Original Article

Human Papillomavirus in Urothelial Carcinoma of Bladder: An Indian study

Abstract

Objectives: To study the role of high-risk human papillomavirus (HPV-16 and HPV-18) types in the causation of urothelial carcinoma of the urinary bladder in Indian population. **Methods:** 50 patients with Urothelial carcinoma of the urinary bladder were included in the study. Another 10 age-matched subjects who were hospitalized for transurethral resection of prostate for benign prostatic hyperplasia and/or ureterorenoscopy for ureteric stone disease were enrolled as controls. The tissue samples were analyzed for the presence of HPV-16 and HPV-18 DNA by polymerase chain reaction (PCR). The histopathology of the tumor tissue was carried out to assess the grade of the tumor. **Results:** The mean age of the patients was 54.1 years. A total of 28 (56%) patients had high-grade tumors and 22 (44%) had low-grade disease. T2 or higher stage disease was observed in 18 (36%) patients. All cancerous specimens and control specimens were found to be negative by PCR for the presence of HPV DNA. **Conclusion:** HPV prevalence in the urothelium is very low irrespective of the stage and grade of the disease, and hence, it is unlikely to be the causative agent for urothelial carcinoma of the urinary bladder in Indian population. However, the role of other HPV types in the etiology of this tumor needs to be clarified.

Keywords: *Human papillomavirus-16, human papillomavirus-18, urinary bladder cancer, urothelial carcinoma*

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Introduction

Association with human papillomaviruses (HPVs) infection has been documented with anogenital tract malignancies, including cervical and vulvar cancer, a subset of oropharyngeal cancers, and certain types of skin cancer. It has been seen that approximately 90% of cervical and vulval carcinomas contain HPVs. Since the urinary bladder is in direct connection with genital area through urethra, HPV may also play a role in the causation of bladder cancer. Although the causal relationship between HPV and urothelial carcinoma of the bladder has been evaluated by several groups, the findings are widely divergent showing the presence of HPV DNA in 0%[1-4] to 35%–52%[5-7] of bladder cancers. Hence, the present study was designed to test for the presence of HPV DNA in urothelial carcinoma of the urinary bladder in Indian population.

Methods

50 patients with Urothelial carcinoma of the urinary bladder were included in the study. Another 10 age-matched subjects who were hospitalized for transurethral resection of prostate for benign prostatic hyperplasia and/or ureterorenoscopy for ureteric stone disease were enrolled as controls. All patients provided written informed consent before enrollment. This study was conducted according to the guidelines set up by the Declaration of Helsinki (modified 2000). The institutional ethics committee approved the protocol. The bladder biopsies were obtained from these individuals and divided into two parts; one part was kept in formalin for histopathological examination and the other part was sent to virology laboratory in viral transport media under aseptic precautions and stored at −20°C in a deep freezer till tested further for the detection of HPV-16 and HPV-18 DNA.

The DNA extraction was carried out from the tumor tissue by the conventional phenol-chloroform method.[8] The HPV-16 and HPV-18 types were detected using type-specific primers as described by Jain *et al*.[9] The type-specific primers for high-risk type 16 were forward primer, 5'AAGGCCAACTAAATGTCAC3'; reverse

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primer, 5'CTGCTTTTATACTAACCGG3' and type 18 were forward primer, 5'ACCTTAATGAAAAACCACGA3'; reverse primer, 5'CGTCGTTTAGAGTCGTTCCTG3' as described earlier. The 25 µg reaction mix consisted of 100–200 ng DNA, 10 mmol/L Tris-Cl (pH 8.4), 50 mmol/l KCl, 1.5 mmol/L $MgCl_2$, 12.5 µmol/L of each dNTP, 5 pmol of each oligonucleotide primer, and 0.5 U of TaqDNA polymerase. The temperature profile used for amplification consisted of initial denaturation at 95°C for 5 min followed by 30 cycles with denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s with final extension of 4 min. To check the adequacy of DNA extraction, beta-globin was used as the internal control. The primers[10-12] used were forward primer, 5'GAG GAA CCA AGG ACA GGT AC 3'; reverse primer, 5'CCA CTT CAT CCA CGT TAC ACC3', which give an amplified product of 268 bp. The amplification product of HPV-16 was obtained at 217 bp and HPV-18 at 100 bp. Negative control sample (double distilled water instead of template DNA in polymerase chain reaction [PCR] mixture) and positive control (plasmid DNA of HPV-16 and HPV-18; positive samples from cervical carcinoma patients) were included in parallel with patient samples in each PCR run. The positive controls used were plasmid DNA of HPV-16 and HPV-18. Precautions were taken to prevent contamination during each experiment such as change of gloves and performing different procedures in separate areas. Analysis of amplified product was performed on 2% agarose gel stained with ethidium bromide [Figure 1].

Results

The age of the patients ranged from 44 to 79 years (mean 54.1 years).Majority of the patients were males (96%) and smokers (75%). On cystoscopy, the mean number of lesions was 2.74 and 74% of the lesions were papillary with a mean size of 2.84 cm.

The histological grade and stage of 50 tumors obtained from cases are shown in Table 1. Out of these 50 patients, 28 (56%) had high-grade tumors and 22 (44%) had low-grade disease. T2 or higher stage disease was seen in 18 (36%) patients.

DNA was isolated from tumor tissue and subjected to PCR for the detection of HPV-16 and HPV-18. Experiments were repeated with positive control for several times. All the 50 transitional cell carcinoma (TCC) bladder tissues were negative for HPV DNA using PCR. Further, HPV DNA was negative in the bladder tissue collected from the control subjects. The adequacy of DNA extraction was checked by amplification of the housekeeping gene globin [Figure 1]. Specific product of beta-globin gene was obtained at 250 bp in majority (47/50) of the samples.

Discussion

The fact that the HPV plays an important role in oncogenesis has been a landmark discovery in the field of medical scienceand accordingly, previous studies have attempted to determine a possible role for the virus in cancers of the urinary bladder. Further, the role of an infectious agent *Schistosoma haematobium* has been well established in the etiology of bladder cancer.

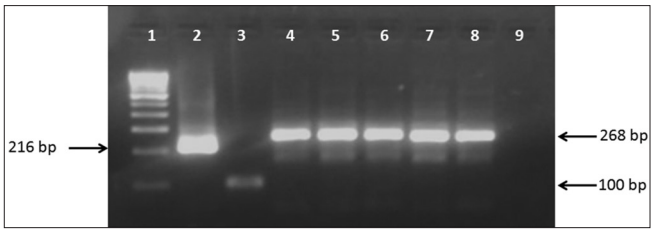

Figure 1: Agarose gel electrophoresis of products obtained by human papillomavirus polymerase chain reaction. Lane 1: Molecular weight markers (100 bp ladder), Lane 2: Positive control (plasmid DNA of human papillomavirus 16 showing 216 bp product), Lane 3: Positive control (plasmid DNA of human papillomavirus 18 showing 100 bp product), Lane 4–8: Representative samples positive for beta globin gene product at 268 bp, Lane 9: Negative control

Table 1: Pathological characteristics

Pathological characteristics	Number of lesions (%)
pT stage	
Ta	5 (10)
T1	27 (54)
T2 or higher	18 (36)
Grade (WHO-ISUP 2003)	
PUNLMP	Nil
Low grade	22 (44)
High grade	28 (56)

The present study aimed to study the role of HPV in the causation of urothelial carcinoma of the bladder in Indian population. HPV DNA could not be detected in any of the 50 tissues tested although 28 of these were high-grade tumors. The result is in agreement with majority of recent reports which suggest that HPV is unlikely to be involved in the etiology of urothelial carcinoma of the bladder.[1-4] We used highly sensitive PCR to detect HPV in a protectively acquired, fresh frozen tumor sample and all precautions were taken to avoid any possible contamination of the specimen. To rule out any problem in nucleic acid extraction, the internal housekeeping gene was amplified in the majority (94%) of the samples. Association of HPV and bladder cancer in previous studies is inconsistent. This variation may be due to multiple reasons such as low number of patients included in the study, lack of homogeneity in patient population, contamination during tissue analysis, and use of different method of detection of HPV such as PCR, immunohistochemistry, and southern blotting. Recently published two meta-analyses by Jimenez-Pacheco *et al.* and Li *et al.* concluded that there is a significant but moderate association between HPV prevalence and bladder cancer and reported an overall prevalence of 16.9%.[13,14] However, other studies have shown no association of HPV with bladder cancer

as described previously.[1-4] Both meta-analyses found geographical variation with the prevalence being highest in Asia (24.6%).[13,14] Our study also negates this observation.

There was no evidence of HPV infection in bladder tissue obtained from 10 control subjects recruited in the study. However, two of the previous studies have reported high HPV detection rate in both tumor tissue and normal urothelium.[15,16] In these studies, it is possible that false-positive results may have arisen as a result of urethral contamination as there is evidence to support the urethra as a reservoir for HPV in literature. Maloney *et al*. minimized the risk of urethral contamination by assessing only bladder tumor samples obtained at radical cystectomy but found no evidence of HPV positivity in any tumors studied using this protocol.[17] Nevertheless, urethral contamination is not the sole explanation as one of the German studies reported detecting HPV in six of 21 (29%) bladder TCC but in only two of 32 (6%) samples derived from urethral swab.[18]

Our study suffered from few limitations. These include a small sample size and the tissue samples were tested for the presence of high-risk HPV types 16 and 18 DNA only. The L1 gene PCR was not performed in our study since the main aim of the study was to see the prevalence of only HPV-16 and HPv-18 as there are no clear-cut studies which have shown the association of HPV with bladder cancer. A recently published study involving Central European population has also failed to find any HPV in bladder cancer tissues in spite of using PCR to detect broad range of HPV.[1]

Conclusion

The results of this study show that HPV-16 and HPV-18 are unlikely to be involved in the causation of urothelial carcinoma of the urinary bladder, especially in Indian patients. However, the role of other HPV types in the etiology of this tumor needs to be clarified.

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Conflicts of interest

There are no conflicts of interest.

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