

Detection of human papillomavirus DNA sequences in cancer of the urinary bladder by in situ hybridisation and polymerase chain reaction

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Abstract

Objective—To evaluate the prevalence of “high risk” human papillomavirus type 16 (HPV 16) in transitional cell carcinoma of the urinary bladder.

Materials and methods—The study included 10 biopsy specimens from male patients of transitional cell carcinoma of the urinary bladder for the detection of HPV DNA sequences. Specimens were collected from the Urology Clinic of the K.G. Medical College Hospital, Lucknow, India. Detection of HPV DNA was carried out by tissue in situ hybridisation (a single copy gene localisation method) using 3H-labelled HPV DNA probe and also by polymerase chain reaction (PCR) techniques using primers to HPV 16 upstream regulatory region (URR).

Results—Out of 10 cases of transitional cell carcinoma of the urinary bladder, “high risk” HPV 16 DNA was detected only in one (10%) by using in situ hybridisation whereas two cases (20%) were found to be positive by polymerase chain reaction.

Conclusion—Our results suggest that the rare occurrence of HPV in bladder carcinoma may not have a causal relation with the viral infection.

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Keywords: HPV; DNA; urinary bladder, carcinoma

Introduction

Although about 70 human papillomavirus (HPV) genotypes have been associated with benign, premalignant and malignant lesions of the anogenital tract and many other organ sites in humans, the involvement of these viruses particularly of “high risk” HPV types 16 and 18 in urinary bladder carcinoma is not clearly understood. Detection of bovine papillomavirus (BPV) in bladder carcinoma of cattle,^{1,2} the presence of HPV specific condylomatous lesions³⁻⁶ and HPV antigens in cancer of the bladder in humans⁷ suggest the presence of HPVs in these carcinomas. This has been further strengthened by experimental evidence that injection of cutaneous BPVs into the submucosa of urinary bladder lead to development of fibroma.^{1,8} Also, HPV DNA has been detected in prostate cancer⁹ and urine samples of men^{10,11} and women.¹² Recently, a low frequency of HPV 16 in tran-

sitional cell carcinoma has been detected by in situ hybridisation¹³ and polymerase chain reaction.¹⁴ But most surprisingly, a very high prevalence of HPV (81%), particularly the high risk (16 & 18) types (62%) in bladder carcinomas has been reported¹⁵ recently from Japan. In India, the incidence of HPV, particularly the type 16 in cancer of the uterine cervix is very high (> 90%) in women.¹⁶ However, so far no report on HPV prevalence in bladder carcinoma has been available from the Indian population. Since genital condylomata can easily extend to the urethra and urinary bladder,^{4-6,17} it is not unlikely that HPV infection will be found in these organs. The present study has been carried out to detect HPV DNA in cancer of the urinary bladder in men using both in situ hybridisation and polymerase chain reaction.

Materials and methods

The study comprised histopathologically confirmed 10 cases of transitional cell carcinoma (TCC) of the urinary bladder in grade II and III subjected to HPV DNA detection by both in situ hybridisation and polymerase chain reaction. Out of 10 cases, 7 were papillary TCC, 2 solid TCC and one was a mixture of both. These cases were registered at the Urology Clinics, K.G. Medical College Hospital, Lucknow. Paraffin embedded tissue sections were used for the in situ hybridisation as well as for the extraction of DNA for the polymerase chain reaction.

Tissue in situ hybridisation

In situ hybridisation for the detection of HPV DNA in paraffin embedded tissue sections was performed according to the protocols described earlier by us.¹⁹ Vector-free 8 kb HPV 16 DNA insert was nick-translated with ³H dCTP, dATP and dTTP (Amersham, England) to a specific activity of 1×10^8 cpm/ μ g DNA and was used as probe. Following hybridisation, slides were coated with Kodak NTB2 nuclear track emulsion (Kodak & Co., USA), incubated for 4 to 6 weeks at 4°C and developed in D19B developer. Haematoxylin-eosin stained slides were examined under oil immersion for the presence of silver grains over the cell nuclei as an index of HPV DNA positivity.

DNA extraction and polymerase chain reaction

DNA extraction from paraffin sections and polymerase chain reaction (PCR) were carried out according to the methods described earlier

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Figure 1 (a, b): ISH of urinary bladder carcinoma tissue sections (a, b) with 3H-labelled HPV 16 DNA probe using the single copy gene localization method. Of 10 bladder cancer tissues analysed only 1 (10%) contained HPV 16 DNA. Presence of HPV DNA was indicated by deposition of silver grains over the nuclei. The sections were stained with a H and E following hybridisation.

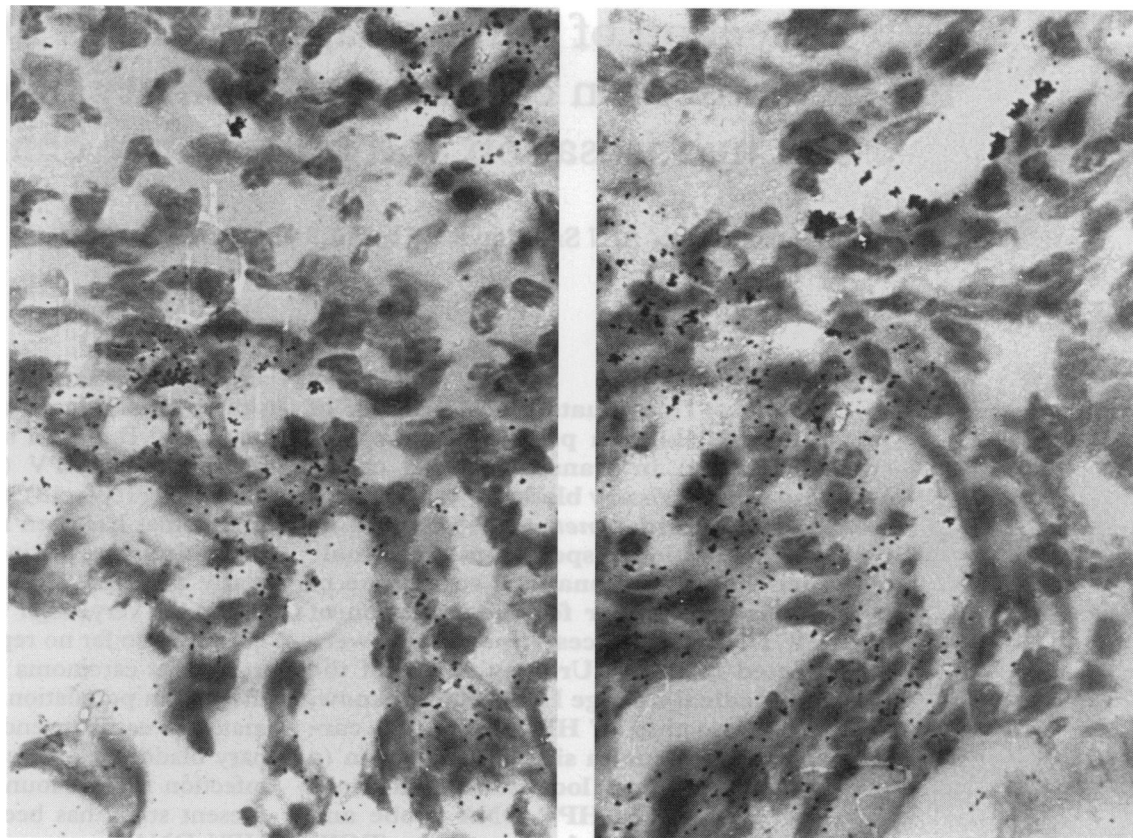


Figure 2 PCR amplification of oligonucleotide primers from the conserved URR region of HPV 16 showing 217 bp amplicon. First lane is Hae III-digested \emptyset X174 DNA size marker. Second lane is HPV 16 plasmid DNA, the lanes 1 to 10 are urinary bladder carcinoma DNA samples showing HPV 16 specific amplification of 217 bp in only 2 samples (lanes 2 and 4).

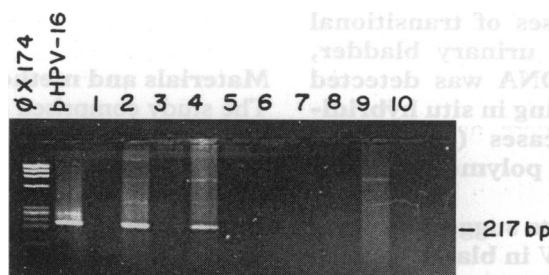


Figure 3 PCR amplification of oligoprimers from human β -globin gene which served as an experimental control. Lanes 1 to 8, all urinary bladder carcinoma DNA, showing 268 bp β -globin gene amplification. Lane 8 is HPV 16 positive bladder carcinoma sample showing amplification of both β -globin gene (268bp) as well as HPV 16 DNA (217bp). First three lanes are Hae III-digested \emptyset X174 DNA size markers, HPV 16 plasmid DNA and distilled water showing no globin gene amplification respectively



by us.¹⁶⁻²¹ Two oligo nucleotide primers from the upstream regulatory region (URR) of HPV 16 have been used. (P1, 5'- AAG GCC AAC TAA ATG TCA C -3' and P2, 5'- CTG CTT TTA TAC TAA CCG G -3'). These primers allow amplifications of the region between nucleotides 7765 and 75 and an amplicon of 217 base-pairs. β -globin gene showing amplification of the 268bp fragment

was used as internal experimental control. Twenty microlitres of PCR amplified products were run on 3% Nusieve agarose gel (FMC BioProducts, Rock Land, USA) along with Hae III-digested \emptyset X174 DNA marker. Gels were stained in ethidium bromide and photographed by a UV transilluminator.

Results

Both in situ hybridisation (ISH) and PCR techniques have been used to detect the presence of HPV 16 DNA sequences in 10 histologically confirmed transitional cell carcinoma of the urinary bladder. Out of 10 cases of transitional cell carcinoma only one was positive by ISH whereas using polymerase chain reaction, HPV 16 DNA was detected in two cases. Figure 1 (a, b) shows the HPV 16 DNA positivity by radioactive ISH in the transitional cell carcinoma of the urinary bladder. The presence of silver grains can be seen in the majority of cell nuclei (arrows).

Figure 2 shows the HPV 16 DNA positivity in two out of ten bladder carcinoma cases analysed by PCR. An amplified PCR product of 217 bp fragment can be seen in the ethidium bromide stained 3% Nusieve agarose gel. Lanes 1-10 are the bladder tumour DNA samples of which lanes 2 and 4 show HPV 16-specific 217bp amplification. The first two lanes on the extreme left represent Hae III-digested \emptyset X174 DNA marker and HPV-16 plasmid DNA amplification respectively. Amplification of β -globin gene (268bp) which served as an internal control for the experiments is shown in fig 3. Lanes 1 to 8 are bladder carcinoma showing amplification of β -globin-specific 268bp fragment. Lane 8 is

an HPV positive sample showing both β -globin and HPV specific bands. Of the three lanes on the left, the first is Hae III-digested OX174 DNA size marker, the other two lanes run with HPV plasmid DNA and distilled water do not show β -globin amplification respectively. In the present study, HPV 16 DNA frequency by ISH was found to be one in 10 as compared with 2 in 10 by PCR. Since the number of the cases analysed was small, no statistical analysis was employed.

Discussion

Carcinoma of the urinary bladder is one of the prevalent cancer among males and has been associated with many risk factors for the development of this disease.²²⁻²⁴ Although immunological demonstration of papillomavirus common structural antigens^{7 25 26} and observation of HPV-specific cytomorphological features of koilocytotic atypia²⁷⁻³⁰ in urinary bladder carcinomas have provided sufficient ground to believe that HPV may be involved in these tumours, several previous studies have failed to demonstrate HPV DNA in them.^{31 32} There have been reports of the presence of condylomatous lesions indicative of HPV 6 and 11 infection^{28 29} and also of a case of bladder carcinoma showing HPV 16 positivity with mild immunodeficiency and genital warts.¹⁷ However, the involvement of "high risk" HPV types 16 and 18 in carcinoma of the urinary bladder has not been established. Recently, there have been two reports showing a low incidence of human papillomavirus type 16 DNA in urinary bladder tumours.^{13 14} In the present study, we have also observed a very low prevalence of HPV 16 in transitional cell carcinoma of the urinary bladder by both ISH and PCR. Our observations are in good agreement with the earlier reports. But in sharp contrast to these findings including this report, recently Anwar *et al*¹⁵ using PCR assay reported a very high prevalence (81%) of HPV in bladder carcinomas as well as in normal urinary bladder specimens (33%). The frequency of "high risk" HPV types—16, 18 and 33 was also found to be very high (62%), a rate much higher than that reported for cervical carcinomas (44%) in Japan.³³ It is difficult at present to ascertain such a high prevalence of HPV in urinary bladder carcinoma. This may be because of either technical problems associated with PCR detection of HPV or geographical variations in HPV prevalence. Further study from Japan and other regions would throw light on this aspect.

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