

DETECTION OF HUMAN PAPILLOMA VIRUS FROM URINARY BLADDER CARCINOMA

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ABSTRACT

Background and Objective: A study was conducted to detect the Human Papilloma Virus (HPV) and to determine its association with other epidemiological factors in bladder cancer. It was a case control study conducted at Pathology Department of King Edward Medical University, Lahore, Pakistan from January 2006 to December 2010. The study may address the possibility of HPV vaccination in future to prevent the risk of bladder carcinoma.

Methodology: A total of 114 biopsies of urinary bladder cancers were collected from different hospitals in Lahore, Bahawalpur, Multan, and Islamabad. One hundred and seven biopsies of non-neoplastic urinary bladder lesions were included as a control. Conventional patient's history and clinical data was collected along with their biopsies. The real time polymerase chain reaction (PCR) was used for detection of Human Papilloma Virus. Data was entered in Window SPSS version 16. Frequency of HPV and relative risk was calculated as compared to control group from different histopathology sections of bladder carcinoma.

Results: Transitional Cell Carcinoma (TCC) was more common in old age (mean age 56.92 ± 13.94 years) and in males (male to female ratio was 4.2:1.0). Use of tobacco was significantly associated with HPV infection and TCC (86/114 [75.5%] cases). DNA was detected from 98 samples (86%) but HPV was isolated in only 16 cases. High risk HPV subtypes 16 and 18 were detected in 11/98 (11%) and 5/98 (5%) cases of TCC respectively. Only 2/107 (1.86%) cases with non neoplastic bladder lesions showed positivity for HPV – 33. The relative risk was found to be 7.5 and 95% CI: 1.7 to 32. A significant association of HPV was seen in TCC ($p = 0.0063$).

Conclusion: The frequency of HPV in our study was not high but it might be one of the important risk factors in this malignancy. No significant association of HPV was seen with other epidemiological risk factors except smoking for induction of urinary bladder carcinoma but its detection may indicate its presence and may be prevented by vaccination.

Key words: Human papilloma virus; Bladder carcinoma, Transitional Cell Carcinoma.

INTRODUCTION

Urinary bladder cancer (UBC) ranks the fourth most common cancer in men and ninth in women in the United States, accounting for an estimated 70,980 new cases in 2009.¹

The incidence in female is about 3 times lesser than in the males but the frequency of this malignancy looks to be increasing.^{1,2} Similarly, this malignancy is not uncommon in Pakistan and its incidence is rising in females using smokeless tobacco in rural areas in our setup.³

The rising incidence of urothelial carcinoma has been seen over the past 3 decades due to a number of etiopathological factors. Some most important associated factors are occupational exposure to certain che-

micals carcinogens (aniline dyes). Some other related agents could be cigarette smoking, bacterial and parasitic infections, human papilloma virus (HPV) infection, stones and radiation.⁴⁻⁶

In a study, it was seen that HPV DNA was positive in 81% urothelial bladder carcinomas and 33% of normal urinary bladder specimens, while the types 16, 18, and 33 were found significantly in 62% cases of carcinoma as compared to 14% of normal.⁷

The choice of specimen and the different techniques performed seem to influence the number of false-positive and false – negative results, e.g., the use of single or multiple tumor fragments, fresh or fixed and paraffin – embedded material, the number of virus genotypes, contamination by plasmid DNA or polyme-

rase chain reaction (PCR) products, and the use of high – or low – sensitivity techniques (Southern blot, PCR, or In situ hybridization).⁸

The high – risk HPV type 16 and 18 genomic sequences have been isolated in the female patients of recurrent urinary tract infection and bladder chronic infections and in transitional cell papilloma and carcinoma of the bladder.^{5,6,9-11}

The current study was designed to detect the HPV-16 and HPV18, by PCR in urinary bladder carcinomas and to see whether there is any association with other epidemiological risk factors.

PATIENTS AND METHODS

Study Design: This was a case control study.

Study Settings: It was conducted with collaboration of two universities, Kagoshima University Japan, and King Edward Medical University, Lahore, Pakistan from January 2006 to December 2010.

Participants: It was conducted on 114 patient's biopsies collected mainly from Mayo Hospital, Lahore but specimens were also collected from Bahawalpur, Multan and Islamabad. One hundred and seven biopsies from non-neoplastic lesions of urinary bladder were used as control cases.

Patients Consent: The Declaration of Helsinki was followed for each case and control. Patients were diagnosed clinically by different urologic symptoms and / or signs, transurethral cystoscopic examination followed by histopathology of biopsies, or resected bladder masses.

Study Protocol: The bladder tissue biopsies (BTB) and clinical data collections were conducted in a prospective manner as the cases were selected. Bladder biopsies were fixed in neutral buffered formalin and

processed for histopathological examination and WHO / ISUP morphological grades were used. After the evaluation and diagnoses on H&E stained sections, the paraffin embedded tissue blocks were sent to Kagoshima University, Japan for the detection of Genotype of "HPV". Detection of HPV16 and 18 DNA in BTB and buffy coat cells made for real time PCR technique. Total cellular DNA was extracted from BTB according to Bukhari, et al.¹²

Statistical analysis: Statistical evaluation was done with SPSS 16 software for data management and analysis. Relative risk, sensitivity, specificity, and predictive values of different assays were calculated against the results of HPV16 and 18 DNA in bladder tissue by PCR. Fisher's exact test was used to examine the statistical significance of the results. P values presented are two sided.

RESULTS

There were 92 males and 22 females with ratio 4.2:1. The malignancy was more common in old age (mean age 56.92 ± 13.94 years). There were 78/114 (68.5%) smokers while none of them was a female. Two females were found to be using dry tobacco while 6 patients were habitual of green chopped tobacco (beera) and 4 patients were betel user. Seven patients gave the history of pesticide exposure during farming. Seventy (61.5%) patients repeatedly got urinary tract infection in their lifetime while 18 (15.8%) patients developed bladder cancer with vesical stones. Twenty nine patients used to dye their hair, 11 (9.6%) patients were professionally exposed to petrochemical agents, and 7 (6%) were associated with pesticides production contact used in agriculture (Table 1 – 2).

DNA was detected from 98 samples (86%) but HPV was isolated only in 16 cases. No virus was detected

Table 1: Clinical features and history of the patients with urinary bladder carcinoma.

Serial number	Age (Years)	Sex – N = 114 (4.2:1)		Tobacco Association 86/114 (75.5%)			Urinary Bladder carcinoma				
		Males	Females	Cigarette Smoking	Green Chopped Tobacco (Beera)	Dry Tobacco (Niswar)	LMP TCC (9.6%)	LG TCC (31.5%)	HG TCC (55%)	AC (1.7%)	SCC (1.7%)
"HPV" Negative	55.98 ± 13.42	78	20	64	5	1	10	30	54	2	2
"HPV" Positive	62.625 ± 16.11	14	2	14	1	1	1	6	9	0	0
Total and Mean	56.92 ± 13.98	92	22	78	6	2	11	36	63	2	2

Key Notes: AC = Adenocarcinoma, SCC = Squamous Cell Carcinoma, TCC = Transitional Carcinoma, LG = Low grade, HG = High Grade, LMP = Low malignant Potential.

Table 2: Etiological features of patients with Urinary Bladder carcinoma.

	Clinical features					Epidemiological					
	Pain	Hematuria	Mass	Dysuria	Obst,	UTI	Hair Dying	PTC	Stone	FH	Pest
"HPV" -ve	7	98	88	13	52	56	25	8	17	0	6
"HPV" +ve	5	16	15	16	7	14	4	3	1	0	1
Total	12 (10.5%)	114 (100%)	103 (93%)	29 (25.5%)	59 (51.7%)	70 (61%)	29 (25.5%)	11 (6.7%)	18 (15.7%)	0	7 (6%)

Key Notes: FH: Family History, UTI = Repeated Urinary tract infection, Obstruction = Urine obstruction, PTC = Petrochemical exposure, Pest: Pesticide exposure

ted in control samples. High risk HPV 16 and 18 was detected in 11/98 (11%) and 5/98 (5%) cases of transitional cell carcinoma (TCC) respectively. The relative risk was found to be 7.5 and 95% CI: 1.7 to 32. A significant association of HPV was seen in TCC ($p = 0.0063$) as compared to control groups (Table 3).

The histopathological grade of tumor specimens considering the sex and HPV positivity are shown in (Table 1). A positive relationship between the tumor grade and the presence of HPV was found as it was more common in high grade tumors as compared to low grade TCC. The HPV was detected in one (0.9%) urothelial tumor of low malignant potential, six (5%) low grade TCC and nine (8%) high grade TCC respec-

Table 3: Positive Samples Analyzed by type specific Primers.

HPV	Positive	Negative	Total
Cases	16	98	114
Controls	2	105	107
	18	203	221

Relative Risk: 7.5 and 95% CI: 1.7 to 32, $P = 0.0063$

Table 4: Clinical features and history of the patients with positive HPV.

Serial Numbers	Age	Males	Female	FH	Profession	Smoking	Green Chopped Tobacco (Beera)	Dry Tobacco (Niswar)	Pesticide
1.	50	0	1	0	House wife	0	0	0	0
2.	60	0	1	0	House wife	0	0	1	0
3.	60	1	0	0	Laborer	1	0	0	0
4.	72	1	0	0	Laborer	1	0	0	0
5.	70	1	0	0	Driver	1	1	0	1
6.	45	1	0	0	Laborer	1	0	0	0
7.	60	1	0	0	Farmer	1	0	0	0
8.	95	1	0	0	Laborer	1	0	0	0
9.	95	1	0	0	Laborer	1	0	0	0
10.	75	1	0	0	Laborer	1	0	0	0
11.	50	1	0	0	Laborer	1	0	0	0
12.	65	1	0	0	Laborer	1	0	0	1
13.	55	1	0	0	Laborer	1	0	0	0
14.	54	1	0	0	Laborer	1	0	0	0
15.	36	1	0	0	Petrochemical	1	0	0	0
16.	60	1	0	0	Teacher	1	0	0	0
Total and Mean	62.625 ± 16.11	14	2	0		14	1	1	1

Table 5: Morphological features of patients Positive for HPV.

Sr. No.	Pain	Hematuria	Mass	Dysuria	Obstruction	Repeated UTI	Hair Dying	Petrochemical	Stone	FH	Profession
1.	1	1	1	1	0	1	0	0	0	0	House wife
2.	1	1	1	1	1	1	0	0	0	0	House wife
3.	0	1	1	1	1	1	1	0	0	0	Laborer
4.	0	1	1	1	1	1	1	0	0	0	Laborer
5.	0	1	1	1	0	1	0	1	0	0	Driver
6.	0	1	1	1	0	0	0	0	1	0	Laborer
7.	1	1	1	1	1	1	1	0	0	0	Farmer
8.	1	1	1	1	1	1	0	0	0	0	Laborer
9.	0	1	1	1	0	0	0	1	0	0	Laborer
10.	0	1	1	1	0	1	0	0	0	0	Laborer
11.	0	1	1	1	0	1	0	0	0	0	Laborer
12.	0	1	1	1	0	1	1	0	0	0	Laborer
13.	1	1	1	1	0	1	0	0	0	0	Laborer
14.	0	1	1	1	1	1	0	0	0	0	Laborer
15.	0	1	0	1	0	1	0	1	0	0	Petrochemical
16.	0	1	1	1	1	1	0	0	0	0	Teacher
Total	5	16	15	16	7	14	4	3	1	0	

ctively. It was not detected from squamous cell carcinoma and adenocarcinoma of urinary bladder. The frequency was more in males (14) as compared to females (7) with ratio of 2:1. The age was same as non-HPV group with mean age 62.62 ± 16.11 (Table 1 – 6).

Histopathology of all sections was done before performing the PCR for HPV (Figure 1).

DISCUSSION

Age, gender, tobacco smoking and infections are the main known associated risk factors to urothelial carcinomas in men and a sizable proportion in women. There is a linear relationship between smoking and risk, and quitting smoking reduces the risk.¹³⁻¹⁴

Gender is the main risk factor in our study, as the tumor is more common in males with a ratio of 4.2:1; the ratio was high in “HPV” group. The findings are consistent with Eslami, et al (2008) who found higher rates in males as compared to females.¹⁵

Another important risk factor in our study was old age because the risk of bladder carcinoma was increased with advancing age. All of our patients were above 30 years and maximum age was 95 years.

Environmental factors like smoking (cigarettes, cigars or pipes) could increase the risk of bladder car-

cinoma by causing harmful chemical carcinogens to concentrate in the bladder that may build-up the risk of developing urothelial tumors. In our study 78/114 (68.5%) patients were smoker while 2 were dry tobacco user and 6 were green tobacco habitual. Our results are consistent with the findings of Mousavi, et al, who observed that the tobacco smoking and infections are the important risk factors for the bladder tumors.¹⁶

The Chronic and repeated urinary infections or inflammations (cystitis), may boost the risk of a urinary bladder cancer.¹⁷ In our study 70/114 (61.5%) cases gave the history of non-specific infection and cystitis and 18/ 114 (15.8%) cases were bearing vesical stone. These findings are also consistent with other studies as the vesical stones and repeated infections are considered risk factors of urinary bladder carcinoma.¹⁷

In our study, 29 patients used to dye their hair, 10 patients were professionally exposed to petrochemical agents, and 7 were associated with pesticides production contact used in agriculture. These risk factors have also been reported by Cassidy, et al.¹⁸

The frequency of HPV in our study is not so high but is one of the important risk factors in this malignancy. In the study, the high risk HPV types 16 and type 18 were isolated from 11% and 5% cases of uro-

thelial carcinomas respectively. The virus was not seen in adenocarcinoma or squamous cell carcinoma. Our findings are consistent with others studies who have isolated that HPV from the bladder carcinoma.¹⁷⁻¹⁹

Our study also correlates with Cassidy, et al that there may be possible relationship because a significant difference was seen between urinary bladder cancer of non “HPV” group and with “HPV” positive patients. Only in 16 patients HPV was detected while 98 bladder cancers were negative.¹⁸

The frequency of HPV in our study is low as compared to other areas and genotypic expression of HPV is also not consistent with the other studies. In a study by Anwar, et al, the presence of high – risk HPV (types 16, 18, and 33) was increased significantly in carcinoma cases (62%) as compared with normal specimens (14%); ($p < 0.01$). Similarly, multiple HPV infections were significantly higher in patients with carcinoma (60%) as compared to the normal tissues (5%) ($p < 0.01$).²⁰

In another study, HPV – DNA was isolated from 48.97% cases and the majority of which (64.6%) were of type 16 (48%) prevalence of HPV in urinaryBladder carcinoma in Egypt.²¹

The frequency is higher than some other investigators, like Mvula²² who found HPV in 3% cases while Tekin found 4.8% frequency in urinary bladder carcinoma,²³ and Simoneau reported low rate of HPV detection (8.5%) in initial tumors.²⁴

The rising incidence of “HPV” in this and many other studies supports a possible etiologic role for HPV in the pathogenesis of bladder carcinoma. Our findings are consistent with Westenend, that HPV has no role in the carcinogenesis of squamous cell carcinoma bladder.²⁵

In our study HPV 16 was more common as compared to “HPV” 18 and no other genotype was seen. Our findings are similar to some other studies where type 16 was more common.²⁶⁻³⁰ It was common in males however no significant difference was seen in the age of both groups.²⁰

It is **concluded** that although the frequency of HPV in our study was not so high yet an association was found as an important risk factor in this malignancy. No association of HPV was seen with other epidemiological factors except smoking.

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Table 6: Morphological features and frequency of “HPV” Genotypes in positive patients.

TCC LMP	LG TCC	HG TCC	B Glo	SP F1-2	SP F10	INNO LIPA	“HPV” 18	“HPV” 16
0	1	0	P	N	P	18	1	0
0	0	1	P	N	P	18	1	0
0	0	1	P	P	N	18	1	0
0	0	1	P	P	N	8	1	0
0	0	1	P	P	N	18	1	0
0	1	0	P	P	N	18	1	0
0	0	1	P	P	N	18	1	0
0	0	1	P	P	N	18	1	0
0	0	1	P	N	P	18	1	0
0	1	0	P	N	P	18	1	0
0	1	0	P	N	N	18	1	0
0	1	0	P	P	N	16	0	1
0	1	0	P	P	N	16	0	1
0	0	1	P	P	N	16	0	1
1	0	0	P	P	N	16	0	1
0	0	1	P	P	N	16	0	1
1	6	9	16	11	4	16	11	5

Key notes:

AC = Adenocarcinoma, SCC = Squamous Cell Carcinoma, TCC = Transitional Carcinoma, LG = Low grade, HG = High Grade, LMP = Low malignant Potential. Innolipa = 165, 000 sites, SPF-10 = 25,500,000 sites, beta – globin = 58,400 sites, Beta globin is a resident gene and beta globin for each sample to know the DNA was present in the sample or not. Those samples with negative B-globin must be cleaned again for DNA detection. SPF-10 PCR is used to detect viral DNA in a sample. Innolipa is the procedure to detect the sub-types of HP – viruses. e.g. HPV-16/18 etc

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Authorship

MA Conceived and Designed the study, MEK reviewed and analyzed the data. MHB helped in manuscript writing and SFI did review and final approval of manuscript. MA takes the responsibility and is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

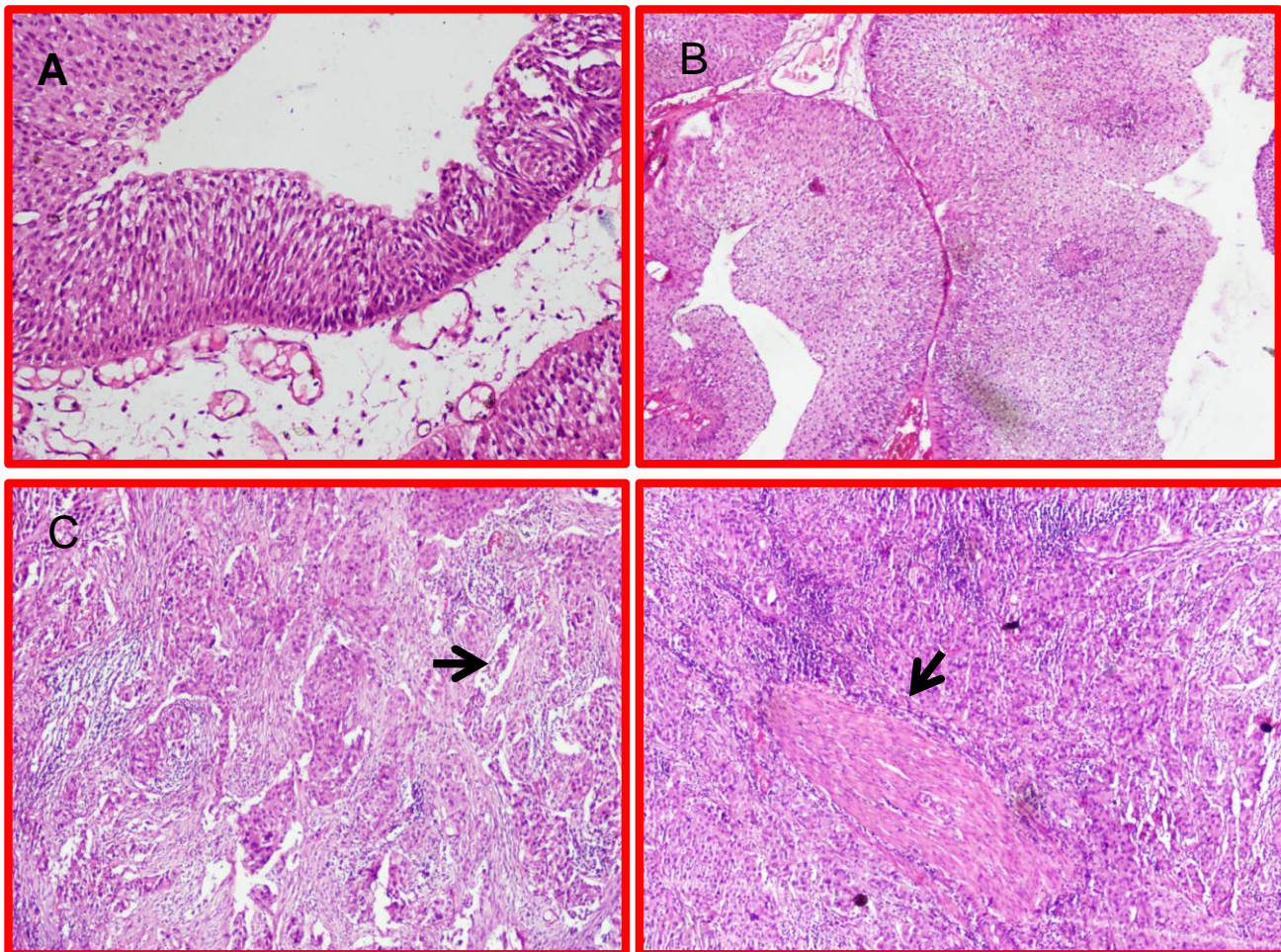


Figure 1: Photomicrographs (H&E;10x) showing: A. Urothelial tumour of low malignant potential, B. Papillary urothelial carcinoma, low grade; C. and D. Papillary urothelial carcinoma, High grade with lymphovascular invasion in C and detrusor muscle in D (arrows).

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