA. The slides were then placed in a humid chamber and incubated over night at 37°C to allow hybridization of the probe to the target nucleic acid. The slides were soaked in protein block at 37°C until the cover slips fell and then treated with conjugate one to 2 drops of conjugate (BCI P/NTB). Positive control reactions were performed by replacing the probe with biotinylated house keeping gene probe. Negative control was obtained by omitting the probe from hybridization buffer. Then substrate was placed on tissue section at room temperature for 30 minutes or until color development was complete. Slides were then counterstained using nuclear fast red and sections were mounted with permanent mounting medium (DPX). Color development was monitored by viewing the slides under the microscope. A blue colored precipitate formed at the site of the probe in positive cells.

The in situ hybridization signal was evaluated under light microscope at oil emersion (X1000) for counting of positive cells. Positive cells were counted in ten different fields for each samples and the average of positive cells of the ten fields was determined as the scope of our research is to qualify the results as positive or negative HPV -16 or -18 ISH reactions. A scale zero was given to these results without detectable ISH reaction whereas the results pointing for >1% were evaluated as positive ISH reaction and without the need to include the scores 1-3 stated by (16), that are referring to low, intermediate, and high infection

Statistical analysis was done by chi-square test, percentage, range, mean and standard deviation. Correlation was considered significant when p<0.05.

Results:
The total Human Papilloma Virus –positive ISH reactions were detected in 10 out of 28 (35.7%) patients with PC and in (55%; 11 out of 20) patients with BPH (Table 1). Among them, 25 % (7 out of 28) of patients with PC showed positive ISH reactions for HPV 16 and HPV 18 (fig 1), separately. In BPH, (fig 2) HPV 16-DNA was detected in 9 out of 20 (45%) whereas HPV18 DNA was found in 35% (7 out of 20) of benign hyperplastic prostatic tissues. Mixed infection of both HPV16 and 18 in the same tissue samples of each PC and BPH were found in 14.3% (4 out of 28 ) and 25% (5 out of 20) respectively. However, the statistical analysis shows non significant differences (P> 0.05).

Table(1) In situ hybridization test results of human papillomavirus in prostatic tissues with PC &BPH.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>HPV 16</th>
<th>HPV 18</th>
<th>combined HPV test</th>
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<tr>
<td>PC</td>
<td>28</td>
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<td>25%</td>
<td>75%</td>
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<td>9</td>
<td>11</td>
<td>7</td>
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<td>55%</td>
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<td>Negative</td>
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<td>9</td>
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<td>13</td>
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<td></td>
<td></td>
<td>55%</td>
<td>45%</td>
<td>Negative</td>
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</table>

The difference between PC&BPH regarding HPV16: P= 0.25 Non Significant
The difference between PC&BPH regarding HPV18: P=0.66 Non Significant
The difference between PC&BPH regarding combined HPV: P=0.29 Non Significant

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Fig.1: Prostatic adenocarcinoma-back to back (moderately differentiated)-Gleason score (6) stained by Hematoxyline and Eosin (10X).
A. In situ hybridization results for human papilloma virus DNA- detection in prostate tumors; BCIP/NBT stained and counter stained by nuclear fast red:
1. Positive ISH reaction (HPV-16) with prostate cancer (40X).
2. Positive mixed nuclear and cytoplasmic ISH reaction (HPV-18) with prostate cancer (40X).

Fig.2: A. Benign postatic hyperplasia stained by Hematoxyline and Eosin (10X)
B. In situ hybridization results for human papilloma virus DNA- detection in; BCIP/NBT stained and counter stained by nuclear fast red:
1. Positive ISH reaction (HPV-16) with benign prostatic hyperplasia.
2. Negative ISH reaction with benign prostatic hyperplasia (10X).
**Discussion:**

The present results were in agreement with the findings of (Ibrahim et al., 1992) (17) who found 25% positivity of HPV-16 in PC by using both PCR & ISH techniques and with the findings of (Serth et al., 1999) (13) who detected HPV16 in 21% PC by using PCR method, too.

The present results are much lower than the results of positivity of HPV16 in the examined prostatic cancerous tissues reported by. (1, 14, 18, 19, 20, 21) where they found (100%, 53%, 51.9%, 50%, 50%, 42.9%), respectively. On the other hand, our results are much higher than those results reported by (22and23) who found positive results of HPV16 in their examined prostatic cancerous tissues in a percentage rate of (13% and 2.3%, respectively). This could be frankly related to the criteria of PCR as the most sensitive technique for DNA amplification than in situ hybridization for detection of viral DNA so as that one particle of viral DNA (or even part of it) in the tissue section could be theoretically detectable by PCR. It is possible that the tissues of prostate cancer with negative results by the present in situ hybridization study may not have an adequate copy numbers of this virus to permit its detection by ISH while it could show positive results on PCR (24).

In addition, the lower numbers of the included prostatic tissues in the present (as well as other studies) which were subjected for molecular testing as well as there was a shortage of knowledge regarding the prevalences of each HPV genotype in the general population of each communities and /or countries precluded any clear and definitive explanation regarding such differences and discrepancies in the reported results of positive percentages of HPV ( this was noted even for those results that were reported by the same researcher and in the same patients of that specific country but at different, even short, time interval of achieving these studies) (1,21).

Although human papillomavirus type16 and type 18 are known to play a role in the development of neoplastic disorders of the urogenital organs, the presence of HPV-16 and HPV-18 in prostatic tissues with benign hyperplasia has been a matter of controversy (25). The present study was extended to include a set of benign prostatic hyperplasia tissues to be tested by ISH technique for these 2 important highly oncogenic HPV genotypes. The results of this study were in agreement with the findings of (17-20) who found 50% of HPV16 in BPH by using PCR method and also consistent with the findings of each (17)(who found 20% of HPV18 in BPH) and (25) (who found 30.8% of HPV-18 in BPH) by using PCR &Southern blot hybridization techniques. However ,our results are lower than those reported by (1) who found HPV16 and HPV18 in BPH in a percentage rate of (93.3% &20%) respectively; those reported by (18) who found HPV16( 60.7%)in BPH by using PCR method; and those reported by (19) who found (82%) positivity of HPV-16 in BPH cases. On the other hand, some investigators have reported negative findings of HPV in BPH samples. In this respect, a pilot study by (17) included a total of 10 BPH samples that were proved to be negative at for HPV by both PCR and in situ hybridization. Also, our obtained results are higher than (18) who found HPV18 (5.4%); (25) who found (15.4%) for co –infection HPV16&HPV18 in their examined benign hyperplastic tissues .The differences in the present obtained percentages are a reflection of low prevalence of HPV in our Iraqi patients and as reported by(26) that may constitutes a probable cause for the differences between all Iraqi studies and worldwide studies. Therefore, other factors and agents might multifactorially or co-factorially play a role in initiation and promotion in prostate carcinogenesis of our country. Although many researches tried to present evidences for liability of conversion of subset of BPH into PC, yet scientists have not confirmed the change of BPH to PC (27). Prostate cancer like that of cervical cancer is also preceded by precursor lesions called prostatic intraepithelial neoplasia (PIN) which are equally paralleled to CIN in cervical cancer (28). In view of these facts &observations, and likewise that of HPV role in cervical carcinogenesis ,the present results could fortify the possibility of changing PIN lesions to PC via the role of highly oncogenic risk HPV types in the course of prostatic carcinogenesis. The detection of such high risk HPV types in BPH would not be interpreted as a chance phenomenon or left without giving a critical importance for the possibility of HPV in initiation or enhancing the conversion of a subset of BPH into the prostatic carcinogenesis to change into PIN and /or PC. Small size of the studied samples compromised the statistical power of this study to detect the effects of these factors under consideration. In addition, the lack of detailed clinical information attached to those prostate tissue samples that were enrolled in this study has deprived the present study to reach to a solid impression for the real role of those mixed viral infections in prostate carcinogenesis and in turn raised a suggestion to compel an integrate team-work study, at molecular and virological levels to elucidate the role of these factors and many other agents in prostate carcinogenesis in this country. Also in the future, it will be interesting to design experimental studies to understand the synergistic effect of HPV with EBV and/or HSV mixed infections on prostate cancer. In view of the clear variations in the results of HPV in BPH from the present study and many other studies, more investigations should be carried out before a possible conclusion that the prostate may be a potential reservoir for the sexual transmission of high risk HPVs can be made. From the results of this study,
a decreasing trend of HPV-16 incidence was noticed with advancing grade of the examined prostatic cancerous tissues. This does not pointing for a possible correlation of HPV-16 infection with the histological aggression of the examined prostate cancer disease. These results are supported by conclusions drawn from earlier investigations by (29 and 30) who found no relationship between HPV-16 infection status and Gleason grade, stage of disease, or combined measure of disease aggressiveness.

The highest percentage of HPV 18 expression (50%; 3 out of 6) was noticed among patients with moderate differentiated prostatic carcinomas, and increasing trend of HPV18 incidence was also evidenced with the advancing of grade so as to indicate for a correlation of HPV18 infection with the aggressiveness of prostate cancer disease. The present results are in disagreement with findings of (29 and 30) that found no relationship between HPV-18 infection status and Gleason grade, stage of disease, or combined measure of disease aggressiveness. On the other hand, the present results are in agreement with finding of Anwar et al (1992) (31), who demonstrated that frequency of HPV-18 infection increased in patients with advanced stages of the tumor and with the higher Gleason score. These results could indicate for a possible early role for HPV-16-18 in prostatic carcinogenesis as initiating agent at an earlier stage (grade) of prostatic cancer disease rather than later enhancing or promoting roles.

Conclusion:
The high percentage of high-oncogenic risk HPV-associated BPH might reflect a crucial role for this important sexually-transmitted disease in the pathogenesis of BPH and their probable transforming role along the pivot of prostatic carcinogenesis. Our results indicate that the oncogenic HPV16&18 might have a crucial role in development, transformation and/or progression of subset prostate cancers and benign prostatic hyperplasia.

References
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