Molecular Detection of Human Papillomavirus Genotype-33 in Tissues from Patients with Prostate Cancer and benign Prostatic Hyperplasia

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Abstract

Background: High oncogenic –risk genotypes of Human Papillomavirus (HPV) are a group of genetically related organisms that infect a wide range of human cells including prostate epithelium and induce proliferative changes in these cells that result in both benign and malignant tumors.

Objectives: This study aimed to detect DNA of HPV genotype-33 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: Fifty(50) formalin-fixed, paraffin embedded prostatic tissue blocks were obtained ,among them (25) tissue biopsies from prostatic carcinoma with different grades and (15) benign prostate hyper plastic tissue blocks as well as (10) apparently normal prostate tissue autopsies which were collected from the archives of Forensic Medicine Institute / Baghdad and used as prostate healthy control groups. Detection and genotyping of HPV was done by highly sensitive in situ hybridization technique.

Results: Signals of in situ hybridization reactions that indicating presence of HPV-33 in prostate cancer tissues were detected in32% (12 out of 40 cases) whereas in BPH, HPV-33 was detected in 13 %(2 out of 15 cases). Non HPV-33 was detected in the apparently healthy control group .The highest percentage (37%) of positive- HPV33- DNA ISH reactions was found in tissues of prostatic carcinoma showing moderate differentiation.

Conclusion: Our results indicate that the oncogenic HPV-33 might contribute to the development of subset of prostate tumors.

Key word: HPV-33; prostate cancer, benign prostatic hyperplasia, in situ hybridization.

الخلاصة

خلفية الدراسة :الفايروسات الحليمية البشرية ذات الخطر السرطاني العالي تصيب طيف واسع من الخلايا البشرية وبضمنها أنسجة البروستات مؤدية الى حدوث أورام حميدة وسرطانات غدية فيها.

هدف الدراسة :التحري عن الفايروس الحليمي البشري ذو نمط الاخطار العالي 33 بالانسجة المأخوذة من سرطانات البروستات وحالات التضخم الحميد في البروستات وتفسير علاقاتهما بعملية حدوث التطور السرطاني في البروستات.

المواد وطرائق العمل :جمعت خمسون عينة من نسيج البروستات المحفوظ بالفورمالين والمطمور بشمع البارافين منها(25)عينة مأخوذة من الخزع الحيويةالنسيجية من سرطان البروستات و 15 أخرى من حالات التضخم الحميد في البروستات بينما أخذ 10 خزع نسيجية من معهد الطب العدلي في بغداد حيث أستخدمت كمجموعة سيطرة /. استخدمت طريقة التهجين الموضعي ذات الحساسية العالية للكشف عن الفايروس الحليمي البشري ذو الاخطار العالي نوع .33

النتائج :أوضحت الدراسة الحالية بأنه نسبة حدوث الفايروس الحليمي البشري ذو الاخطار العالي 33في حالات سرطان البروستات كانت12) 32% من اصل40 حاله.(بينما 2)13% من اصل 15 حاله (نسبة حدوثهما في حالات التضخم البروستاتي الحميد بفحص التهجين الموضعي .لم تسجل حالة تفاعل تهجين موضعي للفيروس في حالات الانسجة في مجموعة السيطرة الطبيعية .

الاستنتاجات :الفايروس الحليمي البشري ذو الاخطار العالي 33 قد يلعب دورا مهما في حدوث وتطور سرطان الخلايا في البروستات و حالات ا التضخم الحميد للبروستات.

الكلمات المفتاحيه: الفايروس الحليمي البشري ذو الاختطار العالي-33 سرطان البروستات, التضخم الحميد للبروستات طريقة التهجين الموضعي.

Introduction

Many DNA and RNA viruses have proved to be oncogenic in animals. However, only a few viruses have been linked with human cancer. Viral factors are the most important class of infectious agents associated with human cancers (Jawetz *et a1*.,2010).

It was estimated that 17-20% of all world-wide incidence of cancers attributable to a viral etiology (Clifford *et a1*.,2003 and Jawetz *et a1*.,2007). Papillomavirus are a group of genetically related organisms, which infect epithelium and infuse proliferation variation in infected cells, which can lead tissues in both benign and malignant tumors (Maghrabi *et a1*.,2007).Viral infection may lead to chronic and recurrent inflammation of the prostate and initiate or promote carcinogenesis (Martinez-Fierro *et a1*., 2010).

The association of Human papilloma virus (HPV) as specific epitheliotropic DNA viruses and (HPV) infection, as sexually transmitted disease, with the risk of prostate cancer may be explained by the role of chronic inflammation and genomic oxidative damage to prostate epithelium (Ferenczy and Jenson 1996) . . (HPVs) are regarded HPVs can persistently infect prostate epithelium in non immunocomprised hosts (Scott *et a1*., 2001).

To date, more than 200 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(Munoz *et a1*., 2003), and have been shown to passes oncogenic potential (Dell and Gaston, 2001; Yahyapour *et a1*., 2012).

High oncogenic risk HPV types may integrate into the host cell chromosome, here they interrupt the integration of E2 gene that regulatesthe transcription& expression of HPV-E6 & E7 oncoproteins.

The carcinogenesis of HPV depends on the expression of viral E6 and E7 oncogenes, which inhibit tumor suppressor proteins p53 and pRb, respectively (Satoshi *et a1*., 2008) and (Ghittoni *et a1*., 2010). These oncoproteins inactivate the cellular tumor suppressor gene products of p53 and Rb, respectively(Satoshi ,2008)(Ghittoni , *et a1*., 2010).

It is clear that continued expression of these viral oncogenes is necessary for histopathologic progression and the malignant phenotype of an HPV-associated tumors (Carole and Gillison,2006). Recent studies suggest that HPV infection may play a role in the development of oral cancers (Blancato *et a1*., 2004);head and neck cancers (Ibrahim *et a1*., 1992 and McNicol & Dodd ,1991);esophageal cancers(Sarkar *et a1*., 1993);lung cancers(Horowitz and Fronk1997); and colorectal cancers(Al-Ahdal *et a1*., 1996);squamous cell carcinoma of the penis(Griffiths and Mellon,2000);transitional cell carcinoma.(Yu *et a1*., 1993).

Recent molecular studies reveal a likely role for HPV infection in skin carcinogenesis (Akgul *et a1*., 2006);non keratinizing squamous cell carcinoma of oropharyngeal region (El-Mofty and Patil,2006). In addition, other reports document the presence of HPV DNA in prostatic tissues (Al Jewari *et a1*., 2007).

The 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 (Moyret-Lalle et al.,1995 and Suzuki etal.,1996).Other study found a strong association with HPV-33 (Adami *et a1*.,2003).

So this study aims to assess the in situ hybridization expression of HPV-33 in Benign Prostatic Hyperplasia(BPH) and prostate cancer and to elucidate the correlation of these two high–risk oncogenic HPV-genotypes with progression of BPH and prostatic carcinogenesis.

Materials and methods

Patients and tissue samples

Fifty(50) formalin-fixed, paraffin embedded tissues were collected from prostate biopsies that were related to (25) prostatic carcinoma,(15) benign prostate hyperplasia and (10) apperarently normal prostate tissues .They were collected from the pathological archives of Teaching Laboratories of Medical City Hospital and Forensic Medicine Institute / Baghdad during the period of 2012 to 2013. 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. Prostate cancer patients were classified into three grades :well differentiation, moderate differentiation, and poor differentiation .

Methods:

Detection of HPV genotype by ISH kit (Maxim biotech Inc, USA) was performed on 4µm paraffin embedded tissue sections using a biotinylated DNA probe for HPV-33(cat. No. IH-60058).One section was mounted on ordinary glass slide and stained with haematoxylin and eosin, whereas other section was mounted on charged slide to be used for in situ hybridization for detecting HPV-33 to perform the In situ hybridization procedure. The slides were placed in 60˚C hot- air oven over night. The tissue sections were deparaffinized and treated by graded alcohols according to the standard methods. The slides were treated then with proteinase K solution.

One drop of the biotinylated cDNA probe for HPV-33 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 -keepine gene probe while negative control reaction was obtaing 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(NFR) 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 score in our research to qualify the obtained results as positive or negative HPV -33 ISH reactions. A score zero was given to these results without detectable ISH reaction whereas the results of (10% , 25% and 50%) were pointing for scores 1-3 stated by (Wilcox e t al ., 1998) are referring to low , intermediate, and high infection, respectively.

Statistical analysis

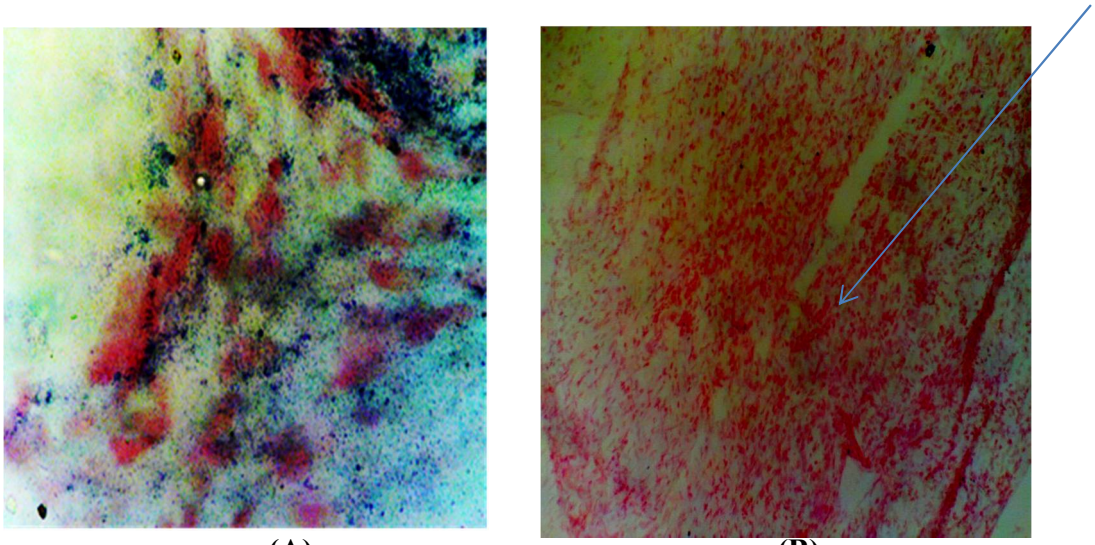
was done by Chi- square test, percentage ,range, mean and standard deviation .Correlation was considered significant when $p<0.05$.

Results

Table 1 and figure 1 shows the positive results HPV33 DNA-ISH detection,where32% (8 out of 25 cases) malignant prostate tumors showed positive signals .The benign group revealed 13.3% positive signals which represented(2 out of 15 cases)in this group. None of control group revealed positive signals for ISH-test . A highly significant differences between the percentage of infection with HPV genotype 33 was detected between patients with prostate cancer and benign prostatic hyperplasia **(**P<0.001**)** (table1).

Table (1) : Frequency distribution of HPV DNA signal scoring among the malignant prostate tumors , benign prostate tumors and healthy prostate tissues.

HPV signal scoring		Malignant Prostate Tumors (n=25)		Benign Prostate tumors (n=15)		Normal Prostate Tissues (n=10)		P
		N	%	N	%	N	%	
Negative		28	68	13	86.7	10	100.0	0.001 significant
Positive		12	32	2	13.3	0	0.00	
Scoring	I	5	41.7	1	50	0	0	
	II	3	25	1	50	0	0	
	III	4	33.3			0	0	
Mean Rank								



(A) **(B)**

Figure (1): In situ hybridization (ISH) for HPV-33 DNA- detection infiltrative prostate cancer using Biotinylated -labeled HPV-33 probe; stained with BCIP/NBT(Blue) stained and counter stained by nuclear fast red (Red); A. prostate cancer with negative ISH reaction(40X). B. positive HPV- ISH reaction with strong score and high signal intensity (40X).

Table 2: shows the correlation of infection with high-risk HPV33 genotype and their scoring with prostate cancer grading . Tweleve cases out of 25 showed positive for HPV33-ISH reaction .Sixteen percent (16 % :4 cases out of 12) of prostate carcinoma that have well differentiated grade . Also , its were found that 20% (5 cases out of 12) and 12% (3 cases out of 12 cases) of prostate carcinoma that showed moderately differentiated grade and poorly differentiated grade , respectively. Statistically, no significant correlations between HPV31 infection and the breast cancer grade .

(Table2): Distribution of HPV33-associated prostate carcinoma according to their tumor grade/differentiation of cancer.

Grade		Type HPV33	
		Positive	**Negative**
Well (I)	Count	**4**	**7**
	% within grade	16%	13%
	% within HPV33	30%	0.00
Moderate(II)	Count	**5**	**14**
	% within grade	%20	26%
	% within HPV33	37.5%	0.00
Poor(III)	Count	**3**	7
	% within grade	12%	%13
	% within HPV33	32.5%	0.00
Total	Count	**12**	**28**
	% within grade	48%	52%
	% within HPV33	100.0%	100.0%

Discussion

Since human papillomavirus (HPV) infection was first identified as a risk factor for cervical cancer, several studies have investigated HPV in relation to prostate cancer with mixed results (Strickler etal ,1998). When Taylor and colleagues (HYPERLINK"HYPERLINK%20%22 http://europepmc.org/abstract/MED/15988645%222"HYPERLINK"http://europepmc.org/abstract/MED/15988645"(Rosenblatt etal,2003). combined the results of ten of these studies, they observed a significant positive association between HPV and prostate cancer.

Many studies showed the association between HPV and PCa .These studies showed that HPV prevalence varies from 2%to 100% in PCa samples, The most reported types of HPVs in prostate cancers were HPV types 16,18,33 and 31 (Anwar *et al.,*1992)and (Leiros *et al,* 2005).

The present results are much higher than the results of positivity of HPV33 in the examined prostatic cancerous tissues reported by(Amitis *et al.,* 2011) and (Leiros *et al.,* 2005) where they found (4.3%, 12%, 32%), respectively.

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 (Al-Ahdal *et al.,* 1996).

By analogy, it was found that the present findings of HPV 16 &31 in an equal proportions are consistent to those studies done in Iraq by Mohammed Ali (Mohammed-Ali,2001;Al-Azzawi,2006;Bakir ,2006; Khashman, 2008; AL-Mahbobi, 2011; Al-Aizzi, 2011;Al-Alwany, 2013) who found that the HPV 16,18 ,31& 33as the prevalent types in their studied group of the cervical ,laryngeal ,esophageal ,oral, prostatic ,ovarian carcinomas and breast carcinoma, respectively. The HPV infection rate has also increased significantly with the increasing Gleason score (Anwar *et a1*., 1992). Al- Maghrabi has demonstrated that the rate of HPV infection has increased in patients with stage promotion of the tumor and with higher Gleason score (Maghrabi *et a1*., 2007).

By analogy ,the results of this study were in disagreement with the findings of (Ibrahim etal,1992). who found 50% of HPV16 in BPH by using PCR method and also consistent with the findings of each (Ibrahim etal,1992).who found 20% of HPV18 in BPH) and (Al-Jewari ,2007) 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 (McNicol & Dodd ,1991). (60.7%)in BPH by using PCR method; and those reported by ((Rotola etal ,1992) who found (82%) positivty 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 (Hisada etal,2000).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 (McNicol & Dodd ,1991). who found HPV18 (5.4%); (Al .Jewari ,2007). 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(Al Jewari 2007).

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 (Wilcox *et al.,* 1998). 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 (Strickler *et al.,* 1998).. 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.

The highest percentage of infection with HPV –genotype 33 was detected in moderate differentiation grade followed by poor grade and well grade (37.5 %,32.5 %,and 30 %) respectively. This result is approximately agreed with a study carried out in Baghdad city/Iraq by (Al_Mahbobi;2011) who mentioned that most of Iraqi patients with prostatic showed that (70.3%) of these cancerous cases have moderate and poor differentiation. Whereas those cases that showed well differentiated grade constituted (29.6%) of the total specimens therefore occupied the least rank in this group. This may be attributed to the fact that late grades of PC are usually associated with immune suppression and increasing the susceptibility to the infection with HPV.

Thos study may provide an evidence for the role of HPV genotype 33 in the induction of prostate cancer.

The detection of viral nucleic acids within tumor cells is one crucial prerequisite to demonstrating an involvement of viral infection in the development of human malignancies (Zur Hausen, 1994). Cancer occurs in people who have infected with HPV for a long time, usually over a decade or more (persistent infection) (Greenblatt, 2005 ; Sinal, 2005). The oncogenic HPV are involved in the pathogenesis of prostate cancer has been a subject of great controversy (Dami *et al.,* 2003; May *et al.,* 2008).

The HPV infection rate has also increased significantly with the increasing Gleason score (Anwar *et al.,* 1992).Al Maghrabi (2007) has demonstrated that the rate of HPV infection has increased in patients with stage promotion of the tumor and with higher Gleason score.

About one –third of patients with prostate cancer in this work were infected with HPV genotype 33 . This may indicates the possible role of this virus in the induction of PC**.**

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.

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