Review Paper

Volume - 1 | Issue - 1 | January - 2017

FREQUENCY OF HUMAN PAPILLOMA VIRUS IN IRANIAN PATIENTS WITH BLADDER CANCER, KERMAN, SOUTHEAST OF IRAN

Reza Malekpour afshar — Stem cell Research center , Kerman University of Medical Sciences,kerman ,Iran.

Arezu Naeim Abadi — Msc In Medical Virology, Department Of Medical Microbiology, Kerman University Of Medical Sciences, Kerman; Iran.

Hamid Reza Mollaei. — Assistant Professor In Medical Virology, Department Of Medical Microbiology, Kerman University Of Medical Sciences, Kerman; Iran

Seyed Alimohammad Arabzadeh. — Associated Professor In Medical Virology, Department Of Medical Microbiology, Kerman University Of Medical Sciences, Kerman; Iran

ABSTRACT To investigate the frequency of human papillomavirus (HPV) in the bladder transitional cell carcinoma (TCC),One hundred twenty paraffin embedded TCC samples were tested for the presence of HPV DNA by Real Time PCR. A consensus MY09/MY11 primer mediated for SYBR Green Real Time PCR technique, and the products were analyzed for specific melting cure. out of 102 samples with TCC, 12 samples (11.76%) were positive for HPV DNA. HPV DNA was detected in patients older 50 years old age. HPV types 16, 18 were high frequency types (32%) in positive samples. The prevalence of HPV DNA in men was almost five times that of women, with ten samples in men (83.3%) and two in women (16.7%) were positive for HPV DNA. These results suggest that HPV can to play an etiological role in the development of urothelial bladder carcinoma. The association of HPV with urothelial bladder carcinoma can vary in different geographical locations. Most studies reporting a high prevalence of HPV in southern Europe or Asia in urothelial bladder carcinoma samples, whereas most carried out in northern Europe and America reported an extremely low rate of HPV in these patients. Therefore, comprehensive and complete studies with more sample size and methods such as microarrays are suggested.

KEYWORDS

Bladder cancer, transitional cell carcinoma, polymerase chain reaction, human papillomavirus

ARTICLE HISTORY

Received : 03 November 2016 | **Accepted : 12 December 2016** | **Published : 05 January 2017**

***Corresponding Author** Hamid Reza Mollaei.
Assistant professor in medical virology, Department of Medical Microbiology, Kerman University of Medical Sciences, Kerman; Iran hrmollaei2015@gmail.com

INTRODUCTION

After deaths with cardiovascular disease, cancer is the second most common cause of non-accidental deaths in Iran , and every six months about 51,000 new cases are detected (1). Bladder cancer is one of the most common types of urinary tract cancer. Not only is it one of a variety of cancers in the umbilical cord blood cells, it is also the deadliest type of urinary tract cancer, and is the second most commonly reported genitourinary tract that grows out in the absence of timely treatment and spreads out of the bladder. The incidence of bladder tumors in men is much higher than in women, but in women, the risk of mortality is 30% higher than in men. Bladder cancer is rare in people younger than 50 years old and the average age of the disease is about 65-70 years (2).The majorities (> 80%) of bladder cancers are of the epithelial transitional type and according to the latest WHO / ISUP pathology classification, this cancer is classified into three categories: Urothelial Neoplasm of Low Malignant Potential (UNLMP), Low Grade Papillary Urothelial Carcinoma (LGPUC) and High Grade Papillary Urothelial Carcinoma (HGPUC),that two last are malignant.(2, 3). In studies in Iran, more than 92% of bladder cancers are transitional type. In the Western countries, up to 80% of bladder cancers are transitional cell carcinoma type (TCC) as the second most common is of Squamus cell carcinoma (Squamus Cell Carcinoma)(4).Several identified risk factors for bladder cancer include environmental carcinogens, smoking, and chronic parasitic infections (such as schistosoma hematobium) . Viral infections in human cancers is an interesting topic, and so far, few viral species have been identified as human tumor viruses, including human papillomavins (HPV), hepatitis C virus (HCV), EBV (Epstein-Barr virus), HTLV1 (Human T Cell Leukemia virus)(5).According to studies, approximately 10% of all cancers in the world, such as bladder cancer, are associated with HPV infection (6). The HPV carcinogenic potential is due to the ability to penetrate and integrate into the host DNA, to stay in the cell cycle, and to stop the function of the P53 and retinoblastoma protein (PRb), which are tumor suppressor proteins (cell cycle inhibitors) (7-9).Human Papilloma virus (HPV) is a small, double stranded containing a circular DNA ,an icosahedra symmetric capsid without envelope , and a size of 52-55 nm(4). HPV has more than one hundred types of viruses and has been found in more than 20 different mammalian species, although many of its types, like type 6 and 61, 34 and 33, cause benign illnesses, but the main and high risk types like 16 and 18 types that play a major role in tumorigenicity(10, 11).The genome of the papilloma virus stays about 10 proteins, the initial protein (E1-E8) and the 2 delayed proteins (L1-L2) .The carcinogenesis of the HPV virus is dependent on two proteins E6 and E7encoded by HPV(12). E6 and E7 proteins in HPV high risk types by binding to P53 and PRb molecules cause them to be inactivated, as a result, cell proliferation will eventually lead to cancer(13). The genome of HPV virus is usually integrated into the host cell genome and the insertion site of the DNA virus is important. The region where the virus DNA is interrupted during the virus integration site is almost constant and is almost identical to the open-reading frame of the E1/E2 viral genome. E2 is a region of the viral genome that suppresses the transcription of the E6 and E7 genes (14, 15).When the virus enters the cell, if the E2 gene is discontinued, it causes high expression of E6 and E7 genes. The E6

protein binds to and suppresses p53. E7 binds to the tumor suppressor protein PRb. So the E6 and E7 proteins from HPV eliminate the two important tumor suppressor proteins that regulate the cell cycle (16-18). Human papillomavirus infection is a necessary but not sufficient condition for the formation of neoplastic processes of various organs after a long period of infection with this virus and the known carcinogenicity of E6 and E7 human papillomavirus with cell cycle interactions and multiple stimulation the molecule results in the malignancy of the virus-infected cell(19).Several previous studies have looked for an association between HPV infection and bladder TCC development. However, the wide range of frequency detected, varying between 0% and 81%, has meant that the role of HPV in bladder carcinogenesis remains controversial(20). For detectection of HPV DNA virus in cancerous tissues, different laboratory techniques with different sensitivity and specificity can be used. In current study, the Real Time PCR molecular method is used, based on viral replication, and very low amounts the virus is even recognizable as a copy of the infectious agent, as well as a quick and sensitive method for diagnosis. If HPV is proven to be a risk factor for bladder cancer, prophylactic measures such as vaccination and the use of appropriate antiviral drugs in the health plan of contaminated communities should be considered. Extensive studies have been done to determine the prevalence of HPV infection and cancer. In current study, we also intend to examine the role of HPV in bladder cancer, and given that no same study has ever been carried out in this geographical area, its results can be important for health authorities, professionals and researcher (3, 21).

MATERIAL AND METHODS

Sample collection:

Samples were provided with 102 paraffin blocks of bladder cancer (Transitional cell carcinoma) paraffin blocks during July2015 to February 2017, in the pathology department of Shahid Bahonar Hospital in Kerman. Paraffin embedded blocks were processed using xylen for remove of paraffin that possibility of isolating DNA for HPV detection assays. This method has received approval for clinical use from the U.S. Food and Drug Administration.

Deparaffination samples

Paraffinated blocks from the 100 tumor samples and 20 no tumor samples were cut in 5 –µm sections and 6 sections, patients were collected in the same micro-centrifuge tube. Samples were de-waxed in 500 µl xylen; all micro-centrifuge tube located for 10 min in a 60 °C heated block and centrifuged at 10,000 rpm for 1 minute, supernatant was removed. This step was then repeated three times. Add 500 µl absolute ethanol, centrifuge at 10,000 rpm for 1 minute, the samples were then dried in a 70°C heated block with open lids for 5-10 min for remove residual ethanol.

Tissue digestion

According to samples (biopsy or Paraffinated blocks), 200-400 µl of Tissue Lysis Buffer was added to each tube [4 M Urea, 200 mMTris, 20 mMNaCl, 200 mM EDTA; PH=7.4 (25°C)]. To all tubes added 20-40 µl proteinase K, Samples were gently vortexes and located for 10 min in a 60°C heated block, and all samples were subsequently incubated at 37°C overnight.

DNA Extraction

The next day, 200 µl of Binding Buffer [6 M Guanidine- Hcl, 10mM Urea, 10mM Tris-Hcl, 20% Tritonx-100 (v/v); PH=4.4 (25°C)] was added to each tube with gently vortex. DNA was isolated using a QIAamp DNA Mini kit (Qiagen, Germany) according to the manufacturer's instructions. Extracted DNA pellets were suspended in 70µl of pre-warmed Elution buffer and stored at -70°C until use.

Qualitative Real Time PCR

After DNA extraction, for detection and screening positive samples with HPV, a qualitative Real time PCR based on SYBR Green was done. The primers used in this study were general primers from L1 region of HPV: MY09 and MY011 pairs (MY09: 5'-CGT CCM AAR GGA WAC TGA TC-3' and MY011: 5'-GCM CAG GGW CAT AAY AAT GG-3').

INNO-LiPA HPV detection and genotyping

After performing a PCR test and identifying positive samples, the Innolippa test was performed to identify the types of HPV. The INNO-LiPA® HPV Genotyping Extra II (Fujirebio Diagnostics, Sweden) kit was used for this experiment, the steps of which have been described previously in detail (22).

Statistical analysis

Chi square and Fisher's exact Tests were used to analyze the data obtained by SPSS 11.5 software (SPSS Inc, Chicago; USA). The differences or association with $p<0.05$ were considered statistically significant.

RESULTS:

Out of 102 confirmed bladder cancer cases, 19.6% (20 cases) were female and 80.4% (82 cases) were male. Mean of age were 63.65±12.9 years old age .The age range was 27 up to 84 years. Of which 2% were less than 30 years old, 2%were 30-40 years, 11.8%, 40-50 years old, 18.6% were 50-60 years old and 65.7% were more than 60 years old (Table1) . Out of 102 samples, 62 cases (60.78%) with low grade bladder cancer and 40 cases (39.21%) were high grade. Out of 62 low grade samples, 10 samples (9.8%) were female and 52 samples (50.98%) were male. Also, out of 40 samples with high grade, 10 samples (9.8%) were females and 30 samples (29.41%) were male. L1 primer was used to isolate HPV infection in all samples of bladder tissues in Kerman province. In this study, the distribution of HPV positive in bladder cancer samples is as follows: out of the 20 samples (19.6%) in the women's group, only two samples (1.96%) were positive for HPV, both of which were high grade and out of 82 (80.39%) samples of bladder cancer in men, 10 samples (9.8%) were positive for HPV, and 8 samples (80%) were in the high grade group. As seen, the incidence of bladder cancer in men of Kerman province (sample collection area) was four times higher than in women. Also, rate of infection caused by HPV in men was five times higher than of women. In this study; there was a significant relationship between bladder cancer and sex (P.Value =0.002), Also, there was a significant relationship between HPV and gender, which was observed in men five times as much as women (P.Value =0.008). The distribution and frequency of different HPV genotypes was shown in Figure 1 and, as observed, HPV genotypes 16 and 18 were the most frequent.There was no significant relationship between type of HPV genotype and bladder cancer (P.Value =0.542).All twelve positive HPV cases were more than 50 years old. There were 4 of 12 positive samples (33.34%)were between the ages of 50 to60 years and 8 of 12 positive samples (66.66%) were older than 60 years old age. There was a significant relationship between age and bladder cancer, as the age increases, the odds of developing bladder cancer increase. Out of 82 male subjects, 10 cases were positive for HPV, and from 20 female samples, 2 cases were positive for HPV. Out of 102 samples, 88.24% were HPV negative and 11.76% were positive for HPV. In different age groups, the results were as follows: less than 30 years old 2 cases of HPV negative (1 case of low grade and 1 high grade), 30-40 years group ; 2 cases were negative for HPV (both of low grade), 40-50 years old, 12 cases were negative (4 cases of high grade and 8 of low grade), 50-60 years old age, 4 cases were positive and 15 cases were negative for HPV (3 high grade and 16 low grade) andin more than 60 years old group , 8 samples were positive and 59 cases were negative for HPV (32 high grade and 35 low grade) There was no significant relationship between HPV and age groups of bladder cancer samples (P.Value =0.84).(Figure2).

DISCUSSION:

Bladder cancer is the most common Urothelial cancer in the world, especially in some parts of the world, such as Africa. It is the fourth most common cancers in men and the eighth most common cancer in women, and is more common in males two to three times(23). In the United States, 38,000 males and 15,000 women have been diagnosed with this cancer per year. Several carcinogens have been reported for this cancer, such as smoking, alcohol, long-term use of drugs, contact with chemicals, schistosoma and genital HPV infection, the best known risk factor is exposure to aromatic amines(24, 25). Among these factors, the role and association of the HPV infection is important because it can make the Condyloma acuminatum(an anogenital wart) in the genital and bladder region. It is a sexually transmitted disease and can be a risk factor for changes in bladder epithelium and as a result of bladder carcinoma(26). The HPV replication cycle depends on specific factors that exist in the sequential epithelial cell differentiation, as a result of which the virus has a particular tendency to epithelial cells and mucous membranes, and by expressing its oncogenes its role is contributing to the creation of many human cancers (15, 27). In our study, we examined the possible etiological role of HPV in bladder carcinogenesis using Real Time PCR. Out of 102 TCC samples, 12 samples (11.76%) were positive against HPV L1 capsid protein by Real Time PCR.Of these, two samples (1.96%) were female

and 10(9.80%) samples were male. Also, 4(3.92%) cases were in the range of 50 to 60 years old, and 8(7.84%) cases were in the age range of more than 60 years. Recent studies have shown that 15% of the existing cancers are due to the infection of the oncogenic viruses, which presence of papillomavirus is commonly found in human tumors, as it can be an etiologic factor of cancers. Also, the presence of HPV DNA in the urogenital system is an important factor for bladder cancer, as one of the most common cancers in developed countries, shows that it's potential as a carcinogenic factor in this cancer (4, 18). Therefore, due to the high prevalence of bladder cancer especially in the elderly population (over 50 years old age) and Severe bladder cancer treatment, we decided evaluate frequency of HPV and its corresponding types in patients with bladder cancer using the Real-Time PCR technique. Several previous studies have looked for an association between HPV infection and bladder TCC development. However, the wide range of frequency detected, between 0% and 81%, which mean role of the HPV in developing bladder cancer remains controversial(11). In a meta-analysis study in 2011 ,an HPV prevalence of 16.88% (95% confidence interval [CI], 15.53%–18.31%) among the bladder cancer cases was shown, also ,a significantly increased risk of bladder cancer was shown for the positivity of overall (20).In 2005, Youshya et al. investigated the role of HPV infection in TCC in the bladder by comparing PCR and IHC in 78 patients with TCC in the bladder. In these two methods, different results were obtained. In the IHC method, 47 out of 78 cases were positive for the HPV antigen, while none were positive for HPV-DNA. They concluded that it is unlikely that HPV would have a role in the TCC etiology (21).In 2011, shigehara and colleagues investigated the etiologic role of HPV infection in bladder cancer. They studied 224 cases of bladder cancer patients between 1997 and 2009. They developed the emergence of HPV-DNA on bladder frozen TUR samples by PCR method And HPV-L1 examined the HPV capsid protein by IHC and finally concluded that high risk HPV types are a cause of some types of lower grade bladder cancer in younger patients (28).In 2012, Barghi and his colleagues examined the relationship between HPV infection and TCC bladder. They examined 59 cases of bladder TCC and 20 normal controls as controls by PCR for HPV-DNA, and eventually concluded that it is likely that the HPV infection in their geographical area contributes to the formation of TCC in the bladder.In 21 samples (35.6%) of the bladder cancer tissue, HPV type 18 was the most common type of virus in these studies(29).In another study in the Department of Dermatology of the University of Northwestern in 2003 about 74 cases of bladder SCC, 10% included subtypes of HPV and according to this study, the presence of HPV in patients with bladder SCC tumors and under Surgical treatment is more likely(30).In 2012, at the Department of Pathology, the University of Indiana Laboratory in the United States, a total of 42 SCC and 27 Urothelial carcinomas were evaluated for expression of the p16 gene expression and also the presence of HPV DNA, although p16 in 13 cases of SCC and 9 cases of Urothelial The isolated HPV DNA was not isolated and it was concluded that there is no correlation between expression of P16 and the presence of HPV infection in SCC of bladder and Urothelial, and P16 should not be used as a control successor marker for HPV infection(31).In 2011, a study was conducted in China on 52 bladder cancer specimens and 19 control samples. This study was performed on the parameters of the study area, the histological type, the HPV DNA sample, and the isolation method. Eventually, an outbreak of 16.88% (with a 95% confidence interval) is among the bladder cancer cases and the HPV virus, especially its high-risk types (especially HPV16 type)(20).As seen in some studies, Many earlier investigations have reported a low prevalence of HPV in TCC and there is no relationship, Nevertheless, a few studies have reported higher incidences of HPV positivity among bladder TCCs, So;there is a significant relationship between HPV and bladder cancer. The dissimilarity in HPV prevalence reported by these investigations suggests that the association of HPV with bladder TCC may vary with different geographical locations. This is supported by the fact that most studies reporting a high incidence of HPV positive samples were performed in southern Europe or Asia, whereas most carried out in northern Europe and America reported an extremely low rate of HPV.These results suggest that in addition of appropriate sample size, use of high sensitivity and specificity diagnostic methods is necessary to examine the role of HPV virus in developing of bladder cancer (4, 12, 32, 33).

CONCLUSION:

Considering the importance role of HPV virus in development of malignancy. The transmission routs of HPV, transmitted through sexuality, could be subject for other studies on the role of the HPV virus in the development of genital / urogenital tumors including bladder, it can be useful and necessary.

ACKNOWLEDGMENT:

The authors of this project are grateful to Physiology research center of Kerman University of medical sciences and staff of Laboratory pathology in Shahid Bahonar Hospital for their cooperation in collecting samples.

Conflict of Interest: There is no conflict of interest.

Funding/Support: This study is a part of a MSc thesis that financially was supported by Physiology research center, Kerman University of Medical Sciences.

Ethics Statement

The protocol of the study was evaluated and approved by the ethics and research committee of Kerman University of Medical Sciences with the approval number IR.KMU.REC.1395.944. Written informed consent was provided by all adult participants, and for minors, it was obtained from their parents.

Table 1:Age Group distribution in HPV positive and HPV negative

Age Group	HPV positive (%)	HPV Negative (%)	Total (%)	P.Value
20-40	0(0%)	4(3.92%)	4(3.92%)	0.446*
40-60	4(3.92%)	27(26.47%)	31(30.4%)	
60-80	8(7.84%)	59(57.84%)	67(65.71%)	
Total	12(11.76%)	90(88.24%)	102(100%)	

*There was not significant relation between age and Bladder cancer (Pearson Chi-Square)

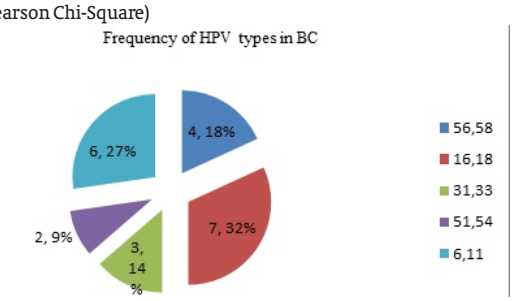

Figure1: Frequency of HPV types in Bladder cancer tissues

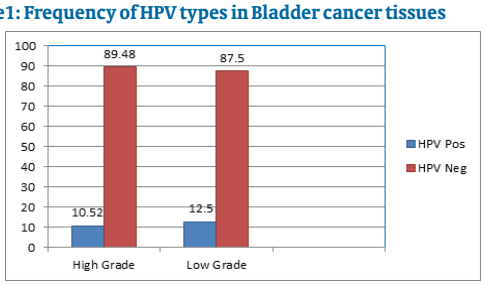

Figure2: Frequency and percent of HPV in different types of Bladder cancer tumor

REFERENCES:

1. Yahyapour Y, Shamsi-Shahrabadi M, Mahmoudi M, Siadati S, Shahryar SS, Shokri-Shirvani J, et al. Evaluation of human papilloma virus infection in patients with esophageal squamous cell carcinoma from the Caspian Sea area, north of Iran. Asian Pac J Cancer Prev. 2012;13(4):1261-6.
2. Sadjadi A, Nouraie M, Mohagheghi MA, Mousavi-Jarrahi A, Malekezadeh R, Parkin DM. Cancer occurrence in Iran in 2002, an international perspective. Asian Pac J Cancer Prev. 2005;6(3):359-63.
3. Afshar RM, Mollaie HR, Fazlalipour M, Arabzadeh SA. Prevalence and type distribution of human papillomavirus infection using the INNo-Lipa assay, Kerman, Southeast Iran. Asian Pac J Cancer Prev. 2013;14(9):5287-91.
4. Loran OB, Sinyakova LA, Gundorova LV, Kosov VA, Kosova IV, Pogodina IE, et al. [High oncogenic risk human papillomavirus and urinary bladder cancer]. Urologiia. 2017(3):60-6.
5. Reza MA, Reza MH, Mahdiyeh L, Mehdi F, Hamid ZN. Evaluation Frequency of Merkel Cell Polyoma, Epstein-Barr and Mouse Mammary Tumor Viruses in Patients with Breast Cancer in Kerman, Southeast of Iran. Asian Pac J Cancer Prev. 2015;16 (16):7351-7.
6. Hojeij R, Domingos-Pereira S, Nkosi M, Gharbi D, Derre L, Schiller

JT, et al. Immunogenic Human Papillomavirus Pseudovirus-Mediated Suicide-Gene Therapy for Bladder Cancer. Int J Mol Sci. 2016;17(7).
7. Khaled HM, Bahnassi AA, Zekri AR, Kassem HA, Mokhtar N. Correlation between p53 mutations and HPV in bilharzial bladder cancer. Urol Oncol. 2003;21(5):334-41.
8. Kim SH, Joung JY, Chung J, Park WS, Lee KH, Seo HK. Detection of human papillomavirus infection and p16 immunohistochemistry expression in bladder cancer with squamous differentiation. PLoS One. 2014;9(3):e93525.
9. Lopez-Beltran A, Escudero AL. Human papillomavirus and bladder cancer. Biomed Pharmacother. 1997;51(6-7):252-7.
10. Moonen PM, Bakkers JM, Kiemeney LA, Schalken JA, Melchers WJ, Witjes JA. Human papilloma virus DNA and p53 mutation analysis on bladder washes in relation to clinical outcome of bladder cancer. Eur Urol. 2007;52(2):464-8.
11. Offutt-Powell TN, Ojha RP, Tota JE, Gurney JG. Human papillomavirus infection and bladder cancer: an alternate perspective from a modified meta-analysis. J Infect Dis. 2012;206(3):453-4; author reply 4-5.
12. Panagiotakis GI, Papadogianni D, Chatziioannou MN, Lasithiotaki I, Delakas D, Spandidos DA. Association of human herpes, papilloma and polyoma virus families with bladder cancer. Tumour Biol. 2013;34(1):71-9.
13. Khaled HM, Raafat A, Mokhtar N, Zekri AR, Gaballah H. Human papilloma virus infection and overexpression of p53 protein in bilharzial bladder cancer. Tumori. 2001;87(4):256-61.
14. Golovina DA, Trofimova OB, Ermilova VD, Matveev VB, Volgareva GM. Complex of molecular genetic and immunohistochemical methods for detection of human papillomavirus in the bladder cancer epithelium. Bull Exp Biol Med. 2014;157(1):70-3.
15. Griffiths TR, Mellon JK. Human papillomavirus and urological tumours: II. Role in bladder, prostate, renal and testicular cancer. BJU Int. 2000;85(2):211-7.
16. Badawi H, Ahmed H, Ismail A, Diab M, Moubarak M, Badawy A, et al. Role of human papillomavirus types 16, 18, and 52 in recurrent cystitis and urinary bladder cancer among Egyptian patients. Medscape J Med. 2008;10(10):232.
17. Bellanger S, Tan CL, Xue YZ, Teissier S, Thierry F. Tumor suppressor or oncogene? A critical role of the human papillomavirus (HPV) E2 protein in cervical cancer progression. Am J Cancer Res. 2011;1(3):373-89.
18. Golovina DA, Ermilova VD, Zavalishina LE, Andreeva YY, Matveev VB, Frank GA, et al. Loss of Cell Differentiation in HPV-Associated Bladder Cancer. Bull Exp Biol Med. 2016;161(1):96-8.
19. Jimenez-Pacheco A. [Relationship between bladder cancer and infection by human papillomavirus]. Rev Med Chil. 2012;140(3):406-7.
20. Li N, Yang L, Zhang Y, Zhao P, Zheng T, Dai M. Human papillomavirus infection and bladder cancer risk: a meta-analysis. J Infect Dis. 2011;204(2):217-23.
21. Youshya S, Purdie K, Breuer J, Proby C, Sheaf MT, Oliver RT, et al. Does human papillomavirus play a role in the development of bladder transitional cell carcinoma? A comparison of PCR and immunohistochemical analysis. J Clin Pathol. 2005;58(2):207-10.
22. Afshar RM, Mollaie HR, Fazlalipour M, Arabzadeh SA. Prevalence and Type Distribution of Human Papillomavirus Infection Using the INNo-Lipa Assay, Kerman, Southeast Iran. Asian Pac J Cancer P. 2013;14(9):5287-91.
23. Scélo G, Brennan P. The epidemiology of bladder and kidney cancer. Nature Clinical Practice Urology. 2007;4:205.
24. Bulbul MA, Husseini N, Houjaij A. Superficial bladder cancer epidemiology, diagnosis and management. J Med Liban. 2005;53(2):107-13.
25. Burger M, Catto JW, Dalbagni G, Grossman HB, Herr H, Karakiewicz P, et al. Epidemiology and risk factors of urothelial bladder cancer. Eur Urol. 2013;63(2):234-41.
26. Cheluvappa R, Smith DP, Cerimagic S, Patel MI. A comprehensive evaluation of bladder cancer epidemiology and outcomes in Australia. Int Urol Nephrol. 2014;46(7):1351-60.
27. Gutierrez J, Jimenez A, de Dios Luna J, Soto MJ, Sorlozano A. Meta-analysis of studies analyzing the relationship between bladder cancer and infection by human papillomavirus. J Urol. 2006;176(6 Pt 1):2474-81; discussion 81.
28. Shigehara K, Sasagawa T, Kawaguchi S, Nakashima T, Shimamura M, Maeda Y, et al. Etiologic role of human papillomavirus infection in bladder carcinoma. Cancer. 2011;117(10):2067-76.
29. Barghi MR, Rahjoo T, Borghei M, Hosseini-Moghaddam SM, Amani D, Farrokhi B. Association between the evidence of human papilloma virus infection in bladder transitional cell carcinoma in men and cervical dysplasia in their spouses. Arch Iran Med. 2012;15(9):572-4.
30. Alam M, Caldwell JB, Eliezri YD. Human papillomavirus-associated digital squamous cell carcinoma: literature review and report of 21 new cases. J Am Acad Dermatol. 2003;48(3):385-93.
31. Alexander RE, Hu Y, Kum JB, Montironi R, Lopez-Beltran A, MacLennan GT, et al. p16 expression is not associated with human papillomavirus in urinary bladder squamous cell carcinoma. Modern Pathology. 2012;25:1526.
32. Pichler R, Borena W, Schafer G, Manzl C, Culig Z, List S, et al. Low prevalence of HPV detection and genotyping in non-muscle invasive bladder cancer using single-step PCR followed by reverse line blot. World J Urol. 2015;33(12):2145-51.
33. Schmid SC, Thumer L, Schuster T, Horn T, Kurtz F, Slotta-Huspenina J, et al. Human papilloma virus is not detectable in samples of urothelial bladder cancer in a central European population: a prospective translational study. Infect Agent Cancer. 2015;10:31.