Lack of detection of human papillomavirus infection by hybridization test in prostatic biopsies

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ABSTRACT

الأهداف: البحث عن وجود الفيروس الحليمي البشري (HPV) في عينات من نسيج غده البروستاتا لعدد من الرجال السعوديين المصابين بالتضخم الحميد أو بسرطان البروستاتا.

الطريقة: إجريت هذه الدراسة في مستشفى الملك عبد العزيز الجامعي – جدة – المملكة العربية السعودية، خلال الفترة مابين مارس 2007م وحتي ديسمبر 2008م. تم الحصول على عينات من غده البروستاتا لعدد 56 رجلاً مصاب بتضخم غده البروستاتا الحميد (BPH) أو سرطان البروستاتا، تراوحت أعمارهم مابين 50–93 عام (متوسط عمر 68 عام). تم إجراء اختبار (DNA) للفيروس الحليمي البشري (HPV) بطريقة الهايبريد كابتشر (HC 2 – Hybrid capture) على عينات من البروستاتا لهؤلاء المرضى للحصول على 18 نوع من الألتهاب الحليمي البشري (HPV)، ولتحديد الفرق بين مجموعتين من (HPV DNA)، النوع شديد الخطورة، والنوعين المتوسط والعالي الخطورة.

النتائج: أثبت الاختبار عدم وجود الإصابة بالفيروس الحليمي البشري (HPV) في أي من العينات التي أخضعت للدراسة.

خاتمة: أوضحت نتائج الدراسة عدم وجود إصابة بالفيروس الحليمي البشري HPV-16 أو HPV-18، في أي من العينات الإضافية التي تقترح الإصابة بسرطان البروستاتا.

Objectives: To explore the possibility of finding human papillomavirus (HPV) infection in the prostate tissue of a cohort of Saudi men presenting with benign prostatic hyperplasia (BPH) or prostate cancer.

Methods: A cohort study on prospectively collected tissue samples was conducted at King Abdulaziz University Hospital (KAUH), Jeddah, Kingdom of Saudi Arabia from March 2007 to December 2008 on a total of 56 male patients, age range 50-93 years (average 68), diagnosed as having BPH or prostate cancer. The HPV DNA hybridization by hybrid capture 2 technology was performed on prostate biopsies of these patients to detect 18 types of HPV infection, and differentiate between 2 HPV DNA groups, the low-risk types, and the high/intermediate risk types.

Results: The tissues of all the prostatic biopsies were negative for HPV DNA.

Conclusions: Our results, using the hybridization test, indicate that it is unlikely that HPV-16 or HPV-18, or the other tested subtypes, enhance the risk of prostate cancer.

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Worldwide, infections are linked to around 15-20% of cancers,[1] as some infections may cause long-term inflammation, which suppresses a person's immune system, or directly affects a cell's DNA. In fact, inflammation is thought to provoke carcinogenesis by causing cell and genome damage, promoting cellular turnover, and enhancing cell replication.[2] Prostate cancer is one of the most common malignancies in males, but little is known about the molecular events involved in its development.[3] The prostate could constitute a target for infection with high-risk (HR) human papillomavirus (HPV) due to anatomical reasons, particularly by direct access of the viral particles through the urethra. Penile and urethral HPV lesions and an increased cancer risk

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associated with sexual behavior have been described,[4] as infection and subsequent inflammation may be an important risk factor in the pathogenesis of prostate cancer as suggested by some researchers.[3,5] Infection with HR HPV is now well established to be linked to the development of cervical cancer.[6] Studies show that HPV DNA is found in 99.7% of all cervical carcinomas and cells derived from that cancer.[6] The recognition of HR HPV as an etiological agent of cervical cancer has increased the demands to use testing for HPV DNA.[7] The HPV subtypes especially 16 and 18 are sexually transmitted, and have been associated with an increased incidence of several anogenital tumors. They can also play a role in the pathogenesis of prostate cancer, as prostate cancer has been closely related to sexual behavior and sexually transmitted diseases (STDs).[8,9] There has been particular attention given to HPV infections as possible prostate cancer risk factors, as these are definite causes of several other forms of cancer, particularly cervical cancer.[6,10] The presence of long-standing atrophy associated with intra-prostatic inflammation has been implicated in the pathogenesis of prostate cancer, as prostatic inflammation can be the source of DNA damage from reactive oxygen and nitrogen species.[11] The HPV, especially HPV type 18, shows a special affinity to the glandular epithelium and it is capable of in vivo replication and can immortalize prostate epithelial cells in vitro.[12] However, in some recent studies, no associations were observed between prostate cancer and HR-HPV.[13,14] Moreover, prostate cancer does not meet some "expected features of HPV-caused cancers", for prostate cancer is not squamous cell in origin, and does not occur at anatomic sites of exposure by direct contact.[15] The carcinogenesis of HPV depends on the expression of viral E6 and E7 oncogenes, which inhibit tumor suppressor proteins p53 and pRb105.[15] However, p53 mutations and deactivation are common in prostate cancer and loss of the Rb gene occurs in at least 50% of prostate cancers.[15] Other epidemiological studies have examined the relationship of HPV infection with prostate cancer, but have shown conflicting results.[5,8,13-23] Other investigators successfully detected HPV DNA in prostate cancer cells.[24] The STDs are theorized to increase the risk of prostate cancer by causing inflammation of the prostate, which may then lead to the initiation of carcinogenesis.[25] A meta-analysis provides evidence of a higher rate of prostate cancer in men with a history of exposure to gonorrhea, HPV, or any STD.[9] Further research is required to confirm this potentially modifiable risk.[9] Sexual behavior, such as first intercourse at early age, and larger numbers of sexual partners are related to an increased risk of prostate cancer,[26] but recent sero-epidemiological studies of these infectious agents in relation to prostate cancer have produced differing results.[24] Adami et al (2003)[16] and Korodi et al (2005)[8] measured the presence of antibodies to the major oncogenic HPV types (16, 18, and 33), and they found that HPV types 16 and 18 were not associated with prostate cancer. Also, other researchers' findings indicated, by testing serum IgG antibodies to HPV-16 and 18, some serologic evidence of HPV-16 and 18 infections and risk of prostate cancer, but noted that the results are inconsistent.[24] Other researchers found increased levels of HPV-16 DNA in a subset of prostate cancers.[25] Although the cause of prostate cancer is still not certain, known risk factors for prostate cancer include older age, sexual behavior, and family history.[26,27] Other risk factors such as diet, physical activity, and occupational exposures have been studied with conflicting findings.[26,27] Treatment consists of surgery (including prostate gland removal) in the early stages, and hormonal and radiotherapy in advanced stages of the disease. An effective HPV vaccine against the 2 most common cancer-causing strains of HPV (16 and 18) has been licensed in the United States to cervical cancers, and could be of benefit for prostate cancer later if a relationship were proven. Hence, the objective of our study was to explore the possibility of finding HPV infection in the prostate tissue of a cohort of Saudi men presenting with benign prostatic hyperplasia (BPH) or prostate cancer, as different studies show evidence and presume that either HPV infections are not related to prostate cancer, or HPV is not implicated in the progression of prostate cancer. The present study is important as prostate cancer is the most common neoplasm of American men,[28] and the second most common cause of cancer-related deaths, even if prostate cancer is considered rare in our country.[29-31]

Methods. In this study, Hybrid Capture 2 (HC2) HPV DNA test (Digene Corporation, Gaithersburg, MD) was used for HPV infection detection from March 2007 to December 2008. Informed consent was obtained from the study participants, after approval of the experimental protocol by the Medical Ethics Committee of King Abdulaziz University Hospital (KAUH), Jeddah, Kingdom of Saudi Arabia. A total of 56 circumcised male patients (n=56), age range 50-93 years, diagnosed with cancer or BPH. The HC2 was applied to detect the presence of HPV infection in prostate biopsies of these patients. The subjects of this study were men attending the KAUH for prostatic biopsy due to a suspected diagnosis of BPH or prostatic cancer. We included all patients where prostate cancer was suspected or surgical management of BPH was indicated to obtain prostatic tissue. The Urology team was responsible for the collection of additional prostatic biopsies from the above-specified patients. Fresh prostatic biopsies with BPH or suspected adenocarcinoma were obtained by biopsy via the TRUS-Guided biopsy

(transrectal prostatic puncture method) and by transurethral resection for benign prostatic hypertrophy up to 5 mm in cross-section then placed immediately into the specimen transport media and transferred to the Virology laboratory at KAAUH, to be stored at -20^{0}C. The prostatic biopsies were processed with HPV DNA HC2 Digene test in the virology laboratory. The HPV DNA tests using HC2 technology are signal amplified hybridization antibody capture microplate assays that utilize chemiluminescent detection of 18 types of HPV DNA in prostatic specimens and can differentiate between 2 HPV DNA groups, the low-risk HPV types (6, 11 42, 43, 44) and the high/intermediate risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68). Specimens containing the target DNA hybridized with a specific HPV RNA probe cocktail. The resultant RNA:DNA hybrids were captured onto the surface of a microplate well coated with antibodies specific for RNA:DNA hybrids. Immobilized hybrids then reacted with alkaline phosphatase conjugated antibodies specific for the RNA:DNA hybrids, and detected with a chemiluminescent substrate, as described by the Digene Corporation instruction manual. Several alkaline phosphatase molecules were conjugated to each antibody. Multiple conjugated antibodies bound to each captured hybrid resulting in substantial signal amplification. As the substrate was cleaved by the bound alkaline phosphatase, light was emitted that is measured as relative light units (RLUs) on a Luminometer. The intensity of the light emitted denoted the presence or absence of target DNA in the specimen. The interpretations of the test result were performed according to the manufacturers' instructions using the Digene hybrid capture system version 2 (DHCS v.2) software and DML 2000 instrument. Briefly, RLU measurements equal or greater than the cutoff value indicated the presence of HPV DNA sequences in the specimen. Whereas, an RLU measurement less than the cutoff value indicated the absence of the specific HPV DNA sequence tested.

Results. Fifty-six histopathological prostatic specimens were studied by HC2. All prostatic lesions were categorized into benign lesion pattern of prostatic hyperplasia, malignant, and various inflammatory disorders as described in **Table 1**. No HPV was detected among the 56 tested biopsies using the HC2, all the participants were circumcised.

Discussion. The possible etiologic role of HPV in prostate cancer[32] is an active focus of research. Our study was conducted to investigate HPV infection in prostate tissue from a small number of Saudi men with BPH and prostate cancer. In the analyzed collected prostate tissues, we did not detect any relationship between HPV DNA positivity and prostate adenocarcinoma, BPH, and BPH with inflammatory changes. Some prior studies[8,21,23-25,28,32] in this field of research reported a positive association between serological evidence of HPV infection and prostate cancer, but there were concerns regarding study methodology and limited study sizes. In our opinion, epidemiological studies of prostate cancer suggesting the existence of a sexually transmitted risk factor, or reporting the presence or association of HPV DNA in prostate-cancer tissue according to a sero-epidemiological evaluation by analyzing serum samples for the presence of IgG antibodies against HPV types are not reliable. Perhaps their results suggest that infection with oncogenic HPV might be involved in the etiology of a minority of prostate cancers. In these studies, dependence on serological data to conclude any relationship between HPV and prostate cancer was not accurate, as integration of HPV DNA in cancer cells were not studied. Other studies confirming the relation between HPV and prostate cancer as an etiological agent is also controversial. The findings of Strickler et al, (1998)[5] by testing specimens from 2 populations at different risks for prostate carcinoma, using 3 different polymerase chain reaction (PCR) assays and 2 serologic assays for HPV, suggested that HPV is not associated with prostate carcinoma, and that HPV DNA is not at all common in the prostate glands of older men. Kulamala et al (2004),[7] and Gazzaz (2007)[33] concluded that PCR testing is not as sensitive for screening HPV as hybrid capture. Almost all the above-mentioned investigators, supporting the relation of HPV and prostate cancer, used PCR or HPV antibody detection,[5,8,15,23,24,33,34] which are not reliable tests. Overall, the association of prostate cancer with sexual history and, particularly, STD has been suggested but not firmly established, thus, it is not possible to entirely rule out a role for HPV or other known sexually transmitted infections in inducing prostate cancers, as consistent association of HPV with cervical cancer is well established.[22,23] Such an interpretation is speculative. The suggestive findings to date are sufficient to prompt efforts to identify viral sequences in prostate cancer specimens and search for an infectious cause of prostate cancer.[22] Updated available existing technologies allow testing for known and unknown infectious agents.

Table 1 - Histological distribution of prostatic biopsies (N=56).

Histological category	n (%)
Inflammatory reactions associated with nodular hyperplasia	29 (51.8)
Benign prostatic hyperplasia	21 (37.5)
Prostatic adenocarcinoma	6 (10.7)
Total	**56 (100)**

Our results using the hybridization test, indicate that it is unlikely that HPV-16, HPV-18, or the other tested subtypes enhance the risk for prostate cancer, as none of the biopsies tested by HC2 showed any positivity to HPV. However, it is possible that other, as yet unrecognized viruses may play a role, or that earlier associations with measures of sexual activity may be somewhat confounded by a man's hormonal milieu, which may differ among men with different sexual practices.[13]

Inconsistent associations with different HPV types reported in the different mentioned studies, suggest, in our opinion that the association may be because of chance, bias, or confounding by some unknown risk factor that may associate with different HPV infections in different populations. Additional studies of the relationship between prostate cancer and other HPV types, notably HPV 33, would be helpful to clarify the possible role of sexual risk factors, as investigated by Korodi (2005).[8]

Reports of low incidence rates of prostate cancer in Saudi Arabia are well documented. The real reasons behind this low incidence may be due to a small cancer pool, as the population is young, and the genetic make up of Saudi's is mostly Asian with low penetration of prostate cancer genes, dietary intake devoid of pork fat, consumption of a protective Mediterranean diet, and the abundance of sunshine. In addition, the most important factor is that all males are circumcised, usually in their early neonatal life eliminating the primary receiving cellular tissue environment, raised in a moral and religious way that strictly prohibits extramarital sexual activities, leading to low prevalence of STD, chronic atrophic prostatitis, and viral infections.

The presence of inflammatory reactions associated with BPH in our study, and BPH in the absence of HPV infection as shown in **Table 1**, raises the question of the etiology of this inflammation. Previous studies effectively ruled out other infections as the cause, which makes this area in need of further extensive research to establish the etiology of this inflammation. In our study, no association was found between the presence of HPV and histological inflammation in the prostate. However, the low number of prostate biopsies was an important limitation of this study.

As a preliminary conclusion, HPV infection looks to not be one of the causes of BPH or prostate cancer in our community. The religious, moral, and ethical behaviors that are associated with neonatal circumcision seem to protect the adult male in our local community from STD, bacterial, and viral infections, and thus data from our current research at KAUH in Jeddah indicated the absence of HPV DNA in several samples of the prostatic tissues examined. Therefore, it may also be the reason why the incidence of prostate cancer in Saudi Arabia is low. A similar study should be conducted on a larger number of cases to identify an infectious cause of prostate cancer, which would be a major advance, providing scientists with a new window into prostate tumorigenesis as well as an excellent target for efforts to prevent and treat prostate cancer. We recommend further studies of the relationship of circumcision, STDs, and prostate cancer in a larger population to examine and confirm the protective role of circumcision against prostate cancer.[34] We also recommend collaboration between epidemiologists and clinicians with laboratory investigators in the collection of appropriate multiple prostate cancer specimens for virology testing.

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